THE EFFECT OF TORBANGUN PLANT (*Coleus amboinicus* Lour) ACTIVE SUBSTANCES ON LACTATION IN MICE

AWALLUDIN RISCH

DOCTOR OF ENGINEERING (BIOPROCESS)
UNIVERSITI MALAYSIA PAHANG

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Date: Date:

THE EFFECT OF TORBANGUN PLANT (*Coleus amboinicus* Lour) ACTIVE SUBSTANCES ON LACTATION IN MICE

AWALLUDIN RISCH

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for the award of the degree of
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STATEMENT OF A WARD FOR DEGREE

Thesis submitted in fulfilment of the requirement for the award of the degree of Doctor of Philosophy (Bioprocess Engineering)

SUPERVISOR DECLARATION

I hereby declare that I have checked this thesis and in my opinion, this thesis is adequate in term of scope and quality for the award of the degree of Doctor of Philosophy in Chemical and Natural Resources Engineering (Bio-Process) Universiti Malaysia Pahang

Signature :

Name of Supervisor : Dr. MIMI SAKINAH binti ABDUL MUNAIM

Position : Chemical and Natural Resources Enginering (Bio-Process), Universiti Malaysia Pahang

Date :

Signature :

Name of Co-Supervisor: Drh. M RIZAL DAMANIK M.Rep.Sc, PhD

Position : Technique in Nutrition Research and Metabolism, Institut Pertanian Bogor-Indonesia

Date:

STUDENT DECLARATION

I hereby declare that the work in this thesis is my own except for quotation and summaries which have been duty acknowledged. The thesis has not been accepted for any degree and is not concurrently submitted for award of other degree.

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Name : AWALLUDIN RISCH

ID Number : PKB.08001

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In the name of ALLAH,

I humbly dedicate to

My beloved wife

Mumung Astuti

My lovely sons

Odith Adi Kusuma and Reza Fauzan

Thank you

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ABSTRACT

The main purpose of this research is to investigate the effects of active ingredients in the *Torbangun* plant to the mice lactation milk glands production. *Torbangun* leaves were extracted using soxhlet. The quantitative analysis of the extracted *Torbangun* leaves were analyzed by Gas Chromatography-Mass Spectrometry (GC-MS).The Fourier Transform Infra Red spectroscopy (FTIR) was applied to examine the functional group of the extracted samples; antibiotic screening tests was performed by the paper disk method; total phenolic content, total flavonoid content and antioxidant activity were analyzed using UV Vis-Spectrophotometer; heavy metals content was determined using ICPMS; Clinical trials were carried out on 36 mice which consisted of six control mice and each six mice were treated with 1%, 5%, 10, 15% and 20% of extracted *Torbangun* leave and mammary glands of mice were observed by histological analysis. The results indicated that the compounds in extracted *Torbangun* leave were phenol (PHE), gamma sitosterol, campesterol, Isocholesteryl methyl ether, Octadecanoic acid, Stigmasterol, Benzoic acid, 1,2-Benzenediol, phenol, 3-methoxy-2,4,5-trimethyl and alpha-amyrin; the functional group of extracted *Torbangun* leave were alcohol, ether, carboxylic acids and esters, aromatic rings and alkenes; antibiotic families determined in extracted *Torbangun* leaves were penicillin, tetracycline, aminoglycoside and macrolide; total phenolic content, total flavonoid content and antioxidant activity were 206.087 mg/L, 82.814 mg/L and 3.68 mg/L, respectively and heavy metals content in extracted *Torbangun* leaves were As (0.1 ppm), Pb (2.39 ppm), Cd (0.07), Mg (23.38 ppm) and Cu (15.42 ppm). The results of clinical trials showed that the differences between control mice and mice consumed with extracted *Torbangun* leave (1%, 5%, and 10%) for milk production were not significant, while the differences of the control mice and mice consumed with extracted *Torbangun* leave (15%, control and 20%) for milk production were significant. The histology of mammary glands for mice treated with 1%, 5% and 10% of extracted *Torbangun* leave exhibited equivalent to control mice.. While the mice treated with 15% and 20% of extracted *Torbangun* leave showed a lot of milk in the lobules (mammary gland). In conclusion, the active substances in extracted *Torbangun* leave influenced the function of lactating mammary gland, which consequently may increase milk production and it was proven safe for human consumption.

ABSTRAK

Tujuan utama kajian ini adalah untuk mengkaji keberkesanan bahan-bahan aktif di dalam tumbuhan Torbangun terhadap penghasilan kelenjar susu laktasi. Daun Torbangun diekstrak menggunakan soxhlet. Analisis kuantitatif terhadap daun Torbangun yang telah diekstrak, dianalisa menggunakan Kromatografi Gas-Spektrometri Jisim (GC-MS). Spektroskopi Jelmaan Fourier Inframerah (FTIR) digunakan untuk mengenalpasti kumpulan berfungsi sampel yang telah diekstrak; ujian saringan antibiotik dilakukan dengan kaedah cakera kertas; Kandungan jumlah fenol, jumlah flavonoid dan aktiviti antioksidan dianalisa menggunakan UV Vis-Spektofotometer; Kandungan logam berat dikenalpasti menggunakan ICPMS; Percubaan klinikal dijalankan terhadap 36 tikus yang terdiri daripada enam tikus kawalan dan setiap enam tikus dirawat dengan 1%, 5%, 10, 15% dan 20% ekstrak daun Torbangun dan kelenjar mamari tikus diperhatikan dengan analisa histologi. Keputusan menunjukkan bahawa sebatian dalam ekstrak daun Torbangun adalah fenol (Phe), gamma sitosterol, campesterol, metil eter Isocholesteryl, asid Octadecanoic, stigmasterol, asid benzoik, 1,2-Benzenediol, fenol, 3-methoxy-2, 4,5 - trimethyl dan alpha-amyrin; kumpulan berfungsi bagi ekstrak daun Torbangun adalah alkohol, eter, asid karboksilik dan ester, cincin aromatik dan alkena; famili antibiotik dikenalpasti dalam ekstrak daun Torbangun adalah penisilin, tetracycline, aminoglycoside dan kumpulan macrolide; kandungan jumlah fenol, jumlah flavonoid dan aktiviti antioksidan masing-masing memberikan nilai sebanyak 206,087 mg / L, 82,814 mg / L dan 3.68 mg / L; kandungan logam berat dalam ekstrak daun Torbangun adalah As (0.1 ppm), Pb (2.39 ppm), Cd (0.07), Mg (23,38 ppm) dan Cu (15,42 ppm). Keputusan percubaan klinikal menunjukkan perbezaan penghasilan susu antara tikus kawalan dan tikus yang telah dirawat dengan ekstrak daun Torbangun (1%, 5% dan 10%) adalah tidak signifikan, manakala perbezaan penghasilan susu antara tikus kawalan dan tikus yang telah dirawat dengan ekstrak daun Torbangun (15% dan 20%) didapati signifikan. Keputusan histologi pada kelenjar mamari tikus yang dirawat dengan 1%, 5% dan 10% ekstrak daun Torbangun adalah sama dengan tikus kawalan. Manakala tikus yang dirawat dengan 15% dan 20% ekstrak daun Torbangun menunjukkan banyak susu dalam lobul (kelenjar susu). Kesimpulannya, bahan-bahan aktif di dalam ekstrak daun Torbangun mempengaruhi fungsi kelenjar susu laktasi di mana ianya meningkatkan penghasilan susu dan ianya juga terbukti selamat untuk dimakan oleh manusia.

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LIST OF ABBREVIATIONS

ATCH = Adeno Thyroid Cortocoids Hormone
AG's = Amino glycoside group
As = Arsenic
BNF = Buffer Neutral Formaldehyde
Cd = Cadmium
CNS = Central Nerves System
Cu = Cuprum
DPPH = 2,2-diphenyl-1-picril hydrazil
ECM = mammary epithelial cells
FDA = Food and Drug Administration
FSH = follicle stimulating hormone
FTIR = Fourier Transform Infra Red
GCMS = Gas Chromatography Mass Spectrophotometeer
GH = Growth hormone
ICPMS = (Inductively Coupled Plasma Mass Spectroscopy)
LTH = luteotropic hormone
Mg = Magnesium
ML's = Macrolide group
Pb = Blumbum/ Lead
PC's = Penicillin group
PRL = prolactin hormone
QE = (Quercetin equivalent)
Se = Selenium
TC's = Tetracycline group
TSH = Tyroid stimulating hormone
UV = (Ultra violet)
WHO = World Health Organization

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CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

Torbangun plant (*Coleus amboinicus* Lour) belongs to the family of Lamiaceae and has been used as a breast milk stimulant (lactagogue) by Bataknese people in North Sumatera, Indonesia for hundreds of years. They have a tradition to consume Torbangun leaves after birth and believe that the consumption of Torbangun leaves for one month after birth caused increases their breast milk production. The leaves of Torbangun plant were being consumed in soup meal form, traditionally (Damanik *et al.*, 2004). Torbangun plan is a plant which of WHO accepted its parameters for the identification as medicinal plants (Kaliappan *et al.*, 2008). The ethno botanical use of Torbangun plant is as medicine, to treat digestive, skin, respiratory, genito-urinary, muscular–skeletal condition, pain, infections, fever and other medical (Lukhobaa *et al.*, 2006).

Torbangun plant containing sources of secondary metabolites such as essential oil (Duke, 2000), consisting of phenol derivatives, which includes carvacrol,isopropyl-o-cresol with high antiseptic capability and cineol chemical substance (Devi, 2006). In addition, the plant contains carvacrol, caryophyllene, patchoulane and flavanoids which have a very large drug application (Zhang *et al.*, 2007). There are several compounds known as antimicrobial, antiviral, anti-inflammatory activity and antioxidant, which makes the product available or the pharmaceutical, food and cosmetics (Hole *et al.*, 2008).

Torbangun plant is reportedly can be edible and used as food and food additive. For example, the leaves of Torbangun containing protein, fat, carbohydrates, fiber,

ash, vitamins A, B1, C and total carotene and minerals such as calcium, phosphorus, ferrous (Mahmud *et al*,1990), potassium (Devi, 2006). Leaves of Torbangun are cut, made into flour balls and fried in oil or butter and used in food field (Purseglove, 1987), for flavouring and marinating beef and chicken (Brown,1997), to mask the odour of strong odours such as the smell of goat meat, fish and shellfish (Morton,1992). The leaves are sometimes eaten raw with bread and butter. In India, they can add to the beer and wine (Morton,1992).

In Asia, Torbangun is the species most frequently cited. Overall, Torbangun plant has the widest geographic range occurs outside the continent of Africa and Asia to Americas (Lukhobaa *et al.*, 2006).

1.2 PROBLEM STATEMENT

Torbangun plant consumed by Bataknese women breeding in the last trimester of pregnancy (Damanik *et al*., 2006). However, the use of traditional Torbangun plant is not being well documented, and limited scientific evidence to establish the effects of the active substance in Torbangun plant on lactating mice. The reason for these new applications have been studied from analysis of the compound and extracted with several solvents using soxhlet equipment and injected into Gas Chromatography Mass Spectrophotometer (GC-MS), identified of compound by PCA program, Fourier Transform Infra Red (FTIR), clinical test using lactation mice and histology description in mice mammary glands.

1.3 OBJECTIVE OF THE RESEARCH

The main objective of this research was to determine the effect of Torbangun plant (*Coleus amboinicus* Lour) active substances on lactation in mice. The other goals researches are:

1. Analysis of compound in the Torbangun leaves extracted with several solvents by using soxhlet and injected into Gas Chromatography Mass Spectrophotometer (GC-MS) and analyzed using PCA programs.

2. Analysis of compound in the Torbangun leaves powder using Fourier Transform Infra Red (FTIR).

3. Analysis of the antibiotics effect in Torbangun plant by screening test using microbial culture of *Bacillus subtilis, Mycobacterium luteus, Bacillus cereus* and *Bacillus stearothermophillus* spore or *Calidolactis* (AOAC,1995).

4. Analysis of the level of total phenolics, flavonoids and antioxidants in Torbangun leaves extract at different temperatures and times for optimization purposes. The total phenolic compound being tested for gallic acid standard, the total flavonoid compound test for quercetin standard and antioxidant test with DPPH method performed by screening test using UV-Vis Spectrophotometer.

5. Analysis of heavy metals present in the Torbangun leaves extracted using Inductively Coupled Plasma Mass Spectroscopy (ICPMS).

6. Clinical trials using lactation mice and histology description of mammary gland organ of control mice and treatment mice for prove the effect of Torbangun plant active substances on lactation in mice (Ressang,1984).

1.4 RESEARCH SCOPE

The present research on the effect of Torbangun plant active substance on lactation in mice performed with extracted leaves of Torbangun, as follows:

1. Determine the active substance of Torbangun plant using GC-MS was performed to determineout the compounds and active substances in Torbangun

leaves and the result were analyzed by principal component analysis (PCA) program.

2. Analysis of compound in Torbangun plant using FTIR performed to determine that the compounds of Torbangun plant could be captured by infrared.

3. Optimization of total phenolic, flavonoid compound and antioxidant activity was carried out with design expert and determined using UV Vis-Spectrophotometer to determine where the temperatures and time for optimum concentration of total phenolic, flavonoid compound and antioxidant activity in Torbangun leave.

4. The screening test of antibiotic to find out that Torbangun leaves as medicinal plant including the assets of anti-inflammatory and antibacterial property.

5. Analysis of heavy metals by using ICP-MS performed to detrmine Torbangun plant as medicinal plant, food and food additive safe for consumed.

6. Clinical tests using lactating mice and histology description in mice mammary gland organs.

1.5 SIGNIFICANCE OF THE RESEARCH

The analysis of Torbangun leave powder by using Gas Chromatography Mass Spectrophotometer (GC-MS) found 46 compounds, and then the compounds performed through processing with the principal component analysis (PCA) program. The compounds were phytochemical (phenol and phenol, 3-methoxy-2,4,5-trimethyl), phytoseroid (gamma sitosterol; campesterol, stigma sterol and isocholesteryl methyl ether), octadecanoic acid, benzoicacid,1,2-benzenediol and alpha-amyrin.

1.5.1 Recovery versus Deleterious of Active Substances on Mammary Glands

Phytosterols contained in Torbangun plant offer protection several types of cancer, including breast, colon and prostate, mainly sitosterol and campesterol (Atif *et al.*, 2003). and were function in the cell as a component of the precursor membrane and

fat-soluble vitamins and steroid hormones (Fahy *et al.*, 2005), and plays a role in cell signaling, whereas steroid hormones regulate the development of the mammary gland (Laron *et al.* , 1989). Phytosterols inhibit intestinal absorption of cholesterol, lowering serum cholesterol, reduced risk of cancer, improve urinary tract symptoms associated with benign prostatic hyperplasia, (Karl, 1997). Phytosteroid can also stimulate apoptosis (cell physiology loss mechanism) in breast and prostate cancer (Rao and Janezic, 1992) and (Holtz and Fink, 1998). Under conditions of breastfeeding has increased steroid hormone metabolism with increased production of milk (Neville *et al.*, 2001).

The fatty acids found in plants Torbangun can affect the development of the mammary gland at some stage and prevention of breast cancer influences submarine life cycle (Russo and Russo, 2004).

Although, other active ingredients directly no role in the mammary gland, such as benzoic acid, and alpha amyrin benzenediol, likely the amount is too little.

Effect of damage due to the active substance contained in Torbangun crops in the mammary gland there is no common, but few reported side effects of sterol compounds high-dose occasional mild constipation or diarrhea (Karl, 1997)and heartburn or indigestion, diarrhoea, and nausea (Rubis *et al.*, 2008).

Loss of fatty acids in the body of the person who is easily injured, and bleeding due to the high doses of fatty acids can increase bleeding, cause gas, bloating, belching, and diarrhea (Steven, 2009). Meanwhile, the man who had a heart attack and stroke, saturated fatty acids are major risk factors. A diet high in fatty acids can lead to increased production of cholesterol and together form a precipitate proteins in the body and can occur obesity.

1.5.2 Beneficial versus deleterious of phytochemical (Total phenolic, flavonoid compound, antioxidant and antibiotic) on Mammary Glands

Antibiotic activity contained in Torbangun plants can prevent mammary gland inflammatory disease caused by bacteria. Giving Torbangun during pregnancy or prenatal effective to eliminate infection of the mammary gland during late pregnancy and to reduce the prevalence of mastitis during early lactation and throughout lactation (Oliver *et al.*, 2003).

Benefits of phenolic compounds in the body, especially in the mammary gland can prevent intra-mammary infections during lactation. Compounds called polyphenols (catechins) found in plant protection Torbangun beneficial for degenerative diseases and prevent breast cancer. In addition, the polyphenolic compounds are also useful for preventing cardiovascular diseases, anti-inflammatory, anti-arthritis, antibacterial, anti-angiogenic, antioxidant, antiviral, nerves, and cholesterol lowering effects (Sabu *et al.*, 2010).

Flavonoids are a group of more than polyphenolic compounds naturally present in fruits, vegetables, and beverages derived from plants. Flavonoids are found in Torbangun plants have anti breast cancer, inhibiting aromatize activity, inhibiting tumor cell proliferation and inhibit the formation of reactive oxygen species (Brian *et al.*, 2006).

Antioxidants serve to the best defence against oxidative stress in the newborn and the mother at birth. In addition, the antioxidant found in the mammary gland, and breast milk colostrums can prevent the occurrence of mastitis, or inflammation of the mammary gland (Justyna *et al.*, 2012) and apoptosis (Su *et al.*, 2002). In addition, the antioxidant may prevent damage to cells caused by cancer, aging, and various diseases (Richard, 2003).

In fact, loss of use of antibiotics on the mammary gland is not directly, but the side effects of antibiotics on the body, especially allergic reactions, contribute to cancer, destruction of microflora in the gut, the development of resistant species of micro-

organism, immune suppression, an overgrowth of *Candida albicans* and more dangerous intestinal infections, chronic fatigue syndrome, diarrhea, leading to the loss of essential minerals (Lawrence, 2012).

Based on data from previous studies and to date, it is likely that the phenolic compounds, flavonoids and antioxidants are not harmful to the body and mammary glands (Richard, 2003) .

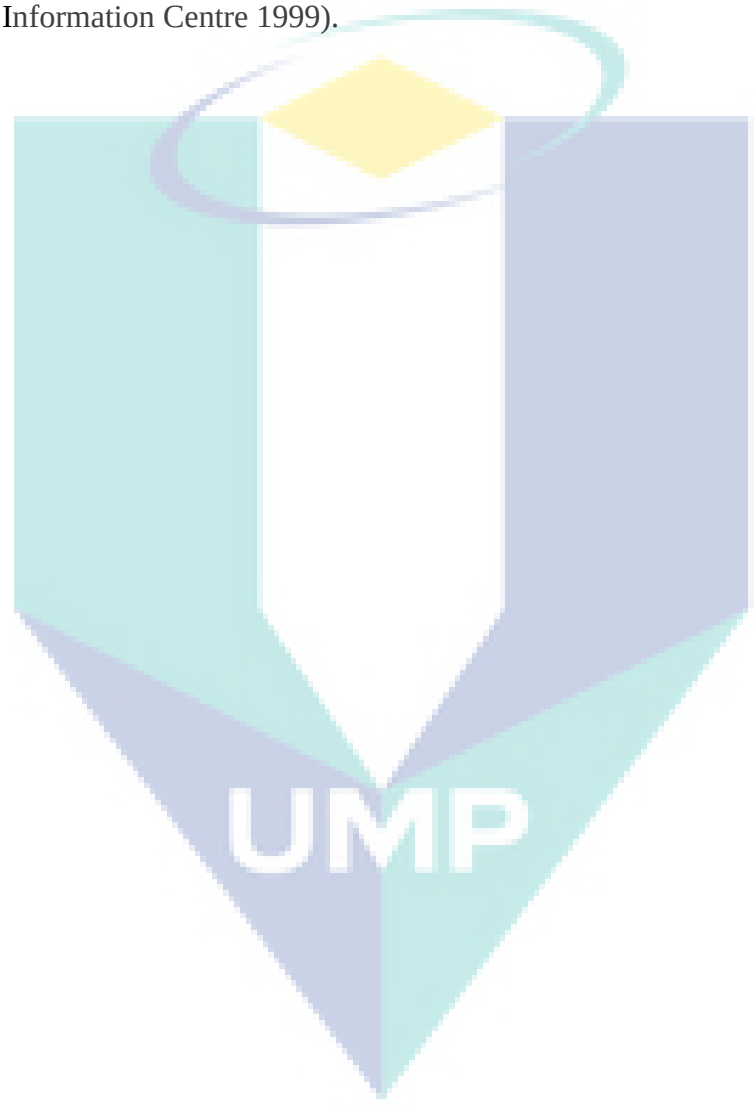
1.5.3 Beneficial versus deleterious of Heavy Metals on Mammary Glands

No heavy metals are beneficial to human health, except some of them referred to as elements eg, iron, copper, manganese, zinc and selenium can be as essential nutrients for a healthy life (International Occupational Safety and Health Information Centre 1999). Especially, magnesium is necessary for biochemical reactions in the body and regulate muscle and nerve function normally, maintaining the rhythm of the heart, the immune system and bone strength manjaga. In addition, the function of magnesium also regulate sugar in the blood, increases blood pressure in normal energy metabolism and protein synthesis. The advantage of magnesium in plants Torbangun to prevent and control hypertension, cardiovascular disease and diabetes (Darius, 2009).

Meanwhile, the copper contained in the plant enzyme cofactor Torbangun useful as antioxidants to protect against oxygen free radicals generated during oxidative stress (Leung 1998), decrease nausea, vomiting, vertigo and seizures caused by chemicals and drugs used in chemotherapy (Pakdaman 1998).

Heavy metals are metallic chemical elements that have a high density and is toxic at low concentrations, eg heavy metals harmful to humans include timbale, arsenic and mercury. In amounts above the threshold of tolerance, heavy metals are very dangerous in the world. Heavy metals become toxic when they are not metabolized by the body and accumulate in the soft tissues (Roberts 1999). Damage caused by heavy metals that reduce vitality and health, sepertipenyakit degenerative neurological diseases, cancer, heart disease, autoimmune diseases and disorders of the skin.

In addition, it can also damage the blood composition, internal organs such as the lungs, liver, kidneys and other vital organs. For a long time, heavy metals in the body can result in physical Progressing slowly, muscular, and Alzheimer's, Parkinson's disease, muscular dystrophy, and multiple sclerosis ((International Occupational Safety and Health Information Centre 1999).



CHAPTER 2

LITERATURE REVIEW

2.1 MEDICINAL PLANT

Medicinal plants are various plants thought by some to have medicinal properties, but few plants or their phytochemical constituents have been proven by rigorous science or approved by regulatory agencies such as the United States Food and Drug Administration or European Food Safety Authority to have medicinal effects. Medicinal plants provide a valuable material base for the discovery and development of new drugs of natural origin (Qinand Xu, 1998). Tropical countries area treasure house of awide variety of medicinal plants. Some species are found wild, while a number of species have been domesticated by the farmers. Many species have been grown in homesteads and become part of traditional home remedies. A limited number of species is commercially cultivated though a few more have been potential for large-scale production. The important tropical and subtropical medicinal plants are discussed here high lighting the importance, medicinal and other uses, distribution, botany, agro technology, chemical constituents and activity. For practical convenience of the discussion in this book, they are classified under the following four broad groups. Of the 250,000 higher plant species on earth, more than 80,000 species are reported to have at least some medicinal value and around 5000 species have specific therapeutic value. They are classified according to the part used, habit, habitat, therapeutic value, etc, besides the usual botanical classification (Joy *et al.*,1989).

India is clearly known to be the richest source of medicinal plants. Four thousand years ago, the medical knowledge of the Indian subcontinent was termed as Ayurveda. Plant alkaloids are the primary active ingredients of Ayurvedic drugs. To day

the pharmacologically active ingredients of many Ayurvedic medicines are being identified and their usefulness in drug therapy being determined. As mentioned in the introduction only acertain percentage of plants is used in traditional medicines. It is roughly estimated that of the discovered 17,000 species, nearly 3,000 species are used in the medicinal field. The therapeutic caption of important medicinal plants and its parts used (Ramar *et al*., 2008).

The active principles in medicinal plants play as trategic role in the phytochemical investigations of crude plant extract and is very important for their potential in terms of the pharmacological effects (Pascual *et al.*, 2002).The medicinally active constituents are present in alkaloid, flavonoid, saponin, tannin, terpenoid/steroid it was present in the medicinal plant, but this compound has been suggested to involve antibacterial and antiviral activity, while tannin and flavonoid are thought to be responsible for anti-diarrheal (Majaw and Moirangthem, 2009; Singh, 2006).The medicinal plant list of great importance to the health of individuals and communities, and these plant passes in some chemical substances that produce a definite physiological action on the human body (Edeoga *et al*., 2005).The plants serve several purposes, whether in health, nutrition, beauty or medicinal. For many of the medicinal plants of current interest, a primary focus of research to date has been in the are as of phytochemistry, pharmacognosy, and horticulture. In the area of phytochemistry, medicinal plants have been characterized for their possible bioactive compounds, which have been separated and subjected to detailed structural analysis. Research in the pharmacognosy of medicinal plants has also involved assays of bio-activity, identification of potential modes of action, and target sites for active phytomedicinal compounds. Horticultural research on medicinal plants have focused on developing the capacity for optimal growth in cultivation. This has been especially pertinent as many medicinal plants are still being harvested in the wild, and conditions for growth in cultivation have not been optimized (Donald, 2000). Many such medicinal plants have hepato-protective, neuro-protective, anti-inflammatory and also antioxidant or radical-scavenging properties (Perry *et al*., 1999; Lin and Huang, 2000).

Many plants synthesize substances that are useful to the maintenance of health in humans and other animals. These include aromatic substances, most of which are

phenol or their oxygen-substituted derivatives such as tannins. Many are secondary metabolites, of which at least 12,000 have been isolated — a number being estimated to be less than 10% of the total. In many cases, substances such as alkaloid serve as plant defence mechanisms against predation by microorganisms, insects, and herbivores. Many of the herb and spices used by humans to season food yield useful medicinal compounds (Lai and Roy, 2004; Tapsell *et al.,* 2006). Plants are the first medicines for mankind and hundreds of plant species are being harvested for their medicinal properties all over the world. In spite of sophisticated pharmaceutical chemicals to treat illnesses, medicinal plants remain an important tool for treating illness. Traditional medicines made from local plants are the only available and affordable source for treating various ailments World Health Organization (2007). Medicinal plants play a vital role to preserve our health. The genus, coleus consists of herbs, that are widespread in all over India and represents highly valuable plant species having therapeutic and nutraceutical importance (Neetuvijay *et al.,* 2009).

The medicinally active constituents are present in alkaloid, flavonoid, saponin, tannin, terpenoid /steroid it present in the medicinal plant, but this compound have been suggested to involve antibacterial and antiviral activity, while tannin and flavonoid are thought to be responsible for anti-diarrheall (Majaw and Moirangthem, 2009; Singh, 2006). The medicinal plant list of great importance to the health of individuals and communities, and this plant passes in some chemical substances that produce a definite physiological action on the human body (Edeoga *et al.,* 2005). The use of medicinal plants in traditional medicine is wide spread and still serve as leads for the development of novel pharmacological agents. Many such medicinal plants have hepato-protective, neuro-protective, anti-inflammatory and also antioxidant or radical-scavenging properties. Therefore, in recent years in the field of clinical medicine, major emphasis is being placed on the use of antioxidants mainly for intervening and sometimes for treating several human ailments (Rao *et al,* 2006). Torbangun plants as medicinal plant have been used to treat malarial fever, hepatopathy, renal and vesical calculi, cough, chronic asthma, hiccough, bronchitis, anthelmintic, colic and convulsions (Table 2.1) (Kaliappan *et al*., 2008).

Table 2.1. Medicinal plant from *Coleus* family species

Plant Name	Active Ingredient	Application	Reference
Torbangun plant (*Coleus amboinicus* Lour)	Flavonoids like quercetin, apigenin, luteolin, salvigenin, genkwanin and volatile oi	Malarial fever, hepatopathy, renal and vesical calculi, cough, chronic asthma, hiccough, bronchitis, helminthiasis, colic, convulsions, and epilepsy.	Kaliappan *et al*., 2008
Torbangun plant (*Coleus amboinicus* Lour)	Butylanisode, β-caryophyllene, quercetin, ursolic acids, triterpenic acids, α-pinene, β-pinene, thymol, eugenol, carvacrol, 1,8-cineole, β-phellandrene, *p* -cymene, salvigenin, crisimaritin and chrysoeriol.	Urolithiasis, antiepileptic, antitumor anti-mu tagenic, neuropharmacoligical, radioprotective effect, antioxidant, antimicrobial, antibacterial, antifungal properties.	Roshan *et al*., 2010
Torbangun plant (*Coleus amboinicus* Lour).	Antioxidants	Aging, arthritis, coronary disease, Alzheimer's disease, cataract and cancer also known to be free-radical mediated	Rao *et al.*, 2006

Table 2.1. Medicinal plant from *Coleus* family species

Plant Name	Active Ingredient	Application	Reference
Torbangun plant (*Coleus amboinicus* Lour)	Essential oils, e.g. thymol, carvacrol and *cis*-caryophylline	Antibacterial, antifungal, antiseptic, antioxidant, antiviral properties. asthma, bronchitis, chronic coughs, sores, burns and insect stings and urinary diseases. For mouthwashes, tooth pastes, soaps, creams, lotions, ointments, throat lozenges and cold remedies.	Hole *at al.*, 2008
Torbangun plant (*Coleus amboinicus* Lour)	Antibacterial activity	Insect bite, head aches, fever, and bronchitis	Kumar *et al.*, 2008
Torbangun plant (*Coleus amboinicus* Lour)	Antimicrobial and anti-fungal activity	Malaria and leptospirosis	Devi, 2008
Torbangun plant (*Coleus amboinicus* Lour)	Antilithiotic, antioxidant chemopreventive, and antiepileptic	Cough, ulcers, boils, swellings, headaches, urogenital	Begum *et al.*, 2009.

Table 2.1. Medicinal plant from *Coleus* family species

Plant Name	Active Ingredient	Application	Reference
Torbangun plant (*Coleus amboinicus* Lour)	Essential oil and antioxidant	Anthemintic and antioxidant activity	Prasenjit *et al.*, 2011
Coleus aromaticus	Antibiotics	Chronic cough, asthma anti-spasmodic, stimulant, stomachic, headache, fever, epilepsy and dyspepsia. To treat indigestion, diarrhea, nervous tension, insect bites, toothache, earache, rheumatism, whooping cough, and bronchitis	Subhas *et al.*,2 010
Coleus aromaticus (Benth)	antimicrobial activity	Reproductive tract infections	*Pritima, and Pandian, 2007;*
Torbangun plant (*Coleus amboinicus* Lour)	Phenolic	Aphthous stomatitis, cure fungus *Oidium albicans* digestive indispositions and vitamin deficiency	Bos *et al.*, 1983

Table 2.1. Medicinal plant from *Coleus* family species

Plant Name	Active Ingredient	Application	Reference
Torbangun plant (*Coleus amboinicus* Lour)	*Essential oil and carvacrol*	Anti-feedant properties	Valera *et al.*, 2003
Coleus blumei Benth.	*Rosmarinic acid*	Fungal and bacterial infections and predators	Bauer *et al.*, 2002
Coleus forskohlii.	*Forskolin*	Antihypertensive, bronchodilatory and anti-glaucoma properties	Moris, 2002
Torbangun plant (*Coleus amboinicus* Lour)	*Acrylic resin*	Denture stomatitis, fungicide effect (*Candida albicanu*)	Devi, 2006
Torbangun plant (*Coleus amboinicus* Lour)	*Antilithiotic, chemopreventive, anti-oxidant and antiepileptic*	Immunostimulant properties	Sunitha *et al.*, 2010

Torbangun is a common plant with medicinal properties. In our study, when the hot, aqueous and ethenolic extract of *C.aromaticus* was tested for antibacterial activity against human pathogenic Gram positive and Gram-negative bacteria like *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Escherichia coli,* and *Salmonella enteritidis* (Subhas *et al*., 2010).

2.2 TORBANGUN PLANT (*Coleus amboinicus* Lour)

Torbangun plant has many synonyms such as Coleus amboinicus Lour, Coleus aromaticus, Plectranthus amboinicus or Spreng, commonly known as country borage, Indian borage (Karpuravalli, Omavalliin Tamiland Patta Kajava, Patharcurin Hindi) is adicotyledonous plant belonging to the family Lamiaceae (Warrier *et al.*, 1995). *Coleus aromatics* Bents, *Coleus carnosus* Hassk, *Coleus suborbiculata* Zolland Mor, *Plectranthus aromatics* Roxb (Heyne, 1987). The plant found almost all area in Indonesia with the different names (Gembong, 2004).

Torbangun plant is a large succulent herb with aromatic leaves, found abundantly in India. The leaves of this plant are traditionally being used for the treatment of severe bronchitis, asthma, diarrhea, epilepsy, renal and vesicle calculi and fever (Warrier *et al.*, 1995). Torbangun plant is used widely as a medicinal and seasoning plant in the Caribbean and other regions. General guidelines for aromatic Torbangun production have been published, dealing mostly with planting material, fertilization, and harvest timing (Acosta *et al.*, 2006). Torbangun plant has been reported to exhibit antilithiotic (Jose *et al*., 2005), chemo-preventive (Prasad *et al*., 2005), antiepileptic (Buznego and Perez, 1999) and antioxidant (Padma *et al.*, 1988) properties, limonene, linalool, myrcene and thymol (Baslas and Kumar,1981),α-Amorphene and β-cubebene (Prudent *et al*., 1995 and Pino *et al*., 1996).

The leaves are bitter, acrid, thermogenic, aromatic, anodyne, appetizing, digestive, carminative, stomachic, anthelmintic, constipating, deodorant, expectorant, lithontriptic, diuretic and livertonic. They are useful lincephalalgia, otalgia, anorexia, dyspepsia, flatulence, colic, diarrhoea and cholera, especially in children, halitosis,

convulsions, epilepsy, cough, chronic asthma, hiccough, bronchitis, renal and vesical calculi, strangely, hepatopathy, malarial fever, antispasmodic and cathartic (Warier and Nambier,1996). Juice of leaves mixed with sugar acts as a powerful aromatic carminative, given in colic and dyspepsia (Chopra *et al*., 1999). Crushed leaves are used as a local application to the head in headache and to relieve the pain and irritation caused by stings of centipedes. Expressed juice is applied around the orbit to relieve the pain in conjunctivitis (Nadkarni, 2002).

The ethno-botanical of Torbangun plant most frequently being used for their medicinal properties, which accounts for over 85% of all uses. The plant is reported as having horticultural uses and grown as ornaments are resistant to diseases, the plant is usually succulent and can survive in dry conditions. The plant is being cultivated in Africa, Asia, Northern and Southern America and Australia (Garden Plants List, 2004).The herb grows easily in a well-drained, semi-shaded position. It grow swell in sub-tropical and tropical locations, but will do well in cooler climates if grown in a pot and brought indoors, or moved to a warm sheltered position in winter. Torbangun plant is well documented, and many people have done researched (Table 2.2).

2.2.1 Classification of Torbangun Plant

Taxonomy of Torbangun is *Coleus amboinicus* Lour international name that be classification (Keng, 1978).

Kingdom	Plantae
Subdivision	Spermatophyte
Ordo	Tubiflorae
Family	Lamiaceae (Labialae)
Sub-Family	Oscimoidae
Gens	Coleus
Species	*Coleus amboinicus* Lour

Table 2.2. Research of Torbangun plant

Extraction Type	Solvent	Temperature	pH	Active Ingredient	References
Maceration; Extraction	Petroleum ether, ethanol, water, chloroform and 95% ethanol	-	-	Diuretic activities	Roshan *et al.*, 2010
Extraction	Dichloromethane	-	-	Essential oil	Bos *et al.*, 1983
Soxhlet extraction	Ethanol	-	-	Anthemintic and antioxidant	Prasenjit *et al.*, 2011
Extraction	Hot water	-	-	Antibacterial	Subhas *et al.*,2010
Extraction	Anhydrous, sodium sulphate	-	-	Essential oil	Valera *et al.*, 2003
Hydroalcoholic extration	Methanol	-	-	Antioxidant, anticlastogenic and radioprotective effect	Rao *et al*, 2006

2.2.2 Biology of Torbangun Plant

The Characteristics of Torbangun leaf are that it has soft bone, space between branches and spherical formis about15mm in diameter, middle and tip part of leaf about 10mm ± 5 mm and easy to combust. The fresh leaf is thick, darkgreen and the superficial of leaf is smooth (Gembong, 2004).The plant consists of hispid villous or torments fleshy stem of about 30 - 90 cm. Leaves are simple, broad, ovate and very thick. Thickly studded with hairs; on the lower surface, the glandular hairs are most numerous and give rise to a frosted appearance. The taste of the leaf is pleasantly aromatic with the agreeable and refreshing odour. Flowers are shortly pedicel led, pale purplish in dense whorls at distant intervals in along slender raceme (Kaliappan and Viswanathan, 2008).

The macroscopic of Torbangun plant is such that it has the characteristics that of soft branch, space between branches and spherical form. The leaf is single,±3.5-6 cm long. Leaf shore is rather waved, ±1.5-3 cm long; bone leaf finned form.Torbangun plants wild growth, rarely flower but possesses ease to reproduction by cutting the branch and plant it on fertilizer land (Heyne,1987). Flowers are shortly pedicel led, 3mm long, pale purplish in dense whorls at distant intervals in along slender race me.Upper calyxes are lipovate, acute, membranous, lower a culminate. Corolla is pale purplish, tube short; throat in flated, lips short. Stamens are shortly exerted (Roshan *et al*, 2009).

Figure 2.1.Leaves of Torbangun plant (*Coleus amboinicus*Lour)

2.2.3 Substances of Torbangun Plant

Torbangun plant contains essential oil that is being composed from carvacrol, isopropyl-o-cresol, phenol and cineole (Wijayakusuma *et al*.,1996). For 120 kg fresh Torbangun leaves, there contain 25 ml essential oil (±0.2%oil), and the antiseptic effect emerged. On the other hand, this leaf also contains C, B1, B2 vitamin's beta-carotene, niacin, carvacrol, calcium, fat acid, oxalate acid and fibers (Duke, 2000)a nd contains 0.043% Eugene oil and 0.2% on dry leaf (Mardisiswojo and Rajak Mangunsudarso,1985). According to Heyne (1987), it had been found that 120 kg fresh Torbangun leaves yielded about more less 25ml Eugene oil, which contained phenol (isopropyl-o-tresol) and basically, he discovered that Torbangun has the antiseptic property of high value. On other hands, it is of atsiri oil origin.This plant contains calcium and essential oils like carvacrol, isopropyl-o-cresol, phenol andcineol. For the example, 120 kg fresh of Torbangun leaves contains 25 ml essential oils. This is equivalent to 0.2 % essential oils, which contain phenol derivate like isopropyl-o-cresol, which has the strong antiseptic effect. Phenol and cresol can kill vegetative cell, fungi and bacteria spore through protein denaturizing (Devi, 2006).

Although, Torbangun is also containing compounds in groups that differences function as active substance, such as phenol, phytosterol, octadecanoicacid, benzoicacid, benzenediols, alpha-amyrin, alkanes and aromatic ring. In plants, it tends to be aminor component only of a complex phytosterol fraction, although there are exceptions, but it is nevertheless importance as a precursor ofsome plant hormones (Christie, 2011). Phytosterols that are gammasitosterol, campesterol, stigmasterol and Isocholesteryl methyl ether and structurally similar to cholesterol orz oosterol (Ostlund *et al.*, 2003). Phytosterols are a group of steroid alcohols, phytochemicals naturally occurring in plant. Phytosterols occur naturally in small quantities invegetable oils, especially sea buckthorn oil, corn oil and soybean oil. Phytosterol is marketed as a dietary supplement and white powders with mild, characteristic odor, in soluble in water and soluble in alcohols. It has applications in medicine, cosmetics and as a food additive taken to lower cholesterol (Li *et al.*, 2006; Pennington and Douglas, 2005).They are currently approved by the U.S. FDA for use as a food additive; however, there is some concern that they may block absorption, not only of cholesterol, but of other important

nutrients as well. At present, the American Heart Association has recommended that supplemental plant sterols be taken only by those diagnosed with elevated cholesterol, and has particularly recommended that they not be taken by pregnant women or nursing mothers. Sterols are also known as steroid alcohols and a subgroup of steroids with a hydroxyl group at the 3-position of the A-ring,They are amphiathic lipids synthesized from acetyl-coenzyme A via the HMG-Co A redutase pathway. The overall molecule is quite flat. The hydroxyl group on the A ring is polar.Therestofthealiphaticchainisnon-polar (Fahy *et al*.,2005).

Sterolis important constituents of all eukaryotes and play the vital role in plant cell membranes. Plant sterols possesses valuable physiological activities. They are biogenetic precursors of many hormones and ovipositor stimulants of some insects (Harborne, 2001). Sterol lipids, such as cholesterol and its derivatives, are an important component of membrane lipids, a long with the glycerol phospholipids and sphingomyelins.The steroids, all derived from the same fused four-ring core structure, have different biological roles as hormones and signalling molecules (Bachand Wachtel, 2003).

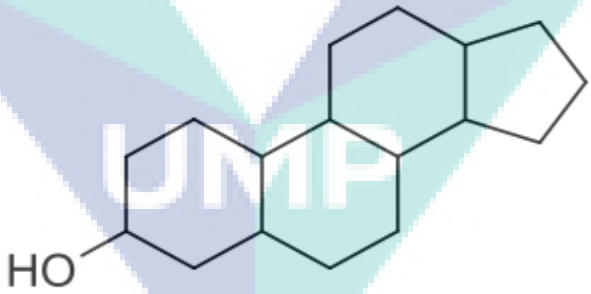


Figure 2.2a. Chemical structure of Sterol

Cholesterol is a ubiquitous component of all animal tissues and of fungi, where much of it is located in the membranes, although it is not evenly distributed. The highest proportion of unesterified cholesterol is in the plasma membrane (roughly 30 to 50%), while mitochondria and the endoplasmic reticulum have very low cholesterol contents, and the Golgi contains an intermediate amount. It may surprise some to learn that the

brain contains more cholesterol than any other organ, where it comprises roughly a quarter of the total free cholesterol in the human body. Of all the organic constituents of blood, only glucose is present in a higher molar concentration than cholesterol. It occurs in the free form, esterifies to long-chain fatty acids (Cholesterol ester), and in other covalent and non-covalent linkages in animal tissues, including the plasma lipoprotein. In plants, it tends to be a minor component only of a complex "phytoserol"" fraction, although there are exceptions, but it is nevertheless importance as a precursor of some plant hormones (Christie, 2011).

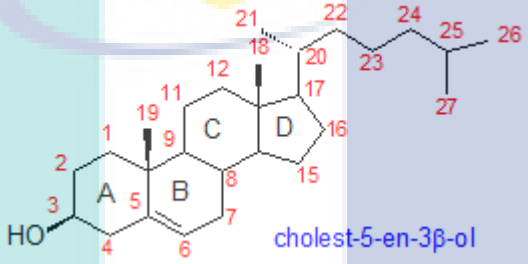


Figure 2.2b. Chemical structure of cholesterol

The main function of phytosterol is to modulate the fluidity of membranes by interacting with their complex lipid components, specifically the phospho lipids such as phosphatidylcholine and sphingomyelin (Christie, 2011). Phytosterols function also to regulate the fluidity of cell membranes in plants, much in same way cholesterol functions in animals and human (Joe, 2011). Phytosterols may offer protection from the most common cancers in Western societies, such as colon, breast and prostate cancer.The effect of phyto sterols on membrane structure and function of tumor and host tissue, signal transduction pathways that regulate tumor growth and apoptosis, immune function of the host and cholesterol metabolism by the host (Atif and Carol, 2000). Cholesterol has vital structural roles in membranes and in lipid metabolism in general. It is a biosynthetic precursor of bile acids, vitamin D and steroid hormones (glucocorticoids, oestrogens, progesterone's, androgens and aldosterone). Inaddition, it contributes to the development and working of the central nervous system, and it has major functions in signal transduction and sperm development. It is found in covalent linkage to specific membrane proteins or proteolipids ('hedgehog' proteins), which have

vital functions in embryonic development. However, because plasma cholesterol levels are a major contributory factor to atherogenesis, media cover age has created what has been termed a ‘cholesterophobia’ in the population at large (Christie, 2011).

Octadecanoic acid (synonymous with stearic acid) is the saturated fatty acid with an 18 carbon chain. It is a waxy solid, and its chemical formula is C18H36O2, or CH3(CH2)16COOH. It occurs in many animal and vegetable fats and oils, but it is more common in animal fat than vegetable fat. The important exceptions are cocoa butter and she a butter whose fatty acids consist of 28–45% stearic acid (Beare *et al.*, 2001) All cells in a plant must produce fatty acids, and this synthesis must be tightly controlled to balance supply and demand for acyl chains. For most plant cells, this means matching the level of fatty acid synthesis to membrane biogenesis and repair (John and Jan, 1997). The octadecanoid 12-oxo-phytodienoic acid (OPDA) is an intermediate in the biosynthesis of jasmonic acid in plants. Synthesized enzymatically by the recombinant allene oxide synthase, as internal standard. The levels of cis-OPDA have been determined in a wide variety of monocotyledonous and dicotyledonous angiosperms and were found to vary considerably among different species. In mechanically stimulated tendrils of Bryoniadioica, the level of cis-OPDA increases several-fold, correlating with the initiation and progression of the free coiling response (Boguslawa *et al.*, 1998).

Octadecanoic acid (synonymous with stearic acid) is the saturated fatty acid. Fatty acids are usually derived from triglyceride's orphospholipids. They are known as "free" fatty acids. Fatty acids are important sources of fuel because, metabolized, they yield large quantities of ATP. Many cell types can use either glucose or fatty acids for this purpose. In particular, heart and skeletal muscle prefer fatty acids. The brain cannot use fatty acids as a source of fuel; it relies on glucose or ketone bodies (Mary *et al.*, 2006). Fatty acids are an important source of energy, which can have an influence on serum lipids. Omega-3 and omega-6 fatty acids, both polyunsaturated fatty acids, have been advocated as a replacement for saturated fat. Omega-3 fatty acids, derived from fish and certain green plants, lower serum triglycerides, but they have also been shown to have a direct effect on myocardial contractility, blood pressure, platelet function,

coagulation factors, cell-mediated immunity and markers of inflammation (Bhatnagar and Durrington, 2003). Research on fatty acid-based signalling systems in plants has focused mainly on the hormonally active compound, jasmonic acid (JA). A rapidly growing body of literature indicates that plant defence responses against insect herbivores, and some microbial pathogens are orchestrated by signalling pathways involving the biosynthesis and subsequent action of JA (Gregg, 2001).

Plant jasmonic acid (JA) and structurally similar animal prostaglandins play pivotal roles in regulating cellular responses against environmental cues, including the innate immune responses. In plants, JA and its immediate precursor 12-oxo-phytodienoic acids (OPDA) are synthesized by the octadecanoid pathway, which employs at least five enzymes (lipase, lipoxygenase, allene oxide syntheses and cyclise, and OPDA reductive, in addition to the enzymes involved in the β-oxidation steps. Until now, no gene has been specifically associated with this pathway. It is therefore, of utmost importance to identify, characterize, and assign the pathway specific genes in rice. In this review, we have surveyed the rice genome, extracted a large number of putative genes of the octadecanoid pathway, and discussed their relationship with the known pathway genes from other plant species. Moreover, the achievements made so far on the rice octadecanoid pathway have also been summarized to reflect the contribution of rice towards extending our knowledge on this critical pathway in plants (Ganesh *et al.*, 2004). Jasmonic acid and its derivatives biochemically belong to a group of cyclic oxylipines derived from C18 unsaturated fatty acids, called octadecanoids. The pathway for the octadecanoid biosynthesis it is generally assumed that octadecanoids are produced from α-linolenic acid and because all enzymes of the octadecanoid biosynthesis are present in un-induced tissue, the substrate – α-linolenic acid – availability can limit metabolite flow in the pathway (Weiler *et al.*, 1999).

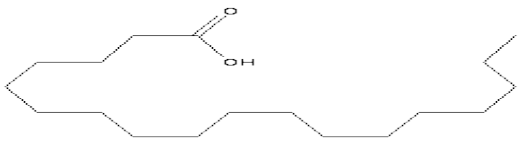


Figure 2.2c. Chemical structure of Octadecanoic or Stearic acid

Benzoic acid is an aromatic acid made up of a carbonoxyl group bonded directly to a benzene ring. It is derived from gum arabic. Aromatic acids are made up of a carbon-oxygen-oxygen-hydrogen component attached to the benzene ring. Benzoic acid is the simplest aromatic acid. At room temperature, Benzoic acid is a white, crystalline organic compound that sublimates at the temperature of boiling water (Ken, 1990). Benzoic acid (C_6H_5COOH), is a colorless crystalline solid and the simplest aromatic carboxylic acid. (Warth, 1991), Benzoic acid is a natural ingredient occurring in many foodstuffs and in plant extracts. Benzoic acid, its salts and esters are used as preservatives in cosmetic products, with a maximum concentration of 0.5 %, (calculated as acid).This request is for use for non-preservative purposes in cosmetic rinse-off products, at a maximum concentration of 2.5 %, and in cosmetic oral-care products, at a maximum concentration of 1.7 %, and in leave-on products, up to 0.5%. In the United States, benzoic acid and sodium benzoate are on the FDA list of substances that are generally recognized as safe. Both may be used as antimicrobial agents, flavouring agents and as adjuvants with a current maximum level of 0.1% in food. The FDA has not determined whether significantly different conditions of use would be GRAS. The FDA hassought fully up-to-date toxicology information. Benzoic acid is used in oral medicines up to 0.15%, in parenteral medicines up to 0.17% and in topical drugs up to 0.2%. Benzoic acid is used as an active ingredient in anti-fungal cream with salicylic acid (3.0%) up to 6%. Sodium Benzoate, expressed as benzoic acid, is permitted in oral medicines up to 0.5%, in parenterally administered up to 0.5%. Benzoic acid is also an intermediate in the synthesis of phenol and caprolactam (SCCP, 2005).

Benzoic acid metabolism, which is primarily a function of liver mitochondria, depending on the concentration of adenosine triphosphate (ATP), coenzymeA(CoA), and glycine in the mitochondrial matrix, was investigated in both rats with long-term cholestasis caused by bile duct ligation (BDL) and sham-operated control rats (Lukas *et al.*, 1997).

Benzoic acid and its salts are used as food preservatives, Benzoic acid inhibits the growth of mold, yeast and some bacteria (Warth, 1991). The efficacy of benzoic acid and benzoate is thus dependent on the pH of the food (Pastrorova *et al*., 1997). Benzoic acid is a constituent of Whitfield ointment, which is used for the treatment of fungal skin diseases such as tine, ringworm, and athlete's foot. As the principal component of benzoin resin, benzoic acid is also a major ingredient in both tinctures of benzoin and Friar's balsam. Such products have a long history of use as topical antiseptics and inhalant decongestants (Charles *et al.*, 2004). Benzoic acid and sodium benzoate, combined with alcohol and water, are sometimes used as a cleaning agent in pharmacies and in hospitals, where their anti-fungal and anti-microbial functions cut down on the transmission of disease. The typical procedure is to spritz down the area to be disinfected with the benzoic acid solution and then with a bit of bleach solution, then wipe it down. Hand sanitizers and anti-microbial soaps largely have benzoic acid or sodium benzoate as their principal anti-bacterial agents, and commercial products are more widely used than 'hand made' mixes of benzoac acid and bleach water in the US; making the solution by hand is still common in Latin America and the less industrialized areas of East Asia (Ken, 1990). However, these purposes are not specified. Other uses for benzoic acid and its salts include regulated use as food preservatives, most suitable for foods, fruit juices, and soft drinks in an acidic pH range. In the EU, there are regulations controlling the maximum levels of benzoic acid and its salts for use in foodstuffs ready for consumption and the specific purity criteria of food additives. The levels are expressed as the free acid (SCCP, 2005).

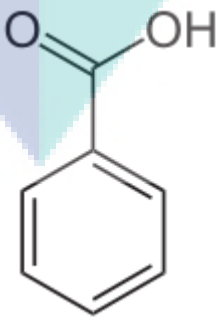


Figure 2.2d. Chemical structure of Benzoic acid

Benzenediols (synonym catechol, dihydroxybenzenes) are organic chemical compound in which two hydroxyl group are substituted onto a benzene ring. These aromatic compounds are classed as phenols, and more specifically as polyphenol. There are three isomers of benzenediol: 1, 2-benzenediol (the ortho, meta and para isomer)) is commonly known as catechol, resorcinol and hydroquinone. Catechol was first isolated in 1839 by H. Reinsch by distilling catechin. Upon heating catechin above its decomposition point, the substance first called "pyrocatechol" (now simply catechol) forms. Catechol occurs in the free form naturally in kino and in beechwood tar; its sulfonic acid has been detected in the urine of horse and humans. Catechol is produced industrially by the hydroxylation of phenol using hydrogen peroxide (Helmut *et al.*, 2002). Catechol is the conjugate acid of a chelating agent used widely in coordination chemistry. Basic solutions of catechol react with iron (III) and catechol can also be conjugated to ruthenium (Almeida *et al.*, 2007).

Approximately, 50% of synthetic catechol is consumed in the production of pesticides, the remainder being used as a precursor to fine chemicals such as perfumes and pharmaceuticals (Helmut *et al.*, 2002) and as common in organic synthesis (Barner, 2004). Several industrially significant flavors and fragrances are prepared starting from catechol. Guaiacol is prepared by methylation of catechol and is then converted to vanilin. Piperonal, a flowery scent, is prepared from the methylene diether of catechol followed by condensation with glyoxal and decarboxylation (Karl-Georg *et al.*, 2005).

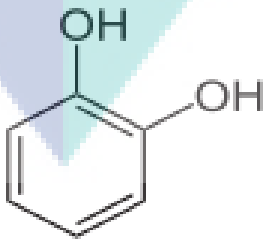


Figure 2.2e. Chemical structure of Benzenediol

Alpha-amyrin (synonym name viminalol) is two crystalline isomeric triterpenoid alcohols C 30H 49OH and has the 29-carbon at the 20 position. Alpha-amyrin is a precursor of Ursolic acid Ursolic acid has anti elastase activity, and like cholesterol, modulates the membrane fluidity. Alpha amyrin has strong anti-inflammatory activity, is a PKA inhibitor as well as a selective protease inhibitor: chymotrypsin is inhibited at an 18-micromolar level. Alpha amyrin are observed as anti-collagenase and anti-edema properties (Monmarche and Laurent, 2001).

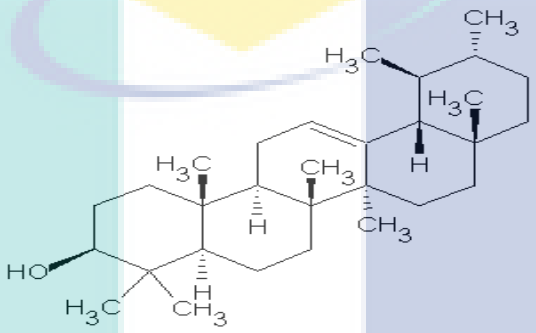


Figure 2.2f. Chemical structure of alpha amyrin

Alkane is a saturated aliphatic hydrocarbon containing no double or triple bonds in the carbon chain, such as propane or any of a group of hydrocarbons that have carbon atoms in chains linked by single bonds and that have the general formula CnH2n+2. It is also called paraffin. The nature of the other lipid constituents can vary greatly with the source of the waxy material, but they include hydrocarbons, sterol esters, aliphatic aldehydes, primary and secondary alcohols, diols, ketones, β-diketones, triacylglycerols, and many more (Christie, 2011). Alkenes are hydrocarbons or organic compounds made up of only carbon-carbon single bonds (in contrast to opposed to double and triple bonds). The simplest alkane is methane. An alkane-assimilating yeast Candida's maltose contains multiple-alkenes-inducible forms of cytochromes, which can be assumed to catalyze terminal hydroxylation ofn-alkanes in the assimilation pathway. Cytochromes are heme-containing monooxygenases that are distributed widely among living organisms (1). Higher eukaryotes generally contain multiple forms of cytochrome P450 catalyzing diverse oxidative reactions in the metabolism of a large number of

endogenous and xenobiotic compounds. Depending on the induction level and substrate specificity of individual cytochromes P450, cellular metabolic processes are often affected by a specific ensemble of isoforms, making it difficult to distinguish their biological function (Nelson *et al.*, 1993).

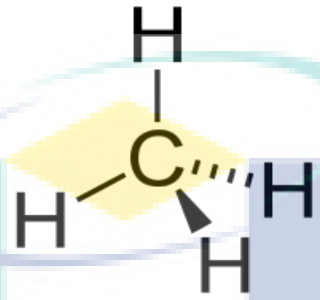


Figure 2.2g. Chemical structure of methane, the simplest alkanes

2.2.4 Benefit of Torbangun Plant

Torbangun leaves also have antimicrobial effects against Candida albicans and Streptococcus mutant. The plant has the effect to cure stomatitis and also fungicidal (Hole *et. al*, 2008). Torbangun leaf as antiseptic has high activity against worm infection (Vasquez *et al*., 2000). Thus, this leaf could be used as against asthma diseases and (Jain and Lata 1996). The leaves also contain potassium, which cleans blood, prevents of infections, decreases pain, emerge to relax fell and furrows membrane mucous. Hot climate could elevate stress cause anorexia, milk secretion and body weigth to decrease (Mepham 1987).

The leaves of the indigenous Torbangunplant possess a distinct aroma and are used in many food preparations to enhance flavor. Apart from this, they also exhibit medicinal properties (Hole *et al*, 2008). This compound is being known to exhibit antimicrobial, antiviral, anti-inflammatory, and antioxidant activities, which make it a valuable product for the pharmaceutical, food and cosmetic industries. The plant of Torbangun is a bushy shrub being cultivated throughout India and is being reported to contain certain essential oils, e.g. thyme, carvacrol and cis-caryophylline, which possess

antibacterial, antifungal, antiseptic, antioxidant and antiviral properties. The extracts are being widely used for the treatment of asthma, bronchitis, chronic coughs, sores, burns and insect stings and urinary diseases. They are also used in mouthwashes, tooth pastes, soaps, creams, lotions, ointments, throat lozenges and cold remedies. Our preliminary investigations on the accumulation of essential oils by tissue cultures of *Coleus amboinicus* showed that the regenerated plants retained the biosynthetic capacity of the parent plant in the production of thyme and carvacrol (Hole *et al.*. 2008). Torbangun plant has scented leaves and these are often rubbed into the hair and body after bathing (Morton, 1992). In the Amazon, the leaves are mixed with sugar and used as an intoxicant (Jain and Lata, 1996), while in Tonga and Martinique the leaves are used in the cleaning of textiles to perfume them (Prudent *at al*., 1995). Torbangun plantisalso used as insect repellants (Omolo *et al.*, 2004).

2.3 MACRONUTRIENT

*Mac*ronutrient is an essential nutrient being required in relatively large amounts, such as carbohydrates, fats, proteins, or water; sometimes certain minerals are included, such as calcium, chloride, or sodium (DMDHC, 2007). Macronutrients are defined as being classes of chemical compounds that humans consume in the largest quantities and which provide the bulk in energy. This is protein, fat and carbohydrate. The list showed the categorization of the most common food components by these macronutrients. Macronutrients can also refer to the chemical elements that humans consume in the largest quantities, while nutrient is organism need to live and grow or a substance used in an organism's metabolism which must be taken in from its environment (Whitney *et al.*, 2005). Nutrients are the substances that enrich the body. They are being used to build and repair tissues, regulate body processes and converts to and use as energy. Methods for nutrient's intake vary, with animals and protist's consuming foods that are being digested by an internal digestive system, but most plants ingest nutrients directly from the soil through their roots or from the atmosphere. Organic nutrients include carbohydrates, fats, protein and vitamins. Inorganic chemical compounds such as dietary minerals, waters, and oxygent may also be considered nutrients (Frances and Ellie, 2007).

The effects of nutrients are dose-dependent and shortages are being called deficiencies. Deficiencies could be due to a number of causes, including inadequacy in nutrient intake called dietary deficiency; or conditions that interfere with the utilization of a nutrient within an organism. Some of the conditions that could interfere with nutrient utilization include problems with nutrient absorption, conditions that cause nutrient destruction, and conditions that cause greater nutrient excretion (Audrey, 1994). Nine species of Coleus are being reported to be edible. Coleus esculentus are rich in macronutrient such as carbohydrates, Vitamin A and minerals and usually are boiled or roasted and eaten as a substitute for sweet potato in most parts of Africa (Lukhobaa *et al.*, 2006). Nutrition composition of 100 grams of Torbangun leaves includes energy, protein, fatty, carbohydrate, fiber, ash, calcium, phosphorous, ferrous, carotene total, Vitamin A, B1 and C. The contain more calcium, ferrous and carotene total than Sourpuss androgynous leave (Mahmud et al., 1990). The macronutrients that contained in Torbangun plant are being listed in Table 2.3.

2.3.1 Protein

Proteins are organic compound made of amino acids arranged in a linear chain and folded into a globular form. The amino acids in a polymer are being joined together by the peptide bond between the carboxyl and amino groups of adjacent amino acid residue. The sequence of amino acids in a protein is being defined by the sequence of a gene, which is encoded in the genetic code (Ridley, 2006). Proteins are polymer chains made of amino acids being linked together by peptide bonds. Amino acids could be divided into either essential amino acids or non-essential amino acids. Proteins and carbohydrate contain four kcal per gram as opposed to lipids, which contain nine kcal per gram. The liver, and to a much lesser extent the kidneys, could convert amino acids used by cells in the protein biosynthesis into glucose by a process known as gluconeogenesis. The essential amino acids, which must be obtained from food sources, are lrucine, isoleucine, valine, lysine, threonine, tryptophan, methionine, phenylalanine and histidine. On the other hand, non-essential amino acids could be made by the body from other amino acids. The non-essential amino acids are arginine, alanine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, proline, serine, and tyrosine

Table 2.3. Macronutrient in Torbangun plant

Type of Macronutrient	Description	References
Mineral : Sodium chloride	Causing greater intracellular availability of calcium	Hole *at al*, 2008
Lactagogue	Stimulate breast milk production	Damanik *et al.*, 2006

(Genton *et al.,* 2010). In general, the genetic code specifies 20 standard amino acids; however, in certain organisms, the genetic code could include selenocycteine and in certain archaea-pyrrolysine. Shortly after or even during synthesis, the residues in a protein are often chemically modified by pos-translational modification, which alters the physical and chemical properties, folding, stability, activity, and ultimately, the function of the proteins. Proteins can also work together to achieve a particular function, and they often associate to form stable complexes (Maton *et.al.,* 1993). Proteins are vital body nutrients, just as fats and carbohydrates, vitamins and minerals. However, proteins, together with dietary fats, are being required by the body in larger amounts than vitamins and minerals because they are the primarily body building block sources for new tissue (Kinsella, 1990).

Protein structures range in size from tens to several thousand residues. Very large aggregates could be formed from protein subunit: for example, many thousands actin molecules assemble into a microfilament. A protein may undergo reversible structural changes in performing its biological function. The alternative structures of the same protein being referred to as different conformations, and transitions between them are being called conformational changes (Brocchieri and Karlin, 2005).

Proteins are assembled from amino acids using information encoded in the genes. Each protein has its own unique amino acid sequence that is being specified by the nucleotide sequence of the gene encoding this protein. The genetic code is a set of three-nucleotide sets being called cordons and each three-nucleotide combination designates an amino acid, for example, AUG (adenine-uracil-guanine) is the code for methionine. Due to DNA containing four nucleotides, the total number of possible codons is 64; hence, there is some redundancy in the genetic code, with some amino acids being specified by more than one cordon. Genes encoded in DNA are first transcribed into pre-messenger (mRNA) by proteins such as RNA polymerase... Most organisms then process the pre-mRNA (also being known as a primary transcript) using various forms of post-transcriptional modification to form the mature mRNA, which is then being used as a template for protein synthesis by the ribosome. In prokaryotes the mRNA may either be used as soon as it is being produced, or be bound by a ribosome

after having moved away from the nucleoid. In contrast, eukaryotes make mRNA in the cell nucleus and then trans locating it across the nuclear membrane into the cytoplasm, where protein synthesis then takes place. The rate of protein synthesis is higher in prokaryotes than eukaryotes and could reach up to 20 amino acids per second (Dobson, 2000).

Protein has a range of essential functions in the body, including the following required for building and repairing of body tissues (including muscle); enzymes, hormones, and many immune molecules are proteins; essential body processes such as water balancing, nutrient transport, and muscle contractions require protein to function; a source of energy; It helps keep skin, hair, and nail healthy; as essential nutrients, is absolutely crucial for overall good health (Emma, 2009). Protein function as a nutrient being needed by the human body for growth and maintenance. Aside from water, protein is the most abundant molecule in the body, and protein is being needed to form blood cells (Hermann and Janice, 2010). Protein is being found in all cells of the body.and is the major structural component of all cells in the body, especially muscle, includes body organs, hair and skin (Hermann and Janice, 2010; Food and Nutrition Board, 2005). Proteins also are being utilized in the membranes, such as glycoprotein. When broken down into amino acids, they are being used as precursors to nucleid acid and vitamins (Food and Nutrition Board, 2005). Hormones and enzymes are also being formed from amino acids in which they help regulate metabolism, support the immune system and other body functions. In the body, including the following are being required for building and repairing of body tissues as well as muscles; enzymes, hormones and many immune molecules are proteins. Essential body processes such as water balancing, nutrient transport, and muscle contractions require protein to function, as a source of energy, helps keep skin, hair, and nail healthy and is absolutely crucial for overall good health (Emma, 2009).

Dietary requirements of protein are also greater during childhood for growth and development, during pregnancy or when breast-feeding in order to nourish a baby, or when the body needs to recover from malnutrition or trauma or after an operation (WHO, FAO, 2007). The excessive intake of protein increases calcium excretion in the

urine. It has been thought that this occurs to maintain the pH inbalance from the oxidation of sulphur amino acids. Also whether if bone resorption contributes to bone loss, and osteoporosis is inconclusive. However, it is also being found that a regular intake of calcium would be able to stabilize this loss (Food and Nutrition Board, 2005).

Protein deficiency is a serious cause of ill health and death in developing countries. Protein deficiency plays a part in the disease kwashiorkor. Famine, overpopulation and other factors could increase rates of malnutrition and protein deficiency. Protein deficiency could lead to reduced intelligence or mental retardation. In countries that suffer from widespread protein deficiency, food is generally being full of plant fibers, which makes adequate energy and protein consumption very difficult (Bodwell, 1979). Protein deficiency is generally caused by lack of total food energy, making it an issue of not getting food in total. Symptoms of kwashiorkor include apathy, diarrhoea, inactivity, failure to grow, flaky skin, fatty liver, and oedema of the belly and legs. This oedema is being explained by the normal functioning of proteins in fluid balance and lipoprotein transport (Jeffery *et al*., 2003).

2.3.2 Fat (Lipid)

Fats are organic compounds that are being made up of carbon, hydrogen, and oxygen. They are a source of energy in foods. Fats belong to a group of substances called lipids, and come in liquid or solid form (Neil, 2009). All fats are combinations of saturated and unsaturated fatty acids. Fats consist of a wide group of compounds that are generally soluble in organic solvents (saturated) and largely insoluble in water (unsaturated). In saturated fat, each carbon atom is typically being bonded to two hydrogen atoms (Maton *et al.,* 1993). Saturated fat should be limited to 10% of calories. Saturated fats are being found in animal products such as butter, cheese, whole milk, ice cream, cream, and fatty meats. They are also being found in some vegetable oils -- coconut, palm, and palm kernel oils. However, unsaturated fats have a lot of calories, so one still needs to limit them. Most liquid vegetable oils are unsaturated with exception that include coconut, palm, and palm kernel oils (Neil, 2009). All lipids are soluble (or dissolvable) in non-polar solvents, such as ether, alcohol, and gasoline. There are three

families of lipids: (Campbell *et al*, 2000) fats, (Must *et al*., 1999).) phospholipids and steroids (Robinson *et al*., 1993).

Fat is essential for the proper functioning of the body. Fats provide essential fatty acids, which are not being made by the body and must be obtained from food. The essential fatty acids are linoleic and linolenic acid. They are important for controlling inflammation, blood clotting, and brain development. Fat serves as the storage substance for the body's extra calories. It fills the fat cells (adipose tissue) that help insulate the body. Fats are also an important energy source. When the body has used up the calories from carbohydrates, which occurs after the first 20 minutes of exercise, it begins to depend on the calories from fat (Neil, 2009). Fats consist of a wide group of compounds that are generally soluble in organic solvents and largely insoluble in water. Chemically, fats are generally triesters of glycerol and fatty acids. Fats may be either solid or liquid at normal room temperature, depending on their structure and composition. Although the words "oils," "fats" and "lipids" are all used to refer to fats, "oil" is usually used to refer to fats that are liquids at normal room temperature, while "fat" is usually used to refer to fats that are solids at normal room temperature. "Lipids" is used to refer to both liquid and solid fats. The word "oil" is used for any substance that does not mix with water and has a greasy feel, such as petroleum (or crude oil) and heating oil, regardless of its chemical structure (Maton *et al.*, 1993).

All cells in a plant must produce fatty acids, and this synthesis must be tightly controlled to balance supply and demand for acyl chains. For most plant cells, this means matching the level of fatty acid synthesis to membrane biogenesis and repair. Depending on the stage of development, time of the day, or rate of growth, these needs can be highly variable, and therefore, rates of fatty acid biosynthesis must be closely regulated to meet these changes. In some cell types, the demands for fatty acid synthesis are substantially greater. Obvious examples are oil seeds, which during development can accumulate as much as 60% of their weight as triacylglycerol. Another example is epidermal cells, which traffic substantial amounts of fatty acids into surface wax and cuticle lipid biosynthesis (John and Jan, 1997). Fatty acids are compounds synthesized in nature via condensation of malonyl coenzyme Units by a fatty acid syntheses

complex. Fatty acids act as building blocks of lipids. In general, they contain even numbers of carbon atoms in straight, although the syntheses can also produce odd- and branched chain fatty acids, to some extent, when supplied with the appropriate precursors; other substituent groups, including double bonds, are normally incorporated into the aliphatic chain later by different enzyme systems. Fatty acids can either be saturated; monounsaturated or polyunsaturated depending on the number of double bonds (Christie, 2011).

2.3.3 Carbohydrate

Carbohydrates are the compounds which provide energy to living cells. They are compounds of carbon, hydrogen and oxygen with a ratio of two hydrogens for every oxygen atom. The carbohydrate's humans use as foods have their origin in the photosynthesis of plants. They take the form of sugar, starches and cellulose. Carbohydrates perform numerous roles in living things. Polysaccharides serve as storage of energy and as structural components (cellulose in plants and chitin in arthropods). The 5-carbon monosaccharide ribose is an important component of coenzymes and the backbone of the genetic molecule known as RNA. The related deoxyribose is a component of DNA. Saccharides and their derivatives include many other important biomoleculer that play key roles in the immune system, fertilization, preventing on of pathogenesis, blood clotting, and development (Maton *et al.*, 1993).

Carbohydrates are being synthesized in the source leaves and translocated to sink tissues in most species in the form of sucrose to sustain heterotrophic metabolism and growth, or to be stored as sucrose or starch. Growth and development of plants are being accompanied by the changes in source–sink relations. Furthermore, plants, as sessile life forms, have developed regulatory mechanisms that enable a flexible response with respect to assimilate partitioning to specific requirements of the habitat such as biotic and abiotic stress factors. In recent years, it has become evident to that sugars, notably sucrose and its cleavage products, are important metabolic signals that affect the expression of different classes of genes (Koch, 1996; Rolland *et al*., 2002) and are being involved in the regulation of development (Wobus and Weber, 1999).

Carbohydrate functions as a primary source of energy, as storage food, as a framework in the body, as the anticoagulant, as antigen, as hormone, and it provides raw material for industries (Xamplified, 2010). Long-distance transport of assimilates from source leaves into sink organs is being driven by differences in osmotic potentials. Thus, cleavage of sucrose by Inv-CW at the site of phloem unloading and metabolization of the cleavage products controls the sink strength to attract sucrose (Eschrich, 1980).

2.3.4 Vitamin

Vitamin is any of a group of organic substances essential in small quantities to normal metabolism, or it is an organic compound required as a nutrient in tiny amounts by an organism (Lieberman and Bruning, 1990). The vitamins are being found in plants and animal's food sources. They have also been chemically synthesized and so could be ingested in their pure form as nutritional supplements. It is not known precisely how much of each vitamin each person needs. The absence of certain vitamins could cause disease, poor growth, and a variety of syndromes. Their actions are different, and though exhaustively being studied; not everything is understood about how they work and what they do. Thirteen vitamins have been identified as necessary for human health, and there are several more vitamin-like substances that may also contribute to good nutrition. The vitamins are named by letters—vitamin A, being vitamin C, D, E, K, and the group of B vitamins. The eight B vitamins were originally being thought to be one vitamin, and as more was being learned about them, they were given numeral subscripts: vitamin B, B2, etc. The B vitamins are now commonly being called more aptly by chemically descriptive names: B, is thiamine, B2 is riboflavin. B6 and B12 retain their numeral names, and the other B vitamins are niacin, pantothenic, biotin, and folic acid (Bender and David, 1992).

Vitamins are being classified as either water-soluble or fat-soluble in humans, there are 13 vitamins: 4 fat-soluble (A, D, E, and K) and 9 water-soluble (8 B vitamins and vitamin C). Water-soluble vitamins dissolve easily in water and, in general, are being readily excreted from the body, to the degree that urinary output is a strong

predictor of vitamin consumption. As they are not readily being stored, consistent daily intake is important (Fukuwatari and Shibata, 2008). Many types of water-soluble vitamins are synthesized by bacteria (Said and Mohammed, 2006). Fat-soluble vitamins are absorbed through the intestinal tract with the help of lipids (fats). As they are more likely to accumulate in the body, they are more likely to lead to hyper vitaminosis compare to water-soluble vitamins. Fat-soluble vitamin regulation is of particular significance in cystic fibrosis (Maqbool and Stallings, 2008).

The B vitamins function as coenzymes that catalyze many of the anabolic and catabolic reactions of living organisms necessary for the production of energy; the synthesis of tissue components, hormones, and chemical regulators; and the detoxification and degradation of waste products and toxin. On the other hand, vitamin C and fat-soluble vitamins do not function as coenzymes. Vitamins C, E and β-carotene (a precursor of vitamin A) act as antioxidants, helping to prevent tissue injury from free-radical reactions. In addition, vitamin C functions as a co-factor in hydroxylation reactions. Vitamin D has hormone like activity in calcium metabolism; vitamin A plays a critical role in night vision, growth, and maintaining normal differentiation of epithelial tissues; and vitamin K has a unique post-transcriptional role in the formation of active blood-clotting factors (McGraw-Hill Science & Technology Encyclopedia).

Side effects of vitamins that tend to be more severe with a larger dosage. The likelihood of consuming too much of any vitamin from food is remote, but overdosing from vitamin supplementation does occur. At high enough dosages, some vitamins cause side-effects such as nausea, diarrhea and vomiting. The doses of vitamins individuals could tolerate varies widely, and appear to be related to aging and state of health (Institute of Medicine Food and Nutrition Board, 2001).

2.3.5 Mineral

Mineral is a naturally occurring solid chemical substance that is being formed through geological processes and that has a characteristic of a chemical composition, highly ordered atomic, and specific physical properties. By comparison, a rock is an aggregate of minerals and/or mineraloid and does not have a specific chemical

composition. Minerals range in composition from pure elements and simple salts to very complex silicates with thousands of known forms (Dana and James, 1985). Minerals like vitamins are essential for the growth, development, maintenance and repair of human bodies. Some minerals such as calcium and sodium (major minerals) are already abundant in the body, while others such as selenium and chromium (trace minerals) are limited. Minerals have a great variety of functions, regardless of the concentration in the body (Pharmavite, 2009). Minerals are defined as inorganic elements that originate in the earth and cannot be made in the body (Brody, 1998). Body requires more than 100 milligrams of per day for proper maintenance of health. Micro or trace minerals are those minerals which the body requires less than 100 milligrams of per day (Juo, 1996). Macro minerals include sodium, calcium, chloride, phosphorus, potassium and sulfur. Meanwhile, micro minerals, otherwise known as trace minerals, include such as iron, zinc, copper, chromium and selenium. Heavy metals are defined as metallic chemical element that has a relatively high density and are toxic or poisonous at low concentrations (Irwandi and Farida, 2009).

Dietary minerals are the chemical elements being required by living organisms, other than the four elements of carbon, hydrogen, nitrogen, and oxygen present in common organic molecules. The term "mineral" is archaic, since the intent of the definition is to describe chemical elements, not chemical compounds or actual minerals. Examples include calcium, magnesium, potassium, sodium, zinc, and iodine. The focus dietary on chemical elements derives from an interest in supporting the biochemical reactions of metabolism with the required elemental components (Lippard *et al.*, 1994).

2.4 PHYTOCHEMICAL

Phytochemicals are chemical compounds being obtained from plant that is with biologically and the chemical substance characteristics of plants such as beta-carotene that occur naturally in plants. The term is generally being used to refer to those chemicals that may affect health, but are not yet being established as essential nutrients. Phytochemical is a term that simply means the plant chemical. It refers to everything in plants - the sugars, fiber, vitamins, nutrients, etc. Many of them act as antioxidants, for

example, lutein, found in tomatoes and other foods, some modify the immune system, and others alter enzymes that metabolize drugs in our system (Jeffrey, 2010). Phytochemicals work synergistically with vitamins, minerals, glynutrients, antioxidant and phytosterol to enhance immunity. For the best immune system support, one needs to use a combination of nutrients that supports functions in one body (Johnson, 2011). Phytochemicals in freshly harvested plant foods may be destroyed or removed by modern processing techniques, and possibly including cooking (World's Healthiest Foods). For this reason, industrially processed foods are likely to contain fewer phytochemicals and may thus be less beneficial than unprocessed foods. Absence or deficiency of phytochemicals in processed foods may contribute to increased risk of preventable diseases (Liu, 2004: Rao and Rao, 2007).

The phytochemical study reveals the presence of various flavonoids like quercetin, apigenin, luteolin, salvigenin, genkwanin and volatile oil in the leaves. Lack of proper standards of medicinal plants may result in the usage of improper drugs which in turn will cause damage not only to the individual consuming it, but also to the respect gained by the well known ancient system of medicine (Kaliappan *et al.,* 2008). Torbangun plant has phytochemical as medicinal plant (Table 2.4).

2.4.1 Antibiotic Activity

Antibiotic is a substance or compound that kills, or inhibits the growth of, bacteria. Antibiotics belong to the broader group of antimicrobial compounds, used to treat infections being caused by microorganisms, including fungi and protozoa (Davey, 2000). Antibiotics are a crucial line of defence against bacterial infections. Nevertheless, several antibiotics are natural products of microorganisms that have as yet poorly been appreciated in terms of their ecological roles in the wider environment. The activity of antibiotics may be concentration-dependent and their characteristic that is antimicrobial activity increases with progressively higher antibiotic concentrations, They may also be time-dependent, where their antimicrobial activity does not increase with increasing antibiotic concentrations; however, it is critical that a minimum inhibitory serum concentration is maintained a certain length of time (Rhee, 2004).

Table 2.4. Phytochemical in Torbangun plant

Type of Extraction	Analytical Method	Phytochemical Analysis	References
Extraction of the oil	The oil was being dried over anhydrous sodium sulfate and stored under nitrogen at 4°- 6°C.	Essential oil	Valera *et al*., 2003
The hydroalcoholic extract	Methanol in water	Antioxidant, anticlastogenic	Rao *et al.*, 2006
The dichloromethane extract	Anhydrous sodium sulfate and distilled water (Dutch Pharmacopoeia method)	Essential oil	Bos *et al.*, 1983
Soxhlet extraction by ethanol	The extract were evaporated to dryness by Rotary-evaporator	Anthemintic and antioxidant	Battacharjee *et al*., 2011
Hot water and ethanol extract	Concentrated using rotary evaporator and filter	Antibacterial activity	Subhas *et al.*, 2010

Table 2.4. Phytochemical in Torbangun plant

Type of Extraction	Analytical Method	Phytochemical Analysis	References
Extracted with petroleum ether, chloroform, ethanol and water by cold maceration	The extracts were being filtered with muslin cloth, the solvent was being distilled off and removed under vacuum.	Antidiuretic activity	Roshan *et al*., 2010
Macerated with distilled water	The extract was then being concentrated on rotary flash evaporator	Antibacterial and anti-inflammatory	Kumar *et al.*, 2008
Cold macerated for 24 hrs	The extract was being filtered and the filtrate being stored in the refrigerator	Anti-leptospiral activity	Devi *et al.*, 2008

and Gardiner, 2004). A laboratory evaluation of the killing kinetics of the antibiotic using kill curves is useful to determine the time- or concentration-dependence (Pankey and Sabath, 2004). Antibiotics could interfere with bacterial cell wall synthesis, increase bacterial membrane permeability and/or inhibit bacterial protein synthesis at the 30S subunit of ribosome (Matsumura *et al.*, 1999).

Antibiotics are substances produced by living organisms that are able to kill or inhibit the growth of microorganisms. Antimicrobials have been used in veterinary practice to control, prevent and treat infection, and to enhance animal growth and feed efficiency (Benito, 2008). An antibiotic is necessary to determine its potential because from the effects of antimicrobial being used increasing resistance, also of its effects a on variety of pathogenic microbes. The effectiveness of the inhibition or killing antimicrobial power is highly dependent on the number and strength of the active substance (Singgih, 2007). The screening of crude plant extracts for synergistic interaction with antibiotics is being expected to provide leads for the isolation of multi drug resistant inhibitors (Stapleton *et al.*, 2004).

Since their introduction in human therapy, 60 years ago, antibiotics have shown to be a remarkable success and constitute one of the most relevant medical inventions for reducing human morbidity and mortality. Unfortunately, the intensive use and misuse of antibiotics have resulted in antibiotic resistance among several human pathogens, reducing the possibilities for the treatment of infection and jeopardizing medical procedures, such as organ trans-plantations or implants of prostheses, where infective complications are common and antibiotic therapy is needed to prevent or treat those infections (WHO, 2003). Antibiotics are commonly being classified based on their mechanism of action, chemical structure, or spectrum of activity. Most antibiotics target bacterial functions or growth processes (Calderon and Sabundayo, 2007). Antibiotics that target the bacterial cell wall (such as penicillin's and cephalosporin's), or cell membranes (for example, polymixins), or interfere with essential bacterial enzymes (such as quinolones and sulphonamides) have bactericidal activities. Those that target protein synthesis, such as the amino glycosides, macrolides, and tetracycline's, are usually bacteriostatic (Finberg *et al.*, 2004).

Antibiotics are microbial metabolites or analogues inspired by them that, in small doses, inhibit the growth and survival of microorganisms without serious toxicity to the host (Lester *et al.,* 2008). Pharmacological industries have produced a number of new antibiotics in the last three decades. Resistance to these drugs by micro organisms has increased, and the antimicrobial activities of plant extract and phytochemical was being evaluated with antibiotic susceptibility and resistance against microorganism (Gislene *et al.*, 2000). Antibiotics were once produced by bacteria and fungi, but now most antibiotics are synthetically being produced (Ernawita, 2008). The new sources of antimicrobial agents were then eventually led to intensive research on antimicrobial compounds from plants. Ever since antibiotics were being discovered, the It researches on antimicrobial compounds from plants were no longer conducted. Due to increasing resistance of bacteria to antibiotics, there is an increasing demand of new antimicrobial agents from plants. Plants are considered a reliable source for the discovery of novel antimicrobial agents (Rangasamy *et al.,* 2007). The antibiotic resistance has become a global concern. The clinical efficacy of many existing antibiotic is being threatened by the emergence of multi drug resistant pathogens (Parekh *et al.*, 2007). Many infectious diseases have been known to be treated with herbal remedies as a natural product, either as pure compound or as standardized plant extracts (Rojas *et al*, 2003).

The amino glycosides (AGs) are a large and diverse class of antibiotics that characteristically contain two or more amino sugars linked by glycosidic bonds to an aminocyclitol component. Structures are presented for over 30 of the most important members of this family of compounds (David, 2000). Although AGs may cause side effects of nephrotoxicity and ototoxicity, they still occasionally are used for the treatment of serious infections. In this study, the development of a method is described for the quantitative determination and confirmation of seven amino glycosides (and relevant isomers) and spectinomycin in animal tissues. Several aminoglycosides are available only as mixtures of isomers, such as neomycin (NEO), kanamycin (KAN) and gentamicin (GNT). Marker residues were established by European Medicines Agency (EMEA) as neomycin B (NEO B), kanamycin A (KAN A) and the sum of GNT isomers

C1, C1a, C2 and C2a (Frédérique *et al.,* 2008). Antibiotics in this family characteristically contain two or more amino sugars joined via glycosidically linkage to deoxystreptamine or streptamine. Presence of several hydroxyl groups makes them hydrophilic in nature. They are valuable in the treatment of serious infections caused by gram-negative bacteria. They inhibit the protein synthesis of microorganism resulting in a rapid concentration dependent bactericidal action (Shalini, 2002).

The aminoglycosides are a large and diverse class of antibiotics that characteristically contain two or more amino sugars being linked by glycosidic bonds to an aminocyclitol component (Umezawa and Hooper, 1982). The amino glycoside antibiotic streptomycin was the first drug being discovered through systematic screening of natural products active in Mycobacterium tuberculosis in 1944 (Miloje *et al.*, 2009), and many aminoglycosides were being isolated from soil bacteria, primarily the Gram-positive actinomycetes of the genera Streptomyces and Micromonospora (Hotta, 1996) and (Suzuki *et al.*, 1994). Some amino glycosides function as antibiotics that are effective for certain types of bacteria. These compounds are very important in treating gram-negative bacilli and tubercle bacillus and are widely being used in medical clinics (Rongjie, 2009). The aminoglycosides are broad-spectrum antibiotics that have bactericidal activity against some Gram-positive and many Gram-negative organisms. They are not active against anaerobic bacteria, possibly because their uptake is being blocked under these conditions (Tanaka, 1982). Antibiotics in this family characteristically contain two or more amino sugars joined via glycosidically linkage to deoxystreptamine or streptamine. Presence of several hydroxyl groups makes them hydrophilic in nature. They are valuable in the treatment of serious infections caused by gram-negative bacteria. They inhibit the protein synthesis of microorganism resulting in a rapid concentration dependent bactericidal action (Shalini, 2002).

Macrolide antibiotics are a very important class of antibacterial compounds widely used in medical and veterinary practices. Erythromycin (EM) and oleandomycin (OM) are 14-membered ring macrolide antibiotics. Josamycin (JM), kitasamycin (KT), mirosamicin (MRM), spiramycin (SPM), tylosin (TS) and tilmicosin (TLM) belong to the class of 16-membered macrolide antibiotics. Macrolide antibiotics are considered to

be medium-spectrum antibiotics. They are highly active against a wide range of Gram-positive bacteria such as Mycoplasma and Chlamydia. The macrolides are the most effective medicine against diseases produced by Mycoplasmas (Masakazu *et al*., 2003). Macrolides are very important class of antibacterials widely used in medical and veterinary practices. These drugs are well absorbed after oral administration and are distributed extensively into tissues, especially in the lungs, liver and kidneys (Salisbury *et al.*, 1995; Aiello, 1998). The most commonly used macrolide antibiotics consist of a macrocyclic lactone ring containing 14, 15 or 16 atoms with sugars linked via glycosidic bonds (Elks and Ganellin, 1991). The clinically useful macrolide antibiotics can be conveniently classified into three groups based on the number of atoms in the lactone nucleus. Erythromycins A, B, C, D, E and F, oleandomycin, roxithromycin, dirithromycin, clarithromycin and flurithromycin are 14-membered macrolides whereas azithromycin is a 15-membered compound. 16-Membered macrolides include josamycin, rosaramicin, rokitamycin, kitasamycin, mirosamicin, spiramycin and tylosin, the latter two compounds being used almost exclusively in veterinary medicine (Reynolds, 1993).

Macrolide antibiotics are a very important class of antibacterial compounds widely being used in medical and veterinary practices. Macrolide antibiotics are being considered to be medium-spectrum antibiotics. They are highly active in a wide range of Gram-positive bacteria such as Mycoplasma and Chlamydia. The macrolides are the most effective medicine against diseases being produced by Mycoplasmas and Rickettsia. (Masakazu *et al.*, 2003). In particular, macrolide antibiotics constitute an important alternative for patients exhibiting penicillin sensitivity and allergy. These agents are generally used to treat infection in the respiratory tract, skin and soft tissues and genital tract caused by gram-positive organisms, mycoplasma species and certain susceptible gram-negative and anaerobic bacteria. The generalized structure is a highly substituted monocyclic lactone (aglycone) to which is attached one or more saccharide units (amino or deoxy sugar) glycosidically linked to hydroxyl groups on either the aglycone or another saccharide. They may be divided into 12-, 14- and 16-membered aglycone rings macrolides. Most of them are derived from various strains of Streptomyces and serve as an alternative for patients exhibiting penicillin sensitivity.

They inhibit growth of bacteria by inhibiting protein synthesis on ribosome's (Shalini, 2002).

Penicillin G (Pen G), or benzylpenicillin, the first β-Iactam to be discovered, is currently produced on a very large scale, world-wide, by fermentation using selected strains of Penicillium chrysogenum (Adlard *et al.,* 1991). Antibiotics, including the â-lactam group, can be detected in animal tissues by a variety of screening tests. The â-lactams have traditionally been differentiated from other antibiotics by degradation with the enzyme penicillinase (Katz, 1986). The analysis of degradation products in commercial penicillin's has two-fold importance; firstly, in pharmacokinetic studies, it is desirable to distinguish between the drug and any degradation products, secondly allergic reactions attributed to penicillin may frequently be caused by such compounds. Accordingly, it is essential to be able to detect the presence of these compounds in the pharmaceutical compounds. HPLC methods have been widely employed for such analysis involving control of purity of pharmaceutical samples. In most of the cases studied C18 columns were used, and UV detection method was preferred. Effect of gamma radiation on both, the combination of amoxycillin sodium–potassium clavulanate and individual components (Valvo *et al.*, 1999).

Penicillin was the first microbial metabolite to distinguish between toxicity to the bacterial cell and toxicity to the mammalian host to permit its use in the systemic treatment of infections caused by gram-positive and -negative organisms in humans and animals. The basic structure of the penicillin nucleus includes a β-lactam ring being fused through nitrogen and adjacent tetrahedral carbon to a second heterocycle, which in natural penicillin is a five-member thiazolidine ring (Shalini, 2002). Penicillin G (Pen G), or benzyl penicillin, the first β-Iactam to be discovered, is currently produced on a very large scale, world-wide, by fermentation using selected strains of Penicillium chrysogenum. The β-1actam antibiotics are a therapeutically important group of antibacterial agents. The large demand for penicillin G arises in part from its direct application in human and animal welfare. However, the main industrial interest lies in its use as a precursor for the manufacture of a wide range of semi-synthetic penicillin, which are more efficacious than penicillin G itself (Adlard *et al.,* 1991). Penicillin and

other β-lactams (cephalosporins) inhibit the synthesis of essential structural components of the bacterial cell wall, i.e. peptidoglycan, which is absent in mammalian cells and. Thus, host cell metabolism remains unaffected and penicillin are regarded as one of the safest and most efficacious classes of antibiotics being used for bacterial infections (Shalini, 2002).

Antibiotic or antibacterial are being screened for any negative effects on humans or other mammals before approval for clinical use and are usually considered safe and most are well-tolerated. However, some antibacterial have been associated with a range of adverse effects. Side-effects range from mild to very serious depending on the antibiotics being used; the microbial organisms targeted, and the individual patient. Safety profiles of newer drugs are often not as well established as for those that have a long history of use (Slama *et al.*, 2004). Adverse effects range from fever and nausea to major allergic reactions, including photodermatitis and anaphylaxis. Common side-effects include diarrhea, which resulted from the disruption of the species composition in the intestinal flora, resulting, for example, in overgrowth of pathogenic bacteria, such as Clostridium difficile (University of Michigan Health System, 2006). Antibacterial could also affect the vagina flora, and may lead to overgrowth of yeast species of the genus Candida in the vulvo-vaginal area (Pirotta and Garland, 2006).

Tetracyclines are broad-spectrum bacteriostatic antibiotics produced by species of the genus's Streptomyces (Nuria, 2004). Tetracycline is an extremely important group of antibiotics having a broad spectrum of activity against gram-positive and -negative bacteria, some large viruses, rickettsiae, spirochetes and mycoplasmas. They are also widely used as growth additives in animal feed. Chemically, tetracyclines contain an octahydronaphthacene ring skeleton, consisting of four fused rings. Besides natural tetracyclines isolated from various strains of Streptomyces, many derivatives (e.g. doxycycline, minocycline) have been prepared by their chemical conversion (Shalimi, 2002). They have been used not only in human medicine for the treatment of infectious disease but also as an additive in animal feed to promote growth (Khairi, 2008).

Tetracyclines possess a wide range of antimicrobial activity against gram-positive and gram-negative bacteria; they have been used not only in human medicine for the treatment of infectious diseases but also as an additive in animal feed to promote growth. Although the development of new antimicrobial agents who are more effective for specific infections, and fewer toxic have declined the indications for their use, tetracyclines are still being used widely in both human and veterinary medicine (Williams and Thomas, 2002). Tetracycline is also being used against some large viruses, rickettsiae, spirochetes and mycoplasmas. Chemically, tetracycline contains an octahydronaphthacene ring skeleton, consisting of four fused rings. Besides natural tetracyclines being isolated from various strains of Streptomyces, many derivatives (e.g. doxycycline, minocycline) have been prepared by their chemical conversion (Liang *et al.,* 1998). The mechanism of tetracycline's action is inhibition of protein synthesis. They are active in many aerobic and anaerobic pathogenic bacteria, mycoplasmas, rickettsias, Chlamydia, and spirochete, and some protozoa. They are especially being used in the treatment of acne, brucellosis, urethritis and acute pelvic infections. Tetracycline are the drugs of choice for treating rickets infections (Rodriguez *et al.,* 2004). They are active in many aerobic and anaerobic Gram-positive and Gram-negative pathogenic bacteria, mycoplasmas, rickettsias, chlamydias, and spirochetae, and some protozoa. They are especially used in the treatment of acne, brucellosis, urethritis and acute pelvic infections. Tetracyclines are the drugs of choice for treating rickettsial infections. Tetracycline or doxycycline (semisynthetic derivative) has been used with quinine in the management of chloroquine-resistant falciparium malaria. Tetracyclines are also the usual treatment of balantidiasis, and they have been used in the treatment of severe amoebic dysentery and in Dientamoebafragilis infections (Chopra and Roberts, 2001; Sweetman, 2002; Yao and Moellering, 2003). Tetracyclines are widely used to treat bovine mastitis. They are also used frequently in veterinary formulations to prevent and control disease, as well as in feed additives to promote weight gain and increase feed conversion efficiency (Farrington and Tarbin, 1991).

These agents are generally used to treat infection in the respiratory tract, skin and soft tissues and genital tract caused by gram-positive organisms, mycoplasma

species and certain susceptible gram-negative and anaerobic bacteria. The generalized structure is a highly substituted monocyclic lactone (aglycone) to which is attached one or more saccharide units (amino or deoxy sugar) glycosidically linked to hydroxyl groups on either the aglycone or another saccharide. They may be divided into 12-, 14- and 16-membered aglycone rings macrolides. Most of them are derived from various strains of Streptomyces and serve as an alternative for patients exhibiting penicillin sensitivity. They inhibit growth of bacteria by inhibiting protein synthesis on ribosomes (Brisson *et al*., 1988 in Shalimi, 2002).

2.4.2 Phenolic Compound

Phenolic compound is any of the various synthetic thermosetting resins, being obtained by the reaction of phenols with simple aldehydes and being used to make molded products and as coatings and adhesives. Also being called phenolic resin, relating to, containing, or derived from phenol.The a compound of phenolic acid being derived from plant, added to food as the antioxidant. Phenols are pollutants of great concern because they are toxic and are known or suspected to be carcinogenic when present at elevated levels in the environment. They occur in wastewater of a number of industries, such as high-temperature coal conversion, petroleum refining, resin and plastics. At present, several methods, such as microbial degradation, adsorption, chemical oxidation, incineration, solvent extraction, reverse osmosis and irradiation, are being used for removing phenols from wastewater. A variety of adsorbents used for removal of phenols from single solute aqueous solutions include activated carbon, bentonite and perlite, rubber seed coat, hydrotalcite and its calcined product, acid-activated bituminous shale, zirconium (IV) arsenate–vanadate ion-exchanger, cross-linked polyvinylpyrrolidone, activated natural zeolites, water-insoluble cationic starch and polymeric XAD-4 resins (Krishnaiah, 2003).

Phenolic compounds form a large group of plants secondary metabolites with many functions related to the acclimation and adaptation of plants to changing environment and to the interaction with other organisms. Interestingly, numerous studies have shown the positive influence of phenolic compounds on human health, and

a higher intake can be considered beneficial (Anttonen, 2007). Phenolic compounds are a class of low molecular weight secondary plant metabolites being found in most land plants. These compounds of great importance for food and drink since they are responsible for their organoleptic properties (Goda *et al.*, 1996 and Alonso *et al.*, 2003). Phenolic compounds include a large class of phytochemicals that are being endowed with interesting biological properties. Among the most important are anthocyanins, flavonoids, catechins, phenolic acids, secoiridoids, stilbenes, coumarins and isoflavones which are widespread in vegetable crops such as fruits, vegetables, herbs, grains and seeds and derived foods such as juices, wines, oils, etc. (Shahidi and Naczk, 2003; Mazza and Miniati, 1993).

Phenolic compounds form a large group of plant secondary metabolites and many functions for the acclimation and adaptation of plants to changing environment and to the interaction with other organisms. Phenolic compounds have positive influence on human health, and a higher intake could be considered beneficial (Anttonen, 2007). Phenolic compounds are also very important constituents of plants. Their free-radical scavenging ability is being attributed to hydroxyl groups. Total phenolic content were being measured using an established method of employing the Folin-Ciocalteu reagent (Prior *et al*., 2005).

Many functions are being related to the acclimation and adaptation of plants to changing environment and to the interaction with other organisms (Anttonen and Mikko, 2007). Phenolic substances such as flavonols, cinnamic acids, coumarins, coffee acids or chlorogenic acids are believed to have antioxidant properties, which are being suggested to play an important role in protecting food, cells and any organ from oxidative degeneration (Ossawa, 1999; Tikkanen *et al.*, 1998; Wiseman *et al*., 2000). Phenolicsposses a wide spectrum of biochemical activity such as an antioxidant, antimutagenic, anticarcinogenic, as well as the ability to modify the gene expression (Nakamura *et al*, 2003 and Tapiero *et al*., 2002).

2.4.3 Flavonoid Compound

Flavonoid is any of a large group of organic compounds or water-soluble plant pigments, including the anthocyanins, that are beneficial to health. Flavonoids are polyphenols and have antioxidant, anti-inflammatory, and antiviral properties. They also help to maintain the health of small blood vessels and connective tissue, and some are being under studied as possible treatments of cancer. Flavonoids are a group of plant phenolics, abundant in the plant kingdom. Much research presented in reviews have confirmed that flavonoids have anti-inflammatory, anti-carcinogenic and other beneficial properties (Irena and Dominika, 2001). Flavonoids are present in most plant tissues and often in vacuoles (Croteau *et al,* 2000). The basic structures of flavonoid molecules are being composed of three rings with various substitutions, including glycosylation, hydrogenation, hydroxylaltion, malonylation, methylation and sulfation (Beecher, 2003; Onyilagha and Grotewold, 2004). Flavonoids are being divided into classes according to their substitutes and oxidation level on the middle ring. For humans, several health beneficial properties of dietary flavonoids are being recognized for their antioxidant and anti-proliferative effects, which may protect the body from various diseases, such as cancers, cardiovascular disease and inflammation (Middleton *et al.,* 2000 and Nijveldt, 2001).

Over 5000, naturally-occurring flavonoids have been characterized from various plants. They have been classified into six subgroups are flavones (luteonin, apigenin, tangeritin); flavonols (quercetin, kaemferol, myricetin, isorhamnetin, pachypodol, rhamnazin); Flavanones (hesteretin, naringenin, eriodictyol); flavan-3-ols: (catechins (catechin, gallocatechin, catechin 3-gallate, gallocatechin 3-gallate) and epicatechins (epicatechin, epigallocatechin, epicatechin 3-gallate, epigallocatechin 3-gallate); isoflavones (genistein, daidzein, glycitein); anthocyanidins (cyanidin, delphinidin, malvidin, pelargonidin, peonidin, petunidin (Manach *et al.,* 2004, Dahan and Altman, 2004).

Biological effects of these compounds are various. They are involved in production of pigmentation in flowers. For example, blue colour results from presence

of anthocyanin in petals. Anthocyanins are, also, responsible for the autumn's colours in many plant species and photo-protection of leaf's cells. Their ability of acting as natural UV filters comes from their absorption in 280-315 nm region. Different plant flavonoids have a role in protection from microbes and insects. Some of them (isoflavones, flavons and flavanones) are recognized as constitutive antifungal plant agents. Others (flavonoids, tannins, etc.) play role in plant's protection from insects and mammalian herbivory (Harborne *et al.,* 2000). Besides, many flavonoids have an ability to alter enzymatic and chemical reactions, and thus impact on human health positively or negatively (Beecher, 2003). Flavonoids in plants could function as color definitions and attractants to pollinators and seed dispersers, as antioxidants to protect plants against UV-radiation, as insect feeding attractants in host-species recognition, as signal molecules to facilitate nitrogen fixation, in inducible defense against bacteria and fungal attack; and as bitter orastringent taste attributes to repelling birds and other animals (Wildman, 2001 and Winkel, 2001).

Flavonoids are present in most plant tissues and often in vacuoles. Flavonoids are polyphenolic plant secondary metabolites, and in plants could function as color definitions and attractants to pollinators and seed dispersers, flavonoid also function as antioxidants to protect plants against ultraviolet radiation, as insect feeding, as signal molecules to facilitate nitrogen fixation, against bacteria and fungal; and as bitter or astringent taste which attributes to repel birds and other animals (Ray *et al.,* 2008). Flavonoids belong to a large group of plant's polyphenols. They have a prominent role in pigmentation of plants and their protection from different external agents. In recent years, there is a rising interest in flavonoids, mostly because of their antioxidant, anti-inflammatory, antiallergenic, antimicrobal and anticancer activity (Jelena, 2007).

2.4.4 Antioxidant Activity

Antioxidant is a chemical compound or substance that inhibits oxidation or any substance that reduces oxidative damage (damage due to oxygen) such as that being caused by free radicals. Certain vitamins, such as vitamin E, are antioxidants and may protect body cells from damage being caused by the oxidative effects of free radicals.

Free radicals are highly reactive chemicals that attack molecules by capturing electrons and thus modifying chemical structures. Antioxidants are vital substances, which possess the ability to protect the body from damages being caused by free radical-induced oxidative stress. A variety of free-radical scavenging antioxidants are being found in plants. Antioxidants are micronutrients that have gained importance in recent years due to their ability to neutralize free radicals or their actions (Varahalarao and Naidu, 2009). Antioxidant characteristics of extracts from medicinal plants and complex plant preparations are used with good effect for the treatment of toxic nephropathies, untimely myocyte aging syndromes, side effects of antibiotics, phenylcyanocreatine phosphate derivatives, and hydroxyl thiasoline immune suppressors (Markaryan, 2006).

The natural antioxidants being discovered recently are expected to replace the synthetic antioxidants that are widely being used at present time. Antioxidants from natural substances such as plants, spices and herbs that are being consumed as foods or ingredients have been widely investigated for several biochemical and pharmacological properties. A number of naturally occurring antioxidant compounds have been found to strengthen the resistance of low-density lipoprotein to oxidative modification in vitro and in vivo (Adaramoye *et al.,* 2005). An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxiditizing agent. Oxidation reactions could produce free radicals. In turn, these radicals could start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibits other oxidation reactions. They do this by being oxidized themselves, so antioxidants often are known as reducing agents such as thiols, ascorbic acid or polyphenols (Sies, 1997). Antioxidants are molecules that relieve oxidative stress by preventing formation and by inhibiting oxidation of free radicals (Halliwell, 1995). They are able to donate one of their electrons or hydrogen to free radicals, stopping their chain reactions (Kaur and Kapoor, 2001). Antioxidants are being found in our diet (e.g. vitamins) or formed inside our body, e.g. enzymes. Antioxidants could protect us from the damaging effects of free radicals (Afzal and Armstrong, 2002). The best way to combat free radicals is to have a diet of fresh fruits and vegetables, red wine, and green tea. These functional foods are rich in phytochemicals with antioxidant properties.

Health supplements being enriched with antioxidants are also now widely available (Percival, 1998; Kaur and Kapoor, 2001).

Although oxidation reactions are crucial for life, they could also be damaging; hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Low levels of antioxidants, or inhibition of the antioxidant enzymes, cause oxidative stress and may damage or kill cells. As oxidative stress might be an important part of many human diseases, the use of antioxidants in pharmacology is intensively (Percival, 1998; Kaur and Kapoor, 2001). Studied, particularly as treatments for stroke and neurodegenerative diseases. However, it is unknown whether oxidative stress is the cause or the consequence of the diseases. Antioxidants are widely (Percival, 1998; Kaur and Kapoor, 2001).used as ingredients in dietary supplements in the hope of maintaining health and preventing diseases such as cancer, coronary heart disease and even altitude sickness. Although initial studies suggested that antioxidant supplement might promote health, later large clinical trials did not detect any benefits and suggested instead that excessive supplementation may be harmful (Baillie *et al.*, 2009; Bjelakovic *et al.*, 2007).

In terms of health benefits, antioxidants are commonly being used as medications to treat various forms of brain injury (Warner *et al*., 2004). These compounds appear to prevent oxidative stress in neurons and prevent apoptosis and neurological damage. Antioxidants are also being investigated as possible treatments for neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (Di Matteo and Esposito, 2003; Rao and Balachandran, 2002), and as a way to prevent noise-induced hearing loss (Kopke *et al.*, 2007). Antioxidants are being found in varying amounts in foods such as vegetables, fruits, grain cereals, eggs, meat, legumes and nuts. Some antioxidants such as lycopene and ascorbic acid could be destroyed by long-term storage or prolonged cooking (Xianquan *et al.*, 2005; Rodriguez, 2003). An antioxidant is defined as a molecule capable of slowing or preventing the oxidation of other molecules, whereas a biological antioxidant has been defined as "any substance that, when present at low concentrations

compared to those of an oxidizable substrate (Halliwell, and Gutteridge, 1995). The oxidation damages various biological substances and subsequently causes many diseases. There are many reviews on the relationships between oxidative damages and various diseases, including cancer (Paz-Elizur *et al.,* 2008), liver disease (Preedy *et al.,* 1998), Alzheimer's disease (Moreira *et al.,* 2005), aging (Liu and Mori, 2006), arthritis (Colak, 2008), inflammation (Mukherjee *et al.,* 2007), diabetes (Naito, *et al*., 2006; Jain, 2006), Parkinson's disease (Beal, 2003; Chaturvedi, 2008), atherosclerosis (Beal Heinecke, 1997), and AIDS (Sepulveda and Watson, 2002). As a result, many diseases have been treated with antioxidants to prevent oxidative damage.

Antioxidant compounds in food play an important role as a health protecting factor. In food science, antioxidant was defined as a substance in foods that when present at low concentrations compared to those of an oxidizable substrate significantly decreases or prevents the adverse effects of reactive species, such as reactive oxygen and nitrogen species (ROS/RNS), on normal physiological function in humans (Huang *et al*., 2005). Plant antioxidants constitute one of the most active food compounds (Kris-Etherton *et al.,* 2002). The first comprises chemical substances, which interrupt the propagation of the free-radical chain by hydrogen donation to radicals or stabilization of relocated radical electrons (Karadag *et al*., 2009). The second group is characterized by a synergistic mode of action. It includes oxygen scavengers and chelators which bind ions involved in free radical formation. Their activity consists of hydrogen delivery to phenoxyradicals that leads to the reconstitution of the primary function of antioxidants. This role is played by substances binding to metal ions, e.g. citric acid, and by secondary antioxidants, such as amino acids, flavonoids, β-carotene, selenium and many others. Some compounds, such as gallates, have strong antioxidant activity, while others, such as the mono-phenols are weak antioxidants (Karadag *et al.,* 2009). According to Akoh and Min (1998), the efficiency of phenolic frees radical scavengers (FRS) depends on additional factors such as volatility, pH sensitivity, and polarity. An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules, when present at low concentrations compared to those of an oxidizable substrate, which significantly delays or prevents oxidation of that substrate. As a result, many diseases have been treated with antioxidants to prevent oxidative damage. Well-

known natural antioxidants, such as vitamin E (R-tocopherol), vitamin C, and polyphenols/flavonoids, have been investigated for their possible use to prevent the diseases being described above. Vitamin E therapy is being reportedly effective in decreasing oxidative stress and the levels of erythrocyte osmotic fragility in patients on dialysis according to (Joon and Takayuki, 2009).

2.5 HEAVY METALS

Heavy metals are chemical elements with a specific gravity that is at least 5 times the specific gravity of water. The specific gravity of water is 1 at 4°C (39°F). Simply stated, specific gravity is a measure of density of a given amount of a solid substance when it is being compared to an equal amount of water (Lide, 1992). A heavy metal is a member of a loosely-defined subset of elements that exhibit metallic properties. It mainly includes the transition metals, some metalloids, lanthanides, and actinides. Many different definitions have been proposed—some based on density, some on atomic number or atomic weight, and some on chemical properties or toxicity (John, 2002). Heavy metals are closely being connected with environment deterioration and the quality of human life, and thus have aroused concern all over the world. More countries have signed treaties to monitor and reduce heavy-metal pollution (But *et al*., 1964). The heavy metal being found are metals accumulate in animal and plant cells, leading to severe negative effects. The transport and accumulation of heavy metals could be by air (Nriagu, 1989), water (Boening, 2000; Barrie *et al*., 1992) and soil (Evans, 1989; Holmgren *et al*., 1993).

Heavy metals are closely connected with environmental deterioration and the quality of human life, and thus have aroused concern all over the world (Yuh-Shan and Mohammad, 2009). They are arsenic (As), cadmium (Cd), cobalt (Co), cyanide (Cn), nickel (Ni), Zinc (Zn) and chromium (Cr), and they are phytotoxic at all concentrations of above certain threshold levels (Varsha *et al*., 2010). The living organisms as bio-indicators for monitoring environmental pollution have been observed for many years in various countries. For permanent observation of diverse kinds of transformations in the environment different vegetable species, are being used of which they are able to absorb

and cumulate which potentially toxic substances are in use. Usually these include moss, lichen, bark, needles of pines or some species of herbs. Among medicinal raw materials include dandelion (Taraxacumofficincale) (Djingovar and Kuleff, 1986; Kabata and Dudka, 1991) and black poplar (Populusnigra) (Djingovar *et al*., 1999).

Arsenic could be present in the terrestrial, marine, and freshwater environments in various chemical forms. Organic arsenic species are less toxic than inorganic species to aquatic plants, animals and humans, and this has been presumed to be also true for terrestrial plants (Meharg and Whitaker, 2002). Arsenic is the most common cause of acute heavy-metal poisoning in adults and is number one on the ATSDR's "Top 20 Lists." Arsenic is being released into the environment by the smelting process of copper, zinc, and lead, as well as by the manufacturing of chemicals and glasses (Roberts 1999).

Lead (Pb) is one of the heavy metals, which is a poison. Lead is a gray metal which readily in the form of salts by combining with anions. It is the most immutable of substances, the production of radioactive decay. The concentration of lead is usually high in the exam area due to the mining activities. The contribution of lead toxicity could affect the red blood cells, narrow system, kidneys, infants, and fetuses also being the most vulnerable. According to the Environmental Protection Agency (EPA), lead is the most common heavy-metal contaminant in the environment. Lead may be toxic to organisms even when being absorbed in small amounts (Watanabe, 1997). Lead could trigger both acute and chronic symptoms of poisoning. Acute intoxications only occur through the consumption of relatively large single doses of soluble lead salts. Chronic intoxications could arise through the regular consumption of foodstuffs even it only slightly being contaminated with lead. Lead is a typical cumulative poison. The danger of chronic intoxications is the greater problem (Codex Alimentarius Committee, 1996). Lead is number 2 on the ATSDR's "Top 20 Lists." Lead accounts for most of the cases of pediatric heavy-metal poisoning (Roberts 1999). Lead is the most common of the heavy elements. Several stable isotopes exist in nature, lead being the most abundant. The average molecular weight of lead is 207.2. Lead is a soft metal that resists corrosion and has a low melting point ($327^{O}C$). From a drinking water perspective, the most universal use of lead compound is in plumbing fitting, and it is important as a solder in

the water distribution system (Quinn and Sherlock, 1990). Lead conc61entrations in Torbangun plant extract was of 2.39 ppm (2.39 mg/kg). The concentration of lead in vegetables exceeded their permissible limit of 3 mg kg-1 (Codex Alimentarius, 2001b).

Cadmium (Cd) is a heavy metal being found as an environmental contaminant, both through natural occurrence and from industrial and agricultural sources. Foodstuffs are the main source of cadmium exposure for the non-smoking general population (Europe Food Safety Authority, 2009). Cadmium is being concentrated particularly in the kidneys, the liver, the blood forming organs and the lungs. It most frequently results in kidney damage (necrotic protein precipitation) and metabolic anomalies being caused by enzyme inhibitions (Codex Alimentarius Committee, 1996). Cadmium tends to be very mobile in soil systems and therefore, very available to plants. Cd 2+ is the main species in soil solution. Accumulation of cadmium in food crops in soil concentrations that are not phytotoxic is a significant concern. Plant species differ widely in their tendency to accumulate cadmium. Absorption/desorption of cadmium is about 10-fold more rapid than that of lead. Chronic cadmium exposures result in kidney damage, bone deformities, and cardiovascular problems (Curtis *et al*., 2002; Fritioff *et al*., 2007). Cadmium is a by-product of the mining and smelting of lead and zinc and is number 7 on ATSDR's "Top 20 lists." It is being used in nickel-cadmium batteries, PVC plastics, and paint pigments. It could be found in soils because insecticides, fungicides, sludge, and commercial fertilizers that use cadmium are being used in agriculture (Roberts 1999). Cadmium is a silvery white, and lustrous. Cadmium concentrations in leaf tissues of Torbangun was at 0.07 ppm (0.07 mg/kg). The concentrations of Cd in plant tissues exceeded the permissible limit of 2 mg/kg (Codex Alimentarius, 2001a). The permissible limit of cadmium concentration set by FAO/WHO (1984) in edible plants was at 0.21 ppm.

Selenium (Se) is a micronutrient for human and animals, and their main source of selenium is food. However, selenium is both essential and toxic depending on its chemical form and concentration, and it has the narrowest tolerance band of any element. Evidence is growing that selenium-enriched derivatives prevent selenium deficiencies and also provide protection against various forms of cancers (Rayman,

1997; Neve *et al.,* 1998 and Yoshizawa *et al.,* 1998). Selenium (Se) concentrations in leaf tissues of Torbangun was at 1.64 ppm (1.64 mg/kg). Selenium is a trace mineral that is essential to good health but being required only in small amounts (Thomson, 2004; Goldhaber, 2003). The concentrations of Se in plant tissues exceeded the permissible limit of 3.50 mg/kg recommended FAO/ WHO (1984).

Magnesium is a macro mineral, essential to the proper utilization of other minerals in the body, including calcium and phosphorus. Magnesium is also critical to energy production and to over 300 enzymatic reactions in the body. It helps metabolize carbohydrates, proteins and fats, plus other minerals and nutrients (Nature's Sunshine Products Follows Numerous Manufacturing Guidelines, 2001). Magnesium is an essential element in biological systems. Magnesium occurs typically as the Mg2+ ion. It is an essential mineral nutrient for life (Lusk *et al.,* 1968; Marschner, 1995). Magnesium (Mg) concentrations in leaf tissues was at 23375.59 ppm (2.3% Mg) in Torbangun plant. Magnesium, a mineral present in most foods, is essential for human metabolism and for maintaining the electric potential in nerve and muscle cells (Nature's Sunshine Products Follows Numerous Manufacturing Guidelines, 2001). Mg is at the core of the chlorophyll molecule, and an essential ingredient for healthy plants, and animals (including humans) that eat those plants. It is needed in more than 300 biochemical reactions in the body. It helps maintain normal muscle and nerve functions, and keep heart rhythm steady (Darius, 2009 and Carolyn, 2009).

Means plan is which can accumulate and tolerate, greater metal concentrations have been reported of the plant member of the Brassicaceae family, the plant is very important in the hyper-accumulator group, with the minimum threshold tissue concentration for Co, Cu, Cr, Pb, should be 0.1% dry weight, while for Zn, Mn threshold 1% (Backer and Brook, 1989). However, phyto remediation of Selenium (Se) is a toxic metal at medium to high concentration, but is essential as a micronutrient for human and animals (Pilon-Smiths *et al.,* 1999). On the order hand, Cd is a toxic element, its concentration greatly being increased by activities such as Zn mining and used as fertilizer (Cobbet *et al.,* 2002). The high concentration of heavy metals in soil is being reflected by higher concentration of metals in plants and consequently, in animal

and human bodies and their health (Buszewski, 2000; Namiesnik *et al*., 2000). The relation between content of metal in soil and their accumulation in different morphological part of a plant is in the concentration of Pb, Zn, Cd, Cu, Fe, Ca, and Mg from the soil and plants (McCrady and Maggard, 1993; Namiesnik *et al.*, 2000). The concentration of the analysis showed from the highest concentration was being observed for macro elements such as Fe, Ca, and Mg from physiological properties of the investigated soil (Buszewski, 2000). An accumulation of heavy metals in plants depends not only on the metal content in soil, but also depends on the soil chemical element, metal type pH of soil and plant species (Darmono, 1995). Cadmium (Cd) being a highly toxic metal pollutant of soil inhibits roots and short growth while yield production is affected in by nutrient uptake and homeostasis. It is frequently being accumulated by agriculturally important crops and impairs animal and human health. Cd is being described as mobile in the soil, to easily absorb by plant than Pb (Alloway, 1995). Cadmium may cause decreased uptake of nutrient element's inhibition of various enzyme activities, induction of oxidative stress, including alterations in enzymes of the antioxidant defence system (Sandalio *et al*., 2001). It is known to be a toxic metal in plants through its incorporation into the carbonic an hydrates enzyme (Price and Morel, 1990). Cadmium is high in soil and is taken up by leafy vegetables in amounts that may affect toxicity in any ages with anaemia, neuropathy, hypertension, cortex of kidney (0.05 to 0.1 mg/10g) and liver dysfunctions and also cancer; WHO has recommended that the provisional permissible intake of Cd cold not exceed 0.4-0.5 mg per week (Ghinwa and Bohumil, 2009)? Heavy metal contamination in vegetables such as cadmium (Cd) and lead (Pb) in the human body will be interaction with enzyme, protein, DNA and other metabolites. Morever, heavy-metal contents in the soil are naturally very low, unless the soil is already contaminated (Charlena, 2004), and being represented the most dangerous metal forms in the environment or plants (Table 2.5).

Copper (Cu) concentrations in leaf tissues was at 15.42 ppm (15.42 mg/kg) in Torbangun plant. Cu concentrations were below the permissible limit of 200 mg kg-1 dry wt. (Food Standards Committee, 1950). Heavy metals such as Cu and Zn are

Table 2.5. Heavy metal in some plant

Plant Name/ Type	Heavy Metals	References
Tomato plant	Lead and Cadmium	Reginawanti *et al.*, 2004
Chinese Brake fern *(Pteris vittata* L.)	Arsenic	Bonada and Lena, 2003
Taraxacum officincale and black Poplar (*Populus nigra*)	Zinc, Cadmium, Lead, Nickel and Molibdenum	Baranowska *et al.*, 2002
Vegetable plant (138 kinds)	Cadmium, Lead, Zinc and Copper	Anthonyand Balwant, 2005
Mangrove plant	Copper, Ferrum, Magnesium, Manganese, Zinc, Mercury, Lead and Strontium	Govindasamy *et al.*, 2007
Ipomoea reptan Poir (Kangkung plant)	Lead	Indrajati *et al.*, 2004

essential for normal plant growth and development since they are constituents of many enzymes and other proteins (Hall, 2001).

2.5.1 Beneficial of Heavy Metals

In small quantities, certain heavy metals are nutritionally essential for a healthy life. Some of these are being referred to as the trace elements (e.g., iron, copper, manganese, and zinc). These elements, or some form of them, are commonly being found naturally in foodstuffs, in fruits and vegetables, and in commercially available multivitamin products (International Occupational Safety and Health Information Centre 1999).

As trace elements, some heavy metals like selenium (Se), zinc (Zn) and copper (Cu) are essential to maintain the metabolism of the human body (Charlena, 2004). In small quantities, certain heavy metals are nutritionally essential for a healthy life. Some of these are being referred to as the trace elements (iron, copper, manganese, and zinc). These elements, or some form of them, are commonly found naturally in foodstuffs, in fruits and vegetables, and in commercially available multivitamin products (International Occupational Safety and Health Information Centre 1999). The metallic elements can be divided into two groups, which are essential for survival, such as iron (Fe) and calcium (Ca), and those that are non essential or toxic, such as cadmium (Cd) and lead (Pb) (Yuh-Shan and Mohammad, 2009). Chromium (Cr), Cooper (Cu) and zinc (Zn) can induce the activity of various antioxidant enzymes and are also non enzymes, like ascorbate and gluthalion (Varsha *et al*., 2010). Heavy metals such as lead are one that is found to contaminate in soil, sediment, air and water. Total annual emission of lead by motor vehicle and industrial plants (Friedland, 1990). Phytoremedian is a green technology for the sustainable remediation of surface soil being contaminated with heavy metals (Gupta *et al*., 2004). It is an innovative, novel, and potentially inexpensive technology using metal polluted soil, sludges and sediments (Salt *et al.*, 1995).

Diagnostic medical applications include direct injection of gallium during radiological procedures, dosing with chromium in parenteral nutrition mixtures, and the use of lead as a radiation shield around x-ray equipment (Roberts 1999). Heavy metals are also common in industrial applications such as in the manufacture of pesticides, batteries, alloys, electroplated metal parts. Textile dyes, steel, and so forth. (International Occupational Safety and Heath Information Centre 1999). Many of these products are in our homes and actually add to our quality of life when properly being used.

2.5.2 Harmful of Heavy Metals

Heavy metals may enter the human body through food, water, air, or absorption through the skin when they come in contact with humans in agriculture and in manufacturing, pharmaceutical, industrial, or residential settings. Industrial exposure accounts for a common route of exposure for adults. Ingestion is the most common route of exposure in children (Roberts, 1999). Heavy metals in environment, soil, water and air with a mechanism in humans, and plants as mediators of the pollutant as the spreading distribution of heavy metal on human is by rhizome and leaves (stomata). By eating those vegetables, it makes the heavy metals move in the human body, such as Pb and Cd (Charlena, 2004). The standards around the world, differences among nations or regulations on importation and exportation of medicinal plants can affect the quality control of herbal product; medicinal plant product may be classified as food, food supplements, and functional food neutraceutical or prescription herbal medicine in the different countries or regions (Kelvin, 2009).

Important toxicity of heavy metals from plants for drugs, are led (Pb), arsenic (As), cadmium (Cd) and mercury (Hg) (Alloway, 1995). Metal toxicity unlike some organic substances, are not metabolically degradable and their accumulation in living tissues can cause death or serious health threats (Ghinwa and Bohumil, 2009). The high concentration of heavy metals in soil is being reflected by higher concentration of metals in plants and consequently, in animals and human bodies (Buszewski *et al.*, 2000). Cadmium is a non essential toxic metal which enters into aquatic environment

through industries like electroplating, batteries, chemical and various other applications, which cause the accumulation of Cd (Garg h *et al.*, 1996). On the other hand, many studies have investigated the presence of toxic contaminants in herbal plants. Trace elements such as zinc, manganese, chromium, copper, iron, lead, nickel and vanadium were being found in a traditional Chinese herb Jingi (Han *et al.*, 2008). In 2007, a 93Year the old hypertensive woman was being reported with severe hypokalemia due to the consumption of licorice-containing herbal medicine for seven years (Yasue, 2007).

There are a number of studies suggesting minerals and heavy metals in plants or foods. Various instruments and methods have been used to determinate minerals and heavy metals of foods, viz., atomic absorption spectrophotometer (AAS), and inductively couple plasma – mass spectrophotometer (ICP-MS). In this study, inductively a couple of plasmas – mass spectrometer was used to determinate minerals and heavy metals. Advantages of using an ICPMS include its ability to identify and quantify all elements except for Argon, multi-element technique, suitable for all concentrations from ultra trace levels to major components; detection limits are generally low for most elements with a typical range of 1 - 100 g/L, high sensitivity; good precision and accuracy, multi elemental analysis can be accomplished, and quite rapidly (Worley and Kvech, 2000).

2.6 LACTATION

Lactation describes the secretion of milk from the mammary gland, the process of providing that milk to the young, and the period of time that a mother lactates to feed her young. The process occurs in all female mammals, and in humans, it is commonly being referred to as breastfeeding or nursing. The chief function of lactation is to provide nutrition and immune protection to the young after birth. In almost all mammals, lactation induces a period of infertility, which serves to provide the optimal birth spacing for survival of the offspring (McNeilly, 1997). Lactation begins after delivery of a baby. The initial secretion of the mammary glands before true lactation begins is being termed colostrums (Thomson, 2006).

Lactation has three stages of lactogenesis, the mammary gland develops the capacity to secrete milk. Lactogenesis includes all processes necessary to transform the mammary gland from its undifferentiated state in early pregnancy to its fully differentiated state sometime after pregnancy. This fully differentiated state allows full lactation (Carol *et al*., 2006). During the latter part of pregnancy, the woman's breasts enter into the Lactogenesis I stage. This is when the breasts make colostrums, a thick, sometimes yellowish fluid. At this stage, high levels of progesterone inhibit most milk production. It is not a medical concern if pregnant woman leaks any colostrum before her baby's birth, nor is it an indication of future milk production (Cregan *et al.*, 2002). The mammary gland becomes competent to secrete milk. Lactose, total protein, and immunoglobulin concentrations increase within the secreted glandular fluid, while sodium and chloride concentrations decrease. The gland is now sufficiently being differentiated to secrete milk, as being evidenced by the fact that women often describe drops of colostrum on their nipples in the second or third trimester. However, high circulating levels of progesterone and estrogen hold the secretion of milk in check (Carol *et al.*, 2006). At birth, prolactin levels remain high, while the delivery of the placenta results in a sudden drop in progesterone, oestrogen, and human placenta levels (HPL). This abrupt withdrawal of progesterone in the presence of high prolactin levels stimulates the copious milk production of Lactogenesis II (Cregan *et al*., 2002).

Lactogenesis III, the hormonal endocrine control system drives milk production during pregnancy and the first few days after the birth. When the milk supply is more firmly being established, an autocrine control system begins. During this stage, the more than milk is being removed from the breasts, the more the breast will produce milk (de Carvalho *et al*., 1985; Hopkinson *et al.*, 1988). In this stage, blood flow, oxygen, and glucose uptake increases, and citrate concentration increases sharply. Progesterone plays a key role in this stage. Removal of the placenta (i.e, the source of progesterone during pregnancy) is necessary for the initiation of milk secretion; however, the placenta does not inhibit established lactation. Progesterone receptors are lost in lactating mammary tissues, thus decreasing the inhibitory effect of circulating progesterone. In addition, maternal secretion of insulin, growth hormone (GH), cortisol,

and parathyroid hormone (PTH) facilitates the mobilization of nutrients and minerals that are being required for lactation (Carol *et al.*, 2006).

In physiological lactation fullness of the breast and galactostasis (milk remaining in the breast without removal) would lead to a decreased milk production. The accumulation of milk in the breast would reduce the binding of prolactin to its membranes. This would happen in any breast that gets overfilled, independent of the status of the other breast. The reduction in the binding of prolactin to membrane receptors would create an inhibitory effect on levels of milk production. In full alveoli lactocytes (milk producing cells) would have a lowered uptake of prolactin from the blood. If the full breast is being emptied, prolactin again would bind to the membrane receptors, thus enhancing milk synthesis. The more empty the alveoli, the higher the milk synthesis rate, slowing down as the breast refills (Cox *et al.*, 1996; Daly *et al.*, 1993; Cregan *et al.*, 2002).

Physiology of lactogenesis is the process of milk secretion, and it occurs as long as milk is being removed from the breast. However, the process of lactation and the act of breastfeeding is quite complex, because a range of factors in the mother's external and internal environment includes her physical and mental health, past experiences and intentions, which would determine breastfeeding efficacy. And most importantly, the quality and quantity of maternal–infant interaction during the early postpartum period, sometimes being described as the fourth trimester, sets the stage for a successful breastfeeding experience (Nancy, 2007).

Milk lactation is breast milk being produced by mammary glands located in the breast tissue. Several hormones regulate the development of the mammary glands as well as the initiation and maintenance of lactation. The most important of these hormones are prolactin and oxytocin, both of which are being produced in the pituitary gland located in the brain. Prolactin, together with other hormones (e.g., oestrogen and progesterone), regulates the final development of the mammary glands during pregnancy. After birth (i.e, parturition), the woman s hormonal environment changes, and in this setting prolactin could initiate milk secretion from the mammary glands

(Julie, 2008). Milk lactation provides many benefits to the newborn as well as the mother. In addition to providing antibodies to the newborn, the mother's milk also contains antibodies that provide important protection from disease. Digestion is being made easier for the baby because of the many digestive enzymes found in the mother's milk. Studies indicate that breast milk also reduces the occurrence of allergies, diarrhoea, and ear infections in babies (Thomson, 2006).

The amount of milk produced depends on the amount of lactations. Successful, exclusively breastfeeding babies show a three-fold variation in the amount of milk they take per day, and in the frequency of breastfeeds and amount of milk consumed during each breastfeed (Jacqueline, 2007). Relaxation is the key for successful lactation. Milk production is responsive to maternal states of well-being. Thus, stress and fatigue adversely affect a woman's milk supply. The mechanism for this effect is the down-regulation of milk synthesis with increased levels of dopamine, nor-epinephrine, or both, which inhibit prolactin hormone synthesis (Carol *et al.*, 2006). However, without pregnancy and simply through nipple stimulation, prolactin begins to be secreted. This also prompts a signal to the brain from the breast to release oxytocin, which is the key to start the ejection of the milk (Cristine, 2007).

Medicinal plant has always been, and would continue to be, an important therapeutic option for kind human, but it has been only recently that herbal products have come under scrutiny for their beneficial properties or potentially toxic components. Of the thousands of plants kind used for medicinal purposes worldwide, very little information exists regarding their effects on lactation (Table 2.6).

2.6.1 Hormone in Lactation

Hormones are chemicals being released by cells that affect cells in other parts of the body. Only a small amount of hormone is being required to alter cell metabolism. It is essentially a chemical messenger who transports a signal from one cell to another. All multi-cellular organisms produce hormones; plant hormones are also being called phytohormone. Hormone in animal often is transported in the blood. Cells respond to a

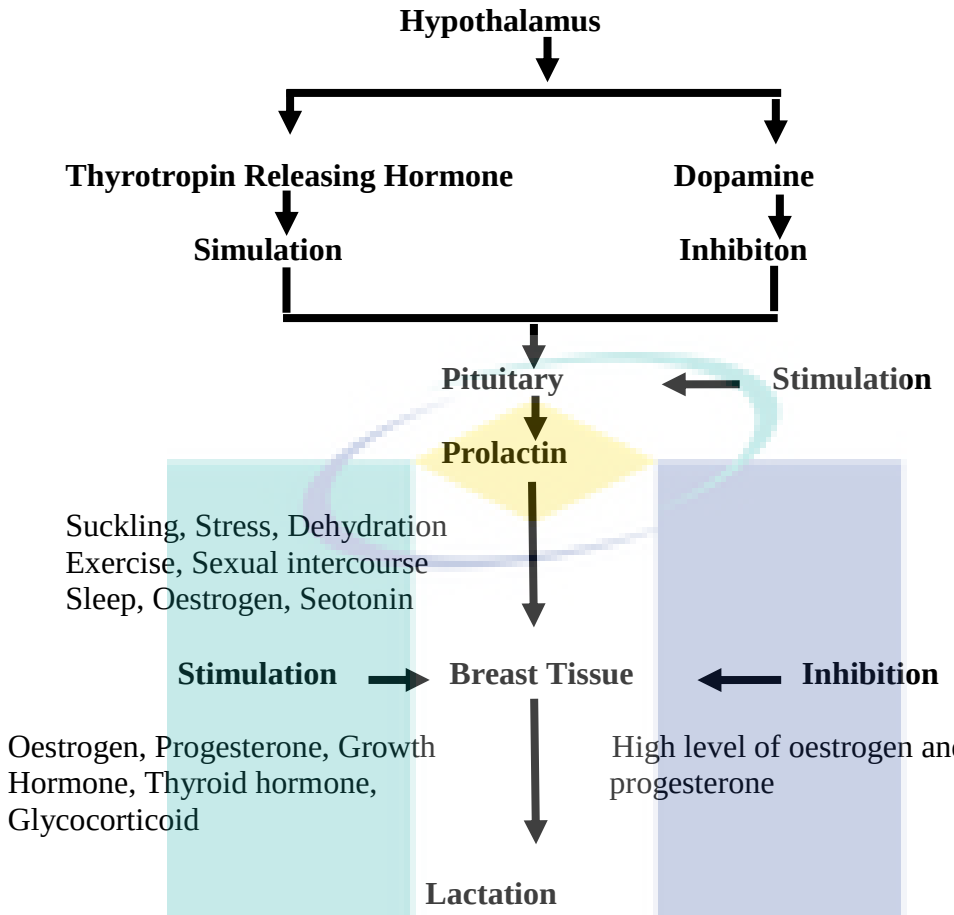


Figure 2.3. Physiology of lactation (Edge and Segatore, 1993; Katznelson and Klibanski,1999; Yazigi *et al.*, 1997)

hormone when they express a specific receptor for that hormone. The hormone binds to the protein receptor, resulting in the activation of a signal mechanism that ultimately leads to cell type-specific responses. The secretion of hormones from successive levels of endocrine cells is being stimulated by chemical signals originating from cells higher up to the hierarchical system. The master coordinator of hormonal activity in mammals is the hypothalamus, which acts on input that it receives from the central nervous system (Mathews and van Holde, 1990; Crisp *et al*., 1998).

Hormones act on multiple organs and affect on other's synthesis and secretion. Estrogens, for instance, control the reproductive tract and the gonads as well as the skeletal system and the cardiovascular system (Cathrin and Malley, 2010 cited in

Table 2.6. The plant as promoted lactation

Plant Name	Active Ingredient	Description	References
Torbangun plant (*Coleus amboinicus* Lor)	Lactagogue	breast milk stimulant	Damanik *et al.*, 2006
Sauropus androgynus (Katuk leaves)	Steroid dan poliphenol	to increase prolactin hormone	Sa'rony, 2004
Trigonella foenum-graecum L. (Fenugreek)			Damanik *et al.*, 2004
Zea maize			Sihombing, 2006; Damanik, 2001

Stampfer *et al*., 1991; McDonnell and Norris 1997; Couse and Korach 1999). They also act on the pituitary gland to stimulate prolactin synthesis and secretion (Cathrin and Malley, 2010 cited in Scully *et al.* 1997). Hormonal influences for lactation are progesterone, oestrogen, follicle stimulating hormone (FSH), luteinizing hormone, prolactin, growth hormone, thyroid-stimulating hormone (TSH), oxytocin, ATCH and glucocortocoids (Mohrbacher *et al.,* 2003).

2.6.2 Essential hormones for lactation

During the second stage of lactogenesis, the breast becomes capable of milk production. For the ongoing synthesis and secretion of human milk, the mammary gland must receive hormonal signals. These signals, which are in direct response to stimulation of the nipple and areola (mammae), are then being relayed to the central nervous system. This cyclical process of milk synthesis and secretion is being termed lactation. Lactation occurs with the help of two hormones, prolactin (PRL) and oxytocin. While PRL and oxytocin act independently on different cellular receptors, their combined actions are essential for successful lactation (Carol, 2006).

Prolactin is a hormone being secreted by the anterior portion of the pituitary gland (sometimes being called the "master gland"). Its role in the females, is that is prolactin promotes lactation, or milk production, after childbirth (Goffin, 2002). Prolactin also known as luteotropic hormone (LTH), a protein that in humans is being encoded by the PRL gene (Evans *et al*.,1989). Prolactin is a peptide hormone, primarily being associated with lactation. In breastfeeding, the act of an infant suckling the nipple stimulates the production of oxytocin, which stimulates the "milk let-down" reflex (Bartholomew *et al.,* 2007). Milk synthesis occurs in the mammary gland epithelial cells in response to prolactin activation of epithelial cell prolactin receptors. PRL, a polypeptide hormone being synthesized by lactotrophic cells in the anterior pituitary, is structurally similar to GH and placental lactogen (PL), which appears to have cytokine functions (Goffin, 2002). Prolactin has many effects, including regulating lactation and stimulating proliferation of oligodendrocyte precursor cells. These cells differentiate

into oligodendrocyte, the cells responsible for the formation of myelin coatings on axons in the central nervous system (Gregg *et al.*, 2007).

Oxytocin is a mammalian hormone that acts primarily as a neuromodulator in the brain. Also known as alpha-hypophamine (α–hypophamine), oxytocin has the distinction of being the very first polypeptide hormone to be sequenced and synthesized biochemically (Vincent *et al.*, 1953). Oxytocin is best known for its roles in the female reproduction: it is being released in large amounts after distension of the cervix and uterus during labor, and after stimulation of the nipples, facilitating birth and breastfeeding. Recent studies have begun to investigate the oxytocin's role in various behaviors, including orgasm, social recognition, pair bonding, anxiety, and maternal behaviors (Lee *et al.*, 2009). Oxytocin receptors are being expressed by the myoepithelial cells of the mammary gland, and in both the myometrium and endometrial of the uterus at the end of the pregnancy. The oxytocin receptor system plays an important role as an inducer of uterine contractions during parturition and of milk ejection. Oxytocin receptors are also present in the central nervous system. These receptors modulate a variety of behaviors, including stress and anxiety, social memory and recognition, sexual and aggressive behaviors, bonding (affiliation) and maternal behaviour (Caldwell and Young, 2006; Kiss and Mikkelsen, 2005; Veenema and Neumann, 2008).

Steroid is any of a large group of fat-soluble organic compounds, as the sterols, bile acids, and sex hormones, most of which have specific physiological action or A group of molecules that include cholesterol. The steroid contains a specific arrangement of four cycloalkane rings that are being joined to each other. Examples of steroids include the dietary fat cholesterol, the sex hormone's estradiol and testosterone, and the anti-inflammatory drug dexamethasone. Steroids vary by the functional groups attached to this four ring core and by the oxidation state of the rings. Sterols is special forms of steroids, with a hydroxyl group at position-3 and a skeleton being derived from cholestane (Moss,1989).

Hundreds of distinct steroids are being found in plants, animals, and fungi. All steroids are being made in cells either from the sterols lanosterol (animals and fungi) or from cycloartenol (plants). Both lanosterol and cycloartenol are being derived from the cyclization of the triterpenesqualene. Some of the common categories of animal steroids those are insects, vertebrate steroids and cholesterol. The vertebrate steroids that are steroid hormone, sex steroids, corticosteroid and anabolic steroids.Whereas, steroids in plant included phytosterols and brassinosterols (Raksha *et al*., 2012).

Steroids include oestrogen, cortisol, progesterone, and testosterone. Estrogen and progesterone are being made primarily in the ovary and in the placenta during pregnancy, and testosterone in the testes. Testosterone is also being converted into oestrogen to regulate the supply of each, in the bodies of both females and males. Certain neurons and glia in the central nervous system (CNS) express the enzymes that are being required for the local synthesis of pregnantneuron steroids, either from peripherally-derived sources. The rate-limiting step of steroid synthesis is the conversion of cholesterol to pregnenolone, which occurs inside the mitochondrion (Rossier, 2006).

2.6.3 Lactagogue

Lactagogue is a substance that increases lactation in humans and other animals. Its may be synthetic; plant derived, or endogenous. At present, people still believe lactagogue from traditional natural material more than products from modern fabric or synthetic. Usually, the case was proven with through anciently experience (Kaliappan, 2008). Herbal galactogogues are being divided into those believe to also have a sedating action on the nursing infant due to their volatile constituents, which could be carried through the breast milk itself, and those seen as promoting milk production without directly affecting the content. This often seems to be linked to anethol content (Macintyre, 2003). Lactagogues are being used during lactation to increase the quantity of breast milk. Common lactogogues include anise, blessed thistle, chaste berry, fennel, fenugreek, hops, marshmallow, milk thistle and nettles. These herbs have been traditionally used by women to increase their milk

supply while nursing with varying results. Proper nutrition, including adequate protein, B- vitamins and essential fatty acids are essential in milk production. If one is having problems with the supply, ones hold check one's diet first, increase fluids and allow the baby to nurse frequently to stimulate increased production. (Gretchen, 2000).Torbangun has been used by the Bataknese people of Indonesia as a galactagogue for hundreds of years. An investigation into this traditional usage found that it increased milk volume produced by 65%, compared to 20% for fenugreek seeds (Damanik *et al.*, 2006). The most effective galactagogues are medications, available usually by medical prescription. These include domperidone and metoclopramide. Domperidone, a dopamine antagonist, is not approved for enhanced lactation in the USA. It is, however, prescribed in the UK. Some drugs, primarily atypical antipsychotics such as Risperdal, may cause lactation in both women and men. Most of those discovered have been found to interact with the dopamine system in such a way to increase the production of prolactin endogenously (Chantry *et al.*, 2004; da Silva and Knoppert, 2004).

2.64 Mammary Gland Organ

The mammary gland is the largest exocrine gland in the animal body. The growth rate of mammary gland is increased following puberty, greatly accelerated during pregnancy, reacheds its greatest development during the lactation period (Dellman and Brown, 1987). The parenchyma is made up of secretory epithelial cells to produce milk from the blood. The epithelial cells function in the synthesis and secretion of milk. Where myoepithel cells assist epithel cells in milk ejection (Holland and Holland, 2005). The relative amount of connective tissue vs secretors tissue varies from animals to animal, stage of mammary gland development and even by location of tissue within the gland. The mammary gland undergoes extensive structural changes during different stage's of lactation including those in heifer and pregnant ones. In view of the importance, it is considerably desired to study the histological aspect of the same in different stages of development (Patel *et al*., 2007).

Mammary glands are the accessory reproductive glands within the breasts that function in milk production. Fifteen to twenty-five lobes of glandular tissues containing

mammary alveoli synthesize and secrete the milk in the mammary glands, a process being known as lactation. The milk is being transferred via a system of lactiferous ducts that converge toward the nipple. Ligamentous and fatty tissues surround the ducts and give support and shape to the breasts (Thomson, 2006). The mammary gland is the largest exocrine gland in the body. The growth rate of mammary gland is being increased following puberty, greatly being accelerated during pregnancies, reaches its greatest development during lactation period and involutes after the lactation (Dellman and Brown, 1987). The mammary gland is a dynamic tissue that undergoes epithelial expansion and invasion during puberty and cycles of branching and lobular morphogenesis, secretory differentiation, and regression during pregnancy, lactation, and involution. The mammary gland is being composed of epithelium, adipose tissue, and connective tissue stoma, which are being invested with blood vessels, nerves, smooth muscle fibres, lymphnodes and lymphatic, and, near the nipple, keratinized epithelium and sebaceous and sweat glands (Patricia *et al*.,2000).

To keep the correct polarized morphology of the lactiferous duct tree requires another essential component mammary epithelial cells extracellular (ECM), which together with adiposity, fibroblast, inflammatory cells, etc. constitute mammary stroma (Edgar and Semken, 1991).Mammary epithelial ECM mainly contains myoepithelial.

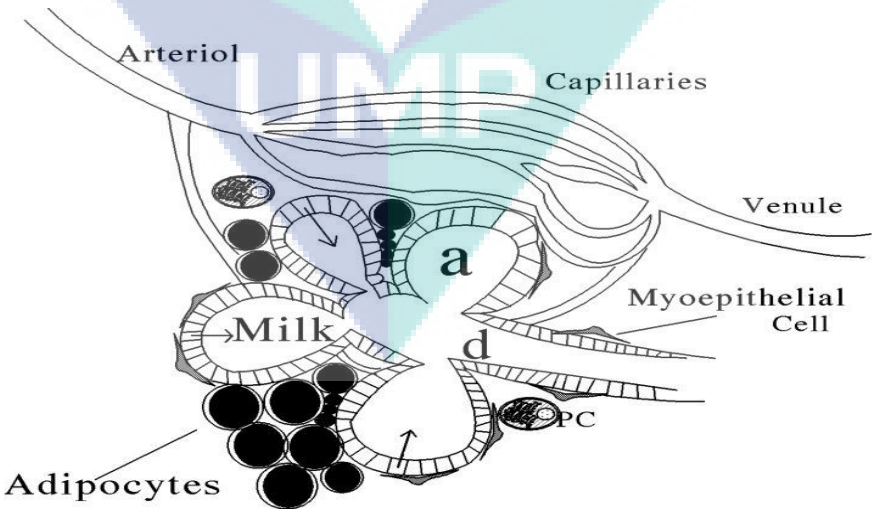


Figure 2.4. Model alveolus (a) with subtending duct (d) showing blood supply, adiposity stroma, myoepithelial cells, and plasma cells (PC).

basement membrane and the connective tissue. They not only help to support mammary basic structure, but also serve as a communicating bridge between mammary epithelials and their local and global environment throughout this organ's development (Bullard *et al*., 1970).

2.7 CLINICAL TEST

Clinical trials are a method being designed to scientifically determine the effectiveness of various treatment regimens. Clinical trials for diseases most often evaluate chemotherapy, radiation therapy, or biologic therapy. These studies consist of four phases being used in the evaluation of investigational drugs that may have therapeutic indications for patients (Terry, 1997). Clinical trial is being designed to test hypothesis and rigorously monitor and assess what happens. Clinical trials could be seen as the application of the scientific method to understanding human or animal biology. The most commonly performed clinical trials evaluate new drugs, medical devices, biologics, psychological therapies, or other interventions. Clinical trials may be required before the national regulatory authority approves marketing of the drug or device, or a new dose of the drug, for use on patients (FDA regulation). Before a new drug, surgical procedure, or therapy becomes available to the public, it must go through a rigorous testing process and be evaluated by the US Food and Drug Administration (FDA). This testing process consists of a series of clinical trials that are being designed to test the safety and usefulness of the new drug as compared to the current standard treatment (Hollon, 2000).

Clinical trials often involve patients with specific health conditions who then benefit from receiving otherwise unavailable treatments. In early phases, participants are healthy volunteers who receive financial incentives for their inconvenience. The durations are of anything from 1 to 30 nights, occasionally longer, although is not always being required. In planning a clinical trial, the sponsor or investigator first identifies the medication or device to be tested. In medical jargon, effectiveness is how well a treatment works in practice, and efficacy is how well it works in a clinical trial. In the U.S., the elderly comprise only 14% of the population, but they consume over

one-third of drugs (Avorn, 2004). Despite this, they often are excluded from trials because they're more frequent health issues, and drug use produces unreliable data. Women, children, and people with unrelated medical conditions are also frequently being excluded (Van Spall *et al.,* 2007).

Clinical trials are only a small part of the research that goes into developing a new treatment. Potential drugs, for example, first have to be discovered, purified, characterized, and tested in labs (in cell and animal studies) before ever undergoing clinical trials. Laboratory animals are not only crucial in understanding diseases; they are also essential in evaluating the safety of drugs, vaccines, food additives, household products, workplace chemicals, cosmetics, water and air pollutants, and many other substances. The FDA oversees this process for drug, vaccine, food additive, and cosmetic safety testing. Other agencies like the Consumer Product Safety Commission, the Environmental Protection Agency, and the Occupational Safety and Health Administration regulate other types of testing. In this research, were being clinical test carried out using animal's laboratory to show histopathology description of mice mammary gland for Torbangun plant effects (Table 2.7).

2.7.1 Histology Description

Histology is the examination of tissues from the body under a microscope to spot the signs and characteristics of disease. A histopathology report describes the tissue that has been sent for examination and what its features are under the microscope. Occasionally, a histopathology report is also called a biopsy report or the microscopic study of abnormal tissue and organs at the cellular level (Mosby's Dental Dictionary, 2008). Histopathology is a specialty concerned with the nature and cause of disease as being expressed by changes in cellular or tissue structure and function caused by the disease process. The classification being offered herein is an attempt to simplify the nomenclature, being based partly on sub-gross and histopathology studies .of whole human breasts which permit the three-dimensional recognition of geographically isolated lesions of hyperplasic, dysplastic, anaplastic and neoplastic character (U.S.

Table 2.7. Clinical test in Coleus family

Plant Name	Method	Result	References
Coleus amboinicus	Isolated perfused frog heart preparation	a positive inotropic effect	Hole et al., 2008
Coleus barbatus B	The doses being used in this experiment corresponded to 10, 20 and 40 times that used for the treatment and distill water for control	880 mg/kg per day of the extract of C. barbatus before embryo implantation caused delayed fetal development and an anti-implantation effect	Almeida and Lemonica, 2000

National Library of Medicine). Histopathology description is a rigorously examination of tissues from the body under a microscope to spot the signs, characteristics of disease and effects of the new drug. A histopathology report describes the tissue that has been sent for examination and what its features are under the microscope. Histopathology which now forms an essential part of modern medicine. In Malaysia, the rising demand for histopathological examinations over the years reflects an increasing recognition of the useful contribution which Histopathology can make question in the diagnosis and management of patients. Like the other branches of pathology, the quality of the histopathology service influences and reflects the quality and standard of patient care. However, unlike some of the other branches of pathology, histopathology demands the direct involvement and attention of the pathologist in every examination, for he has to personally read and report on each slide. It is a branch of pathology, which is particularly taxing on the energy and time of the pathologist. Without the pathologist, the histopathology service ceases. This paper gives an idea of the state of the histopathology services in Malaysia today and the problems unique to it (Looi and Path, 1983).

2.8 MOUSE (*Mus musculus*)

Mice are common experimental animals in biology and psychology primarily because mice are mammals, and also because mice share a high degree of homology with humans. Mice are the most commonly used mammalian model organism, more common than other animal's Laboratory. Mouse (*Mus usculus albinos*) is a rodent (rodentia), which multiplies quickly, easily kept in the number of lots. The genetic variation is quite large and the nature of its anatomical and character physiologic are well (Malole and Pramono, 1989). In addition, mouse also has a life cycle that is relatively short, the number of child births per lot. The nature of variation is high, and easily being treated (Moriwaki *et al.,* 1994). So what matters is that the basic of mice election as animal experiments in this research. According to Malole and Pramono (1989), the mice who are the most frequently being used in laboratories for a variety of research is of the Swiss albino kind (Figure 2.8.1), and mice have always been used as laboratory animals for several researchers (Table 2.8).

Table 2.8. Animal Laboratory from some Coleus family

Laboratory Animals	Plant Name	References
Mouse	*Coleus barbatus* B	Almeida and Lemonica, 2000
Mouse	Torbangun plant (*Coleus amboinicus* Loru)	Kumar *et al.*, 2007; Jose *et al.*, 2005; Roshan *et al.*, 2010;
Frog (*Rana tigrina)*	Torbangun plant (*Coleus amboinicus* Lour)	Hole *et al.*, 2008
Fish	Torbangun plant (*Coleus amboinicus* Lour)	Sunitha, *et al.*, 2010
Chinese hamster	*Coleus aromaticus*	Rao *et al*., 2006
Chicken	Torbangun plant (*Coleus amboinicus* Lour)	Presilla, 2005

2.8.1 Classification of Mouse

Kingdom	Animalia
Phylum	Chordata
Class	Mammalia
Order	Rodentia
Suborder	Myomorph
Super family	Muroidea
Family	Muridae
Subfamily	Murinae
Genus	*Mus*
Species	*Musmusculus*

Figure 2.5a**.** Scientific classification of Laboratory mouse **(**Arrington (1972).

Figure 2.5b. Mouse(*Musmusculus albinos*)

2.8.2 Morphology of Mouse

The mouse has short smooth hairs and white colour and its tail is reddish colour with longer body than the head. The colour of the mouse hairs differs due to the differences of blood proportion compare to wild mouse who allows production and reproduction being refracted (Nafiu, 1996). Laboratory mouse has the body weight of approximately the sum as the wild mouse. Its body weight is 18-20 grams at four weeks old but after selective care for over eighty years, it now many hairs, and different body weight (Smith and Mangkoewidjojo 1988).

Table 2.9. Biology Data of Mouse (*Mus musculus albinos*). Smith and Mangkoewidjojo 1988).

Criteria	Estimate
Life circle	1 - 2 years, usualy 3 years
Gestation period	9 months
Long pregnant	19-21 days
Wean and mature age	21 days and 35 days
Breeding age	8 weeks
Estrus circle	4-5 days
Long estrus	12-14 hours
Mature weight	Male : 20-40 grams and Female : 18-35 grams
Birth weight	0,5 - 1,0 grams
Children amount	Averages 6, until 15
Rectal temperature	35-39ºC (averages 37,4ºC)
Breath	140-180/m,anestecy condition to become decrease
Pulse	80/min and if stress up 230/min, 600-650/min, anestecy condition to become decrease 350/minute and if stress up 750/minute.
Nipple	10 nipples, 3 pair in breast and 2 pair in stomach
Growth speed	1 gram/day.
Passive Immunity	Via intestine until 17 days old, and via ovum

2.9 OPTIMIZATION

The discovers the best way in which a product or process can be optimized. Some commonly used optimization experiments include response surface methodology, evolutionary operation, simplex optimization, and self-directed (Brereton, 1990). Optimization is a procedure being used to make a system or design as effective or functional as possible, especially when the mathematical techniques are being involved. The function that is to promote the optimization and diversification of agricultural products. The optimization is well known that least-squares and linear programming problems have a fairly complete theory, arise in a variety of applications, and could be

solved numerically very efficiently (Stephen and Vandenberghe, 2004). Optimization is one of many desirable goals in software engineering, and is often antagonistic to other important goals such as stability, maintainability, and portability. At its most cursory optimization is beneficial and should always be applied (Hsieh, 2004).

Optimization techniques are one way to obtain operation (decision making) that would, to the extent possible, approach goals that have been set in response to a given problem. As a result, solutions such as design for minimal operating cost, optimal product quality, smallest device size and the like could be realized. The development of optimization techniques began during World War II. Subsequently, mathematical programming has been developed to realized optimization through the application of mathematical techniques (Kitagawa *et al.*, 2011). The most basic advantage is that the problem could then be solved, very reliably and efficiently, using interior-point methods or other special methods for convex optimization (Stephen and Vandenberghe, 2004). In this research, optimization techniques were being carried out on total phenolic, flavonoid compound and antioxidant activity analysis.

Experimental optimization could be carried out in several ways. Most popular is the one-variable-at-a-time approach. This approach is, however, extremely inefficient in locating the true optimum when interaction effects are present, simultaneous design's experiment, the choice depends on the purpose of the study (Walters *et al.*, 1991).

2.9.1 Process optimization

Process optimization is an activity used to increase the efficiency of a process. A process that is completely efficient is one that does not create any unintentional work nor does it have any non value-added work or activities within it. The act of doing something that changes a process with the express purpose of improving the efficiency of the process in respect to the processes intended outcome (International Process and Performance Institute, 2008).

An optimization process is a process that systematically and the discipline of adjusting a process to optimize some specified set of parameters without violating some constraint.

2.10 Inductively Coupled Plasma – Mass Spectrometer (ICP-MS)

The principle of this method is measurement of ions produced by a radiofrequency inductively coupled plasma. Species originating in a liquid are nebulized and the resulting aerosol is transported by argon gas into the plasma torch. The basic instrumental components of the ICP-MS are shown in Figure. 2.6a.

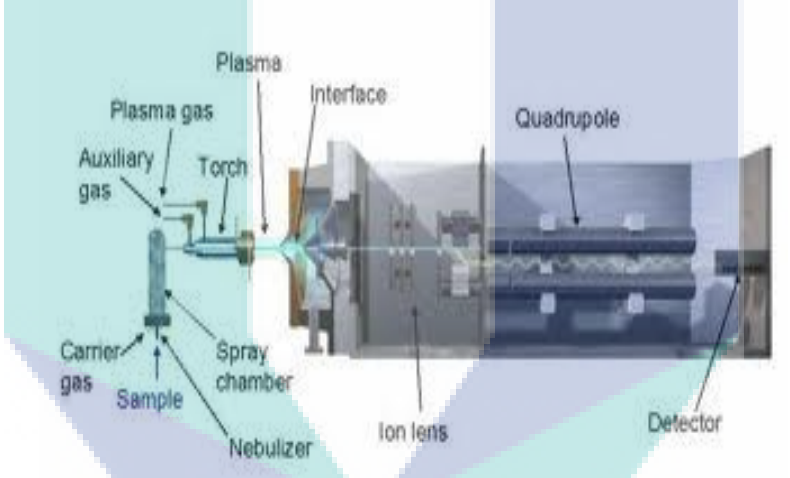


Figure 2.6a. Basic instrumental components of ICP-MS. (Wilbur & Morton, 2005)

The ions produced by high temperatures are entrained in the plasma gas and introduced, by an interface, into a mass spectrometer. The ions produced in the plasma are sorted according to their mass-to-charge ratios and quantified with a channel electron multiplier.

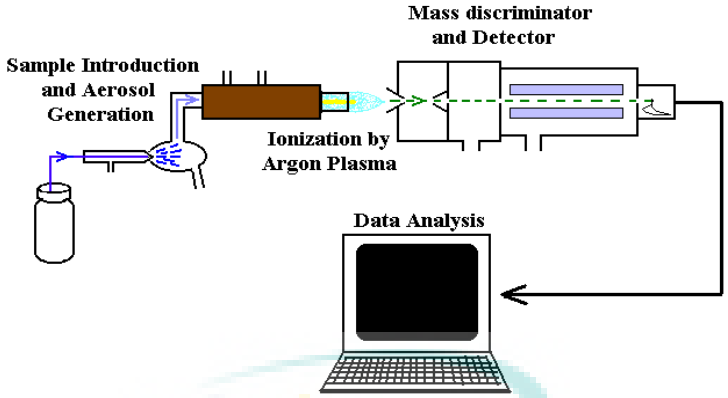


Figure 2.6b. **S**chematic of ICP-MS. (Worley and Kvech, 2000)

Figure 2.9b shows the schematics of an ICP-MS main process. Interferences must be assessed, and valid corrections applied. Interference correction must include compensation for background ions contributed by the plasma gas, reagents, and constituents of the sample matrix (van der Wiel, 2003).

2.11 Gas Chromatography Mass Spectrophotometer (GC-MS)

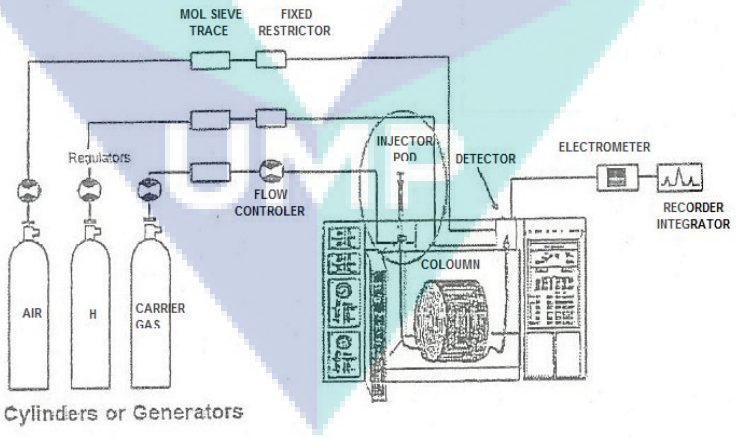


Figure 2.7. **S**chematic of GC-MS (Agilent Technology).

2.12 Fourier Transform InfraRed (FTIR)

FTIR is the preferred method of infrared spectroscopy In infrared spectroscopy. Infrared radiation is passed through a sample. Some of the infrared

radiation is absorbed by the sample, and some of it is passed through (transmitted). Infrared spectroscopy is one of the most common spectroscopic techniques used by organic and inorganic chemists. It is the absorption measurement of different infrared frequencies by a sample positioned in the path of an infrared beam. The main goal of infrared spectroscopic analysis is to determine the chemical functional groups in the sample. Different functional groups absorb characteristic frequencies of infrared radiation (Sherman, 2011).

Infrared spectroscopy is a simple and reliable technique widely used in both organic and inorganic chemistry, in research and industry. It is used in quality control, dynamic measurement, and monitoring applications (Lau, 1999). The qualitative aspects of infrared spectroscopy are one of the most powerful attributes of this diverse and versatile analytical technique. Over the years, much has been published in terms of the fundamental absorption frequencies (also known as group frequencies) which are the keys to unlocking the structure–spectral relationships of the associated molecular ibrations. Applying this knowledge at the practical routine level tends to be a mixture of arts and cience. This is achieved by recognizing characteristic shapes and patterns within the spectrum, and by applying the information obtained from published group frequency data, along with other chemicals and physical data from the sample (John, 2000).

CHAPTER 3

MATERIALS AND METHODS

3.1 INTRODUCTION

This chapter presents the materials and methods used for the entire experiments. The works aim to researching “the effect of Torbangun plant (*Coleus amboinicus* Lour) active substances on mammary gland in lactating mice". The research was conducted which prepares of Torbangun plants, animals or mice (*Mus musculus*) and cages, preparation of animal research feeding, the research procedure, the preparation of chemicals and equipment. The methods of research are compound analysis by using GC-MS and FTIR, phytochemical test (total phenolic, flavonoid compound, antibiotic and antioxidant activity), heavy metal's analysis, clinical test by using lactating mice and histology description of mammary gland.

3.2 DURABILITY ANALYSIS

The research was conducted in the Laboratory of Chemistry, Faculty of Chemical and Natural Resources Engineering, Universiti Malaysia Pahang and Veterinary Laboratory in Agriculture Institute Bogor, National Veterinary Drug Assay Laboratory, Bogor and Quality Control of Product Livestock Laboratory Bogor, Indonesia. Durability analysis began from July 2009 until July 2010.

3.3 EQUIPMENT AND INSTRUMENTATION

Cage (36x28x12cm), drum for water, 265ml measurement bottle and suckling from metal pipette, plate for feed, gloves, masker, thermometer and barometer, brush, scisssors, spuite and napkins, machine for mouse meals, clean bench, tissue processor,

embedding center, microtome, microscope with camera, autopsy and histology slide equipment.

Glassware that is petri dish, reaction tube, Baker's glass, volumetric flash, volumetric glass, erlenmeyer, balance bottle, volumetric pipette and graduate pipette, funnel, centrifuge tube and soxhlet.

Other equipments included digital balance, UV-Vis Spectrophotometer Hitachi 1800 (Hitachi, Japan), hotplate, vacuum rotary evaporator (Buchi, Germany), centrifuge, homogenizer, micropipette 100ml-1000 ml, blender (Waring, USA), Gascooker, waterbath, caliper, clean bend, refrigerator, homogenizer, freezer -18^{O}C, Ultrasonic, Incubator 30^{O}C; 36^{O}C and 55^{O}C (Memmert, Germany), autoclave, paperdish, pH meter, balance, hot-plate and magnetic stirrer, freeze dryer, ICPMS 7500a Agilent (Agilent Technologies, USA), microwave digester (MILESTONE Ethos-E,Italy), and GC-MS.

3.4 MATERIALS AND REAGENTS

Torbangun plant (Coleus amboinicus Lour) bought at Senen Market in Jakarta, Indonesia. The sample were identified and confirmed by Indonesia Institute of Sciences, Research Center for Biology No.1033/IPH1.02/If.8/VIII/2010. The 36 mice (*Mus musculus*) and cages from Kullyah Science, International Islamic University Malaysia, Kuantan, Pahang.

The chemicals utilized were alcohol, xylene, 10% buffer neutral formalin, liquid paraffin, haematoxylin–eosin and entelan reagents. The agar Mediums: BactoPeptone, D(+)Glucose, Bacto Agar, Tryptone, Beef Extract and Yeast Extract (Merck); KH2PO4 p.a, KOH p.a, Na2HPO4 p.a, H3PO4 p.a, HCl p.a, NaOH p.a, K2HPO4, NaCl p.a(ChemAR).

Micrococcus luteus ATCC 9341for Macrolide group, *Bacillus subtillis* spore ATCC 6633 for Aminoglycoside group, *Bacillus cereus* spore ATCC 11778 for

Tetracyclineand *Bacillus steorothermophillus* spore ATCC 7953 for Penicillin group. Comparison standard solution being used were Na-PC, KM-SO4, TS-tartrat and OTC-HCl.

3.5 METHODOLOGY and METHODS

3.5.1 ANALYSIS ACTIVE SUBSTANCES BY GC-MS

(1) Preparation of sample extract

Fresh leaves of Torbangun plants mixed with used distilled water, blended and then dried using a freeze dryer to turn it into a powder. Twenty grams powdered of Torbangun leaves mixed with 100 ml of various solvent (distilled water, ethanol and acetone solution). The plant extracts were prepared by using soxhlet apparatus collected for eight hours and stored in a vial bottles (Alade and Irobi, 1993). In addition, the plant extracts ng prepared using Rotary Evaporator equipment to dried and collected with a solution of ethanol added and stored in a vial bottles (Adam, 1995).

(2) GC-MS Analysis

TheGC-MS analysis of the Torbangun leave extracts was carried out using a Claruss 500 Perkin Elmer gas chromatography equipped with an Elite-5 capillary coloum (5% Diphenyl 95% dimethylpolysiloxane) (30nm x 0.25mm ID x 0.25µmdf), and mass detector turbo mass gold of the company which was being operated in electron ionization (EI) method. Helium was being carried gas at a flow rate of 1ml/ min, the injector was operated at 200^{O}C, and the oven temperature was being programmed at 60^{O}C for 15 minutes, then gradually increased to 280^{O}C at three minutes (Figure 3.1).

Table 3.1. Experiment of Torbangun plant active substance Performance.

THE PERFORMANCE OF TORBANGUN PLANT (COLEUS AMBOINICUS LOUR) ACTIVE SUBSTANCES EFFECTED ON LACTATION MAMMARY GLAND IN MICE

SEPARATION PROCESS/ OPTIMIZATION	CHARACTERIZATION OF MATERIALS	CLINICAL TEST
1.Total Phenolic Compound 1.Optimization of Sample temperature and minute. 2.Preparation of Standards 3.Folin-Ciocalteu's reagent put into all tubes. 0, 1, 2, 3, 4, 6, 8 and 10 ml of 20 ppm gallic acid standard solution, 1 g sample added 20 ml hot water(80^{O}C). Procedure: 9 ml sample extract added 1ml diluted Folin Ciocateu's reagent, shake and added 1 ml 20% Na carbonat, shake and stand on room temp. for 30 minutes. Determined on UV-Vis Spectrophotometer **2. Total Flavonoid Compound Screening Test** 1.Preparation of Standard Quercetin as a standard. Using a seven point standard curve (0 – 50 mg/l).	**1.Active Compound Analysis by GC-MS** 1.Preparation of sample extract 20 g sample powder mixed with 100 ml ethanol by using soxhlet 2.GC-MS Analysis Injec into GC –MS with flow rate 1 ml/min, the injector 200^{O}C and the oven temperature was at 60^{O}C for 15 min, then gradually increased to 280^{O}C at 3 min. **2.Compound analysis by FTIR** Preparation of sample and KBr pellet 1 part smooth sample to 100 part KBr Procedure: KBr pellet put in sample holder and placed the sample holder in the spectroscopy, Obtain the infrared spectrum of the sample	**1. Histology Description** 2. Preparation of Sample 3. Preparation of Histology Slides:trimmed, fixated dehydrated, impregnation, embedded, cutting, 4. Staining process by xylol, alcohol and haemotoxyline-eosin (H & E) 5. Mounting by entelan reagent and covered with the glass 6. Examined under a light microscope

Table 3.1. Experiment of Torbangun plant active substance Performance

THE PERFORMANCE OF TORBANGUN PLANT (COLEUS AMBOINICUS LOUR) ACTIVE SUBSTANCES EFFECTED ON LACTATION MAMMARY GLAND IN MICE

SEPARATION PROCESS/ OPTIMIZATION	CHARACTERIZATION OF MATERIALS	CLINICAL TEST
Procedure: 0.1 ml of the sample added 1 ml DW 0.5 ml sample added 1.5 ml of 95% alcohol, 0.1ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of DW Incubated at room temp. for 40 minutes, measured at 415 nm on UV Vis-Spectrophotometer **3.Antioxidant Activity Determination** **1. Procedure** 1 ml alcohol 0.3 mM solution of DPPH added 2.5 ml samples with different concentrations and kept at room temperature in the dark and after 30 min the optic density was being measured at 518 nm.	**3.Antibiotic Activity Screening Test** 1. Preparation of Agar medium *B.subtilis* spore medium (NV3); *M. luteus* medium (NV8); *B. Cereus* spore medium (MX) and *B. Stearothermophillus* spore medium (Calidolactis) 2. Preparation of phosphate buffer :No.2; No. 3 and No.8 3. Standard stock solution and working standard solution 4. Medium culture Sample analysis: Agar medium culture put into Petri-dish, to put 3 paper disc on Agar medium culture and pipette with sample and 1 paper disc pipette with standard, The medium culture put on room temperature for 1-2 hours. To incubate for 16 – 18 hours. **4.Heavy Metal Analysis by ICPMS** Procedure: 0.5 g sample into a Teflon digestion vessel, added HNO_3 (65%, 7 mL) and H_2O_2 (30%, 1 mL). capped and fitted into rotor segments and inserted into the microwave cavity. The samples were radiated for 20 minutes. Upon cooling, the vessels were being uncapped and solutions transferred into 50 mL volumetric flasks.	**2. Clinical Test** Procedure a. 36 mice female and three male were mated b. After pregnant, put of mice in one cage one mouse (36 cages). c. Torbangun extracts give on birth (0 day) via oral everyday. d. The mother and pups weighed before and after suckled for 15 days and recorded. e. And then finished, the mother carried out autopsy and took mammary gland and weighed and recorded. f. The mammary organs processed histology description.

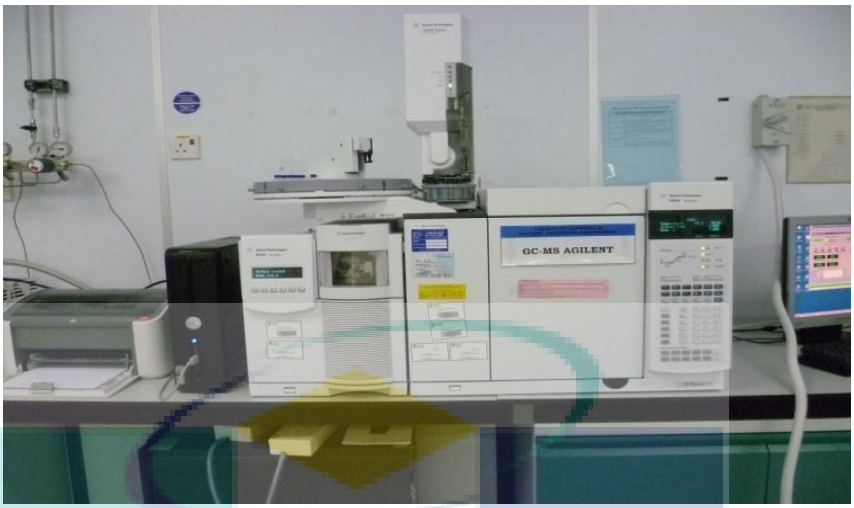


Figure 3.1. Gas Cromatography Mass Spectroscopy (GC-MS) equipment unit

3.5.2. INFRARED ANALYSIS IN TORBANGUN LEAVES

(1) Preparation of sample and KBr pellet

Fresh leave of Torbangun plants blended until smooth with used distilled water and then dried using by freeze dryer to become powders. The pellet of KBr is a dilute suspension of solid. It is usually obtained by grinding the sample in anhydrous KB rat the ratio approximately one part sample to100 parts KBr.

(2) Procedure

The mixtures are ground up using mortar and pestle and then placed on apart of stainless steel die. The dies are then placed in a hydraulic press and subjected to pressure of 15000 psi for about 20 seconds. The powder will results in KBr pellet that is reasonably transparent both to visible light and infrared radiation. After preparing the KBr pellet to put in sample holder and placed the sample holder in the spectrometer (Figure 3.2), obtains the infrared spectrum of the sample (Helal, 2009).

Figure 3.2. FourierTransform InfraRed (FTIR) equipment unit

3.5.3 ANTIBIOTIC SCREENING TEST (BIOASSAY)

(1) Preparation of Agar Media

B. subtilis spore media (NV3): 5 g Peptone, 3 g beef extracts and 15-16g Bacto agar put into 1000 ml aquadest, pH.8,5± 0.1 and boiled. The medium was being sterilized and put in to the autoclave at 121^{O}C temperature and 15 psi pressure for 15 minutes.

The *M. luteus* medium (NV8): To soluble of 6 g Peptone, 1.5 g beef extracts, 3 g yeast extracts, 1g D(+)Glucose H_2O and 15-16 g bacto agar were put into1000 ml distilled water, pH 8.5± 0.1, and boiled. The media were sterilized and put into the autoclave at 121^{O}C temperature and 15 psi pressure for 15 minutes.

The *B.cereus* spore medium (MX): To soluble of 6 g Peptone, 1.5g beef extracts. 3 g yeast extracts, 1.35g KH_2PO_4 and 15-16g bacto agar into 1000 ml distilled water, pH 5.7±0.1, and boiled. The media were sterilized and put in to the autoclave at 121^{O}C temperature and 15 psi pressure for 15minutes.

B. Stearothermophillus spore media (Calidolactis): To soluble of 12g yeast extracts, 5g Tryptone,1g Dextrose and 15-16 g bacto agar into 1000 ml distilled water, pH 7.0±0.1 and boiled. The media were sterilized and put into the autoclave at 121^{O}C temperature and 15 psi pressure for 15 minutes.

(2) Preparation of phosphate buffer solution

(a) Phosphate buffer No.2 pH.6,0±0,1 for KM-SO_4: To soluble of 3.5g KH_2PO_4 and 3 g Na_2HPO_4 into 1L distilled water, pH.6,0±0.1, Sterilized into the autoclave at 121^{O}C temperature with 15 psi pressure for 15minutes.

(b) Phosphate buffer No.3 pH.6,0±0,1 for Na-PC: 7g KH2PO4 and 6g Na_2HPO_4 into 1L distilled water, pH.6,0+0.1,Sterilized into the autoclave at 121^{O}C temperature with 15 psi pressure for 15 minutes.

(c) Phosphate buffer No.8 pH.7,0+0,1 : 6.4g KH_2PO_4 and 18.9 g Na_2HPO_4 into 1L distilled water, pH.7,0+0.1, Sterilized into the autoclave at 121^{O}C temperature with 15 psi pressure for 15 minutes.

(3) Standard stock solution

Each antibiotic standard such as Na-PC, KM-SO_4 and OTC-HCl were weighed and diluted with buffer solution except for Tylosine, which was being (TS-tartrat) dissolved previously with 10% Methanol in distilled water.

(4) Working standard solution

Each antibiotic was made 3 series dilution of working standard solution with phosphate buffer. No.8 for KM-SO_4, TS tartrate and OTC-HCl until get 1 µg/ml concentrate for Na-PC could produce 5 series dilutions until 0.01 unit/ml concentrate was obtained.

(5) Media culture

The agar media to provide water at a temperature of 100 ° C, and placed in the waterbath at 56^{O}C temperature. *B.subtilis* spore was being inoculated on NV3 agar media. *M. luteus* NV8 agar medium. *B. cereus* spore on MX agar media, *B.*

stearothermophillus spore on calidolactis agar media and became homogeneous. Each of the 7ml media culture pipette and poured into a sterile petri-dish and frozen.

(6) Sample preparation

The fresh leaves and stem of Torbangun as samples, each sample was added the water, mixed and blended until smooth and drying performed using freeze dried epuipment. After drying, each sample extracted with methanol with concentration (1:10) and water(1:10).

(7) Procedure

In order to prepare culture media for each group of antibiotics, paper dishes are placed in shallow culture media. There are four paper-discs on each petridish. Three paper-dishes pipette with the sample, and one paper-dish was pipette as comparison standard. The the sample was placed at room temperature for 1-2 hours and incubated. Culture media for Tetracycline group was being incubated at 30^{O}C, Macrolide and Amino glycoside group at 36^{O}C and Penicillin group at 55^{O}C for 16–18 hours. Sample testing was performed 3 times repeatedly. The result of antibiotic screening with measured inhibition zone diameter was performed by utilizing the calliper. Positive result will be obtained if there was an inhibition zone around the paper-disc, minimal l2 mm larger than the paper-disc diameter (AOAC,1995).

3.5.4 PHENOLIC COMPOUND DETERMINATION

(1) Optimization of Sample

Fifty grams of powdered Torbangun leaves was added 500 ml of distilled water and shake until smooth. The sample filtered using Whatman No.1 filter paper, and take a 10 ml sample into a test tubes and made 36 samples. The sample were heated from 50, 60, 70, 80, 90 and 100^{O}C temperature for 10, 20, 30, 40, 50 and 60 minutes.

(2) Preparation of Standards

Arrange eight test tubes were being on the tube rack, labelled respectively as 0 (0ppm), 1 (2ppm), 2 (4ppm), 3 (6ppm), 4 (8ppm), 6 (12ppm), 8 (16ppm) and 10 (20ppm). Transfer 1ml of Folin-Ciocalteu reagent was transferred into all tubes.0, 1, 2, 3, 4, 6, 8 and 10ml of 20ppm gallic acid standard solution, were added consecutively, followed consecutively by 10, 9, 8, 7, 6, 4, 2 and 0ml of distilled water. They were well shaken using vortex mixer and 1ml of 20% solution carbonate solution was added. Shake well and allow to stand for 30 minutes to get the color development. The blue color absorbance (A) was being by UV Vis-Spectrophotometer at 760 nm.

(3) Procedure

Prepare 100 ml glass beakers on a shelf and labelled with the name of the sample. 1 g of each sample was accurately weighed and given into each glass the. 20 ml of hot water ($80^{O}C$) was let stand for 5 minutes with 3 times of stirring using a glass rod. They were filtered through using whatman filter paper into 100 ml volumetric flask. The above extractions was repeated 2 times. Let the extract was allowed to cool, and made up to volume with distilled water. 25 ml test tubes were prepared and labelled as sample size. Into each test tube, add 1 ml of diluted Folin-Ciocateu reagent was added followed by 1 ml of the sample extract and 9 ml of distilled water. It was shaken well using a vortex mixer. 1ml of 20% sodium carbonate solution was added, shake well and allow to stand at room temperature for 30 minutes. The absorbance (A) was measured by a UV Vis-Spectrophotometer (Figure 3.3).

(4) **Calculation Total Phenols**

$$\% \text{ Total Phenolic} = \frac{Extract\ Vol\ (ml)}{1000} \text{ x ppm x } \frac{100}{\text{Samp0le weight x 1000}}$$

(Resource: Waterman and Mold, 1994)

3.5.5 FLAVONOID COMPOUND DETERMINATION

(1) Optimization of Sample

Fifty grams of powdered Torbangun leaves was added 500 ml distilled water and shaken until smooth. The samples were filtered using filter paper and take 10 ml sample into a test tubes and made 36 samples. The samples were heated from 50, 60, 70, 80, 90 and 100^{O}C temperature for 10, 20, 30, 40, 50 and 60 minutes.

(2) Preparation of Standards

Quercetin are as a standard. Using a seven point standard curve (0 – 50 mg/l), the total flavonoid content of samples was determined in triplicate, respectively. The data were expressed as milligram quercetin equivalent (QE)/g. The data in converted into milligram of quercetin (QE)/100 fresh material from the sample based on the moisture content of lyophilized powder and fresh sample material.

(3) Procedure

The total flavonoid content determined in accordance with the aluminium chloride with colorimetric method described by (Chang and Chern, 2002). Mainly, aliquots of 0.1 ml of the optimization sample respectively, dissolved in 1 ml of distilled water. This solution (0.5ml) was mixed with 1.5 ml of 95% alcohol , 0.1 ml of 10% aluminium chloride hexahydrate ($AlCl_3$), 0.1 ml of 1M potassium acetate (CH3COOK), and 2.8 ml of deionised water after incubation at room temperature for 40 minutes, there action mixture absorbance was being measured at 415nm against a deionised water blank on UV Vis-Spectrophotometer (Figure 3.3).

3.5.6 ANTIOXIDANT ACTIVITY TEST BY DPPH METHOD

(1) Optimization of Sample

Fifty grams of powdered Torbangun leaves was added 500 ml distilled water and shaken until smooth. The samples were filtered using filter paper and take 10 ml

sample into a test tubes and made 36 samples. The samples were heated from 50, 60, 70, 80, 90 and 100^{O}C temperature for 10, 20, 30, 40, 50 and 60 minutes.

(2) Procedure

The radical scavenging ability was determined as described by (Mensor *et al.*, 2001). Briefly, 1.0 ml from alcohol 0.3mM solution of DPPH was added to 2.5 ml of samples optimization with different temperature and time. The samples were storedt at room temperature in the dark and after 30 minutes, the optical density was measured at 518 nm. The antiradical activity (AA) was determined by the following formula:

AA% = 100-{[(ABS sample – ABS empty sample) x 100]/ABS control}

Where blank samples – 1 ml ethanol + 2.5 ml from various concentrations of extract; control sample – 1 ml 0.3 mM DPPH + 2.5 ml ethanol. The optical density of the samples, control and blank samples were measured in comparison with ethanol. One of the synthetic antioxidant, butylhydroxy toluene, was being used as positive control.

Figure 3.3. UV Vis-Spectrophotometer equipment

Solution A is a dilute alkali solution: 2N Folin and Ciocalteu's Phenol Reagent containing hydrochloride and phosphoric acids. 2.8598 g NaOH added 14.3084 g Na_2CO_3.

Solution B : 1.4232 g $CuSO_4.5(H_2O)$.

Solution C: 2.85299 g Na_2Tartrate.$2(H_2O)$.

3.5.7 HEAVY METALS ANALYSIS

(1) Determination of heavy metals with ICP-MS

Mineral content was determined using an Inductively Coupled Plasma–Mass Spectometer (ICP-MS) (Figure3.5.2b). The samples were analyzed for heavy metals (Pb, As, Cd, Se, Cu and Mg),

A"three-point calibration curve" was constructed using five concentrations (0-500µg/L) of standards prepared in-house.HNO_3 being used as a blank and all the analyzes were performed in triplicates.

(2) Sample preparation for mineral analysis

Lyophilized plant extracts were being weighed, approximately but accurately (0.5 g) into a Teflon digestion vessel, to which was being added exactly HNO_3 (65%, 7 mL) and H_2O_2 (30%, 1 mL). The vessels (Fig. 3.5.2c) were then being capped, and fitted into rotor segments and inserted into the microwave cavity (Fig. 3.4). The samples were radiated for 20 minutes. Upon cooling, the vessels were being uncapped and solutions transferred into 50 mL volumetric flasks.

Figure 3.4. Inductively Coupled Plasma Mass Spectroctroscopy (ICPMS) equipment

(3) ICP-MS operating conditions

An Inductively Couple Plasma–Mass Spectrometer (ICPMS) was being used with the following operating conditions for determination of the minerals as being summarized in Table 3.2.

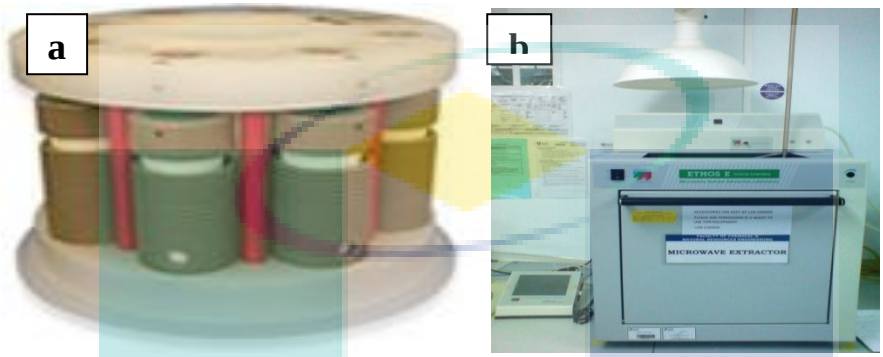


Figure 3.5. Sample vessels (a) and rotor (b) Microwave digester

Table 3.2. Operating conditions for ICP-MS

Instrument	**Agilent 7500a**
Nebulizer	: Babington type
Spray chamber	: Scott-type
Plasma	**:** RF generator Frequency; 10 Mega Herzt, Power output1300 W
	Air flow rate (1/min) : Plasma; 15, auxiliary: 0.9, nebulizer: 1-1.1 Sample uptake rate: 1.8 ml/min
Interface	
Samper cone	: Nickel, id: 1.1 mm
Skimmer	: Nickel, id: 0.9 mm
Vacuum	: Interface: 4 torr, quadrupole: 2 x 10^{-5}torr
Data acquisition	: Peak hoping, replicate time 200 ms, dwell time 200 ms, sweep reading 3, readings/replicate 3.

3.5.8 CLINICAL TEST

(1) Procedure

The procedure of clinical trials with 36 mice (*Mus musculus albino*) strain is as follows:

a. Thirty six multifarious mice female and three male were mated respectively.

b. The mice were housed in standard plastic cages (36x28x12cm).

c. Water as their drinking and food were provided prepared (ad-libitum).

d. Bedding and nest material consisted of wood shaving.

e. After pregnant, separated and put of mice in one cage one mouse (36 cages). for example:

Cage A_{1-6}: Mice control only gave drinking and food.

Cage B_{1-6}: Mice treatment gave 1% Torbangun extracts.

Cage C_{1-6}: Mice treatment gave 5% Torbangun extracts.

Cage D_{1-6}: Mice treatment gave 10% Torbangun extracts.

Cage E_{1-6}: Mice treatment gave 15% Torbangun extracts.

Cage F_{1-6}: Mice treatment gave 20% Torbangun extracts.

f. The Torbangun extracts give on birth (0 day) via oral by using syringe everyday.

g. The mother and pups weighed before and after suckled for 15 days and recorded.

h. The research finished, the mother carried out autopsy and took mammary gland and weighed and recorded.

i. The mammary organs processed histology description.

Figure 3.6. Freeze dryer

(2) Preparation of Histology Slides

The mammary organs were being soaked in 10% buffer neutral formalin, trimmed, fixated and dehydrated. Dehydration involved a process where the tissue was being soaked in alcohol and cleared, and then to be soaked again in xylol solution by using the tissue processor (Figure 3.7a).

Figure 3.7a. Tissue processor equipment

Impregnation was soaked of tissue in liquid paraffin and then the tissue embedded into the embedding cassette by using the embedding center (Figure 3.7b).

Figure 3.7b. Embedding center equipment

The tissue was cut using a microtome (Figure 3.7c). and put on a glass slide. Furthermore, staining technique was being used by using haemotoxyline-

eosin (H and E), and the mounting was being used by using entelan reagent and covered with the glass on the deck glass and then was being examined under a light microscope (Ressang,1984).

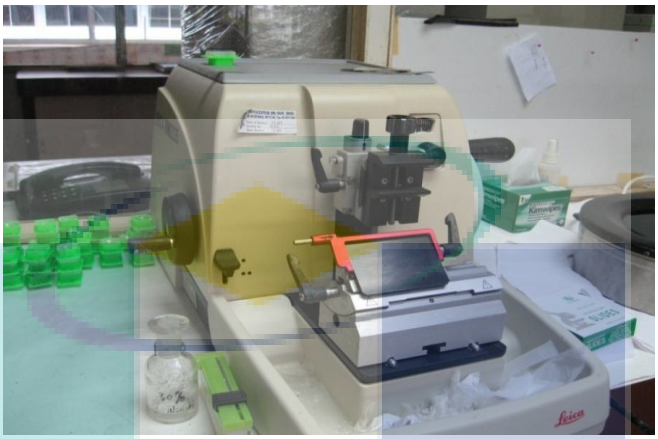


Figure 3.7c. Microtome equipment

3.5.9 DATA STATISTICAL ANALYSIS

All data were analyzed as follows:

a. Data from the phytochemicals analysis (total phenolics, flavonoids and antioxidant activity) were analyzed using design expert optimization and variance (ANOVA).

b. Data from active substance test by using GC-MS and PCA analysis with the program Microsoft Excel 2007-XLSTAT 2010.

c. Data from clinical trial analized by correlation and ANOVA in SPSS statistic program.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Introduction

The presents chapter describes the results and discussion of the active substances identified using GC-MS, result of compounds in Torbangun leave using FTIR, result of antibiotic using bioassay method, result of total phenolic, flavonoid compound and antioxidant activity using UV Vis-Spectrophotometer, result of heavy metals analysis using ICPMS and result of clinical test and histology description of lactation mammary gland organ.

4.2 Result of Research

4.2.1 Result of Active Substances in Torbangun Leaves Using GC-MS

The result of active substances analysis in Torbangun leave with GC-MS (Figure 4.1).

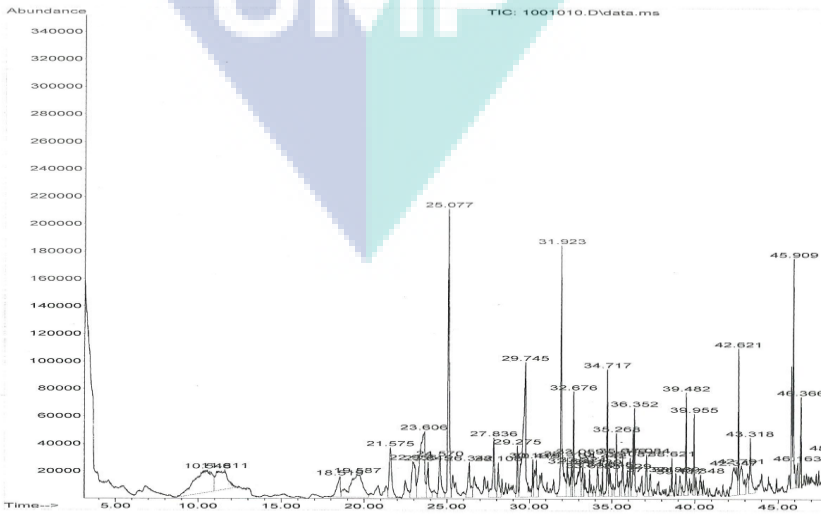


Figure 4.1. The result of Torbangun leave with ethanol extract by using GC-MS test

The result of Torbangun leave powder analysis were found 54 compounds (Table 4.1). The compounds were performed processing with principal component analysis (PCA) program.

Table 4.1. Result of compounds in Torbangun leave powder using GC-MS.

No	Compound	Quality (%)	Abreviation	Peak Area
1	Phenol, 2-methyl-5-(1-methylethyl)	94	PM	8.091
2	2-Methoxy-4-vinylphenol	96	MV	0.126
3	Cyclohexasiloxane, dodecamethyl-	91	CCHX	0.130
4	Caryophyllene	99	CYP	0.439
5	Alpha.-Caryophyllene	98	ACP	0.608
6	Cycloheptasiloxane	90	CHSX	0.183
7	Cyclohexene	97	CH1	0.071
8	Phenol, 2,4-bis(1,1-dimethylethyl)	96	PHE.2	0.248
9	Tetradecanoic acid	97	TDA	0.115
10	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-	90	BCD	0.643
11	Pentadecanoic acid,	98	PTDA	0.592
12	n-Hexadecanoic acid	99	NHA	2.414
13	Hexadecanoic acid, ethyl ester	96	HA	1.061
14	Phytol	95	PTOL	3.976
15	9,12,15-Octadecatrienoic acid,	99	OTA	4.205
16	1-Phenanthrenecarboxaldehyde,	90	PTAL	2.639
17	1-Phenanthrenecarboxylic acid,	96	PTCA	3.356
18	1,2-Benzenedicarboxylic acid,	91	BZCA	0.795
19	2,6,10,14,18,22-Tetracosahexaene,	99	TCH	3.513
20	Vitamin E	99	VITE	0.418
21	Gamma.-Tocopherol	99	GT	0.229
22	3-Methyl-4-isopropylphenol	94	MI	13.587
23	1,3,6,10-Dodecatetraene, 3,7,11-trimethyl-,	95	DTM	0.456
24	Cyclopentanol,1-(1-methylene-2-propenyl)-	92	CCP	0.169
25	1H-Indole-3-carboxaldehyde	96	ICD	0.196
26	Heptadecanoic acid	98	HAD	0.722
27	1-Octadecene	97	OTDC	0.420
28	Nonanoic acid,	97	NNA	3.323
29	Decanedioic acid,	94	DCA	0.504
30	1,2-Benzenediol	94	BZD	0.084
31	2,5-Cyclohexadiene	96	CH	0.029

32	Benzeneacetic acid	90	BZAA	0.21
33	Indole-5-aldehyde	97	IDA	0.191
34	Hydroxy-1-propenyl)-2-methoxyphenol	95	HPM	0.095
35	4-Hexadecen-6-yne,	94	HXC	1.505
36	7,10,13-Hexadecatrienoic acid,	93	HXA	3.449
37	Gamma.-Sitosterol	99	GS	3.361
38	alpha.-Amyrin	92	AA	3.506
39	beta.-Tocopherol	90	BT	60.427
40	di-.alpha.-Tocopherol	92	DAT	0.144
41	Benzoic acid	90	BZA	3.456
42	Phenol, 3-methoxy-2,4,5-trimethyl-	91	PMT	0.361
43	Stigmasterol	99	SS	4.927
44	Octadecanoic acid,	98	OA	0.743
45	Isocholesteryl methyl ether	96	IME	2.085
46	Campesterol	97	CS	1.625

4.2.2 Identification Active Substances inTorbangun by Principal Component Analysis (PCA)

A principal component analysis often is performed before are aggression, to avoid using correlated variables. PCA was being used to classify samples and find variables and all compounds of Torbangun leaves that would contribute to the differentiation. Factor analysis was being used to understand the correlations between variables instead as the dimensions of the variables were small. One group of compounds were being separated from other groups and these group compounds that were responsible as active substances in Torbangun plant:

Summary statistics:

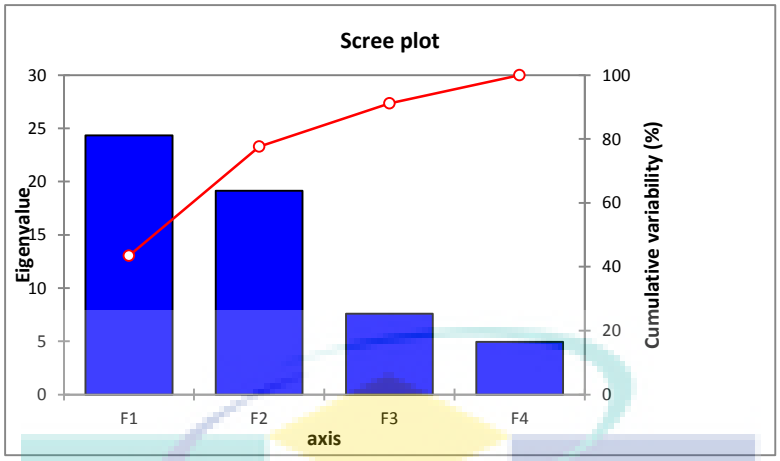
Variable	Obser-vations	Obs. with missing data	Obs. without missing data	Minimum	Maximum	Mean	Std. deviation
PM	5	0	5	0.000	11.545	4.855	6.126
MV	5	0	5	0.000	0.126	0.050	0.069
CCHX	5	0	5	0.000	0.130	0.052	0.071
CYP	5	0	5	0.000	0.439	0.088	0.196
ACP	5	0	5	0.000	0.718	0.365	0.340
CHSX	5	0	5	0.000	0.183	0.073	0.100
CH1	5	0	5	0.000	0.071	0.028	0.039

PHE2	5	0	5	0.144	0.356	0.248	0.102
TDA	5	0	5	0.000	0.144	0.069	0.067
BCD	5	0	5	0.000	0.677	0.386	0.355
PTDA	5	0	5	0.000	1.022	0.473	0.363
NHA	5	0	5	0.905	2.955	2.414	0.869
HA	5	0	5	0.000	2.523	0.849	1.006
PTOL	5	0	5	0.000	5.116	3.181	1.909
OTA	5	0	5	0.885	5.888	4.205	2.216
PTAL	5	0	5	0.000	5.356	2.116	2.485
PTCA	5	0	5	0.000	5.346	2.111	2.480
BZCA	5	0	5	0.000	0.992	0.477	0.498
TCH	5	0	5	0.000	4.793	2.119	2.476
VITE	5	0	5	0.000	0.629	0.328	0.316
GT	5	0	5	0.000	0.230	0.046	0.103
MI	5	0	5	0.000	17.618	5.435	7.969
DTM	5	0	5	0.000	0.574	0.182	0.263
CCP	5	0	5	0.000	0.170	0.034	0.076
ICD	5	0	5	0.000	0.196	0.039	0.088
HAD	5	0	5	0.000	0.985	0.289	0.437
OTDC	5	0	5	0.000	0.420	0.084	0.188
OTDA	5	0	5	0.000	7.677	1.535	3.433
NNA	5	0	5	0.000	3.323	0.665	1.486
DCA	5	0	5	0.000	0.504	0.101	0.225
BZD	5	0	5	0.000	0.084	0.017	0.038
CH	5	0	5	0.000	0.029	0.006	0.013
BZAA	5	0	5	0.000	0.210	0.042	0.094
IDA	5	0	5	0.000	0.191	0.038	0.085
HPM	5	0	5	0.000	0.095	0.019	0.042
HXC	5	0	5	0.000	1.505	0.301	0.673
HXA	5	0	5	0.000	3.449	0.690	1.542
GS	5	0	5	0.000	5.872	1.344	2.557
AA	5	0	5	0.000	6.531	2.104	2.870
BT	5	0	5	0.000	60.427	12.085	27.024
DIT	5	0	5	0.000	0.144	0.029	0.065
BZA	5	0	5	0.000	3.456	0.691	1.546
PMT	5	0	5	0.000	0.361	0.072	0.161
SS	5	0	5	0.000	4.927	0.985	2.203
OA	5	0	5	0.000	0.743	0.149	0.332
IME	5	0	5	0.000	2.085	0.417	0.932
CS	5	0	5	0.000	1.625	0.325	0.727

Principal Component Analysis:

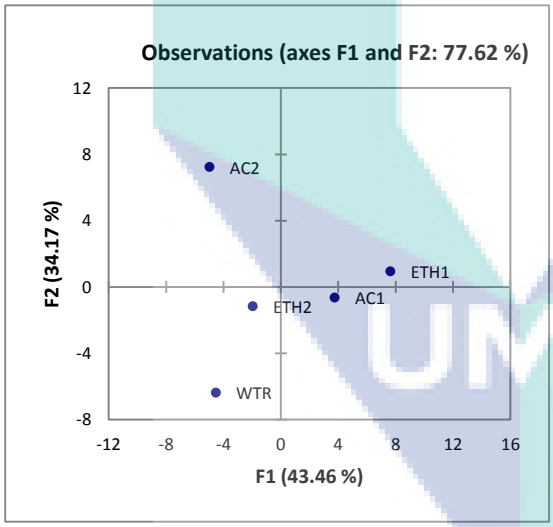
Eigenvalues:

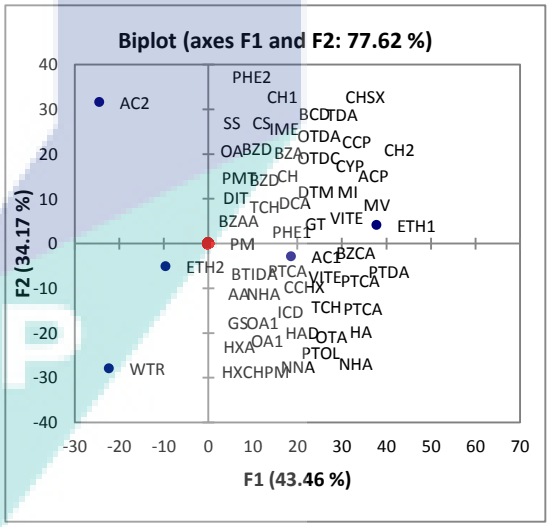
	F1	F2	F3	F4
Eigenvalue	24.336	19.133	7.588	4.943
Variability (%)	43.457	34.166	13.550	8.827
Cumulative %	43.457	77.623	91.173	100.000



Factor scores:

Observation	F1	F2	F3	F4
ETH1	7.658	0.947	-2.312	-2.036
ETH2	-1.942	-1.157	4.405	-2.454
AC1	3.767	-0.645	1.960	3.779
AC2	-4.963	7.233	-1.271	0.445
WTR	-4.520	-6.379	-2.781	0.266

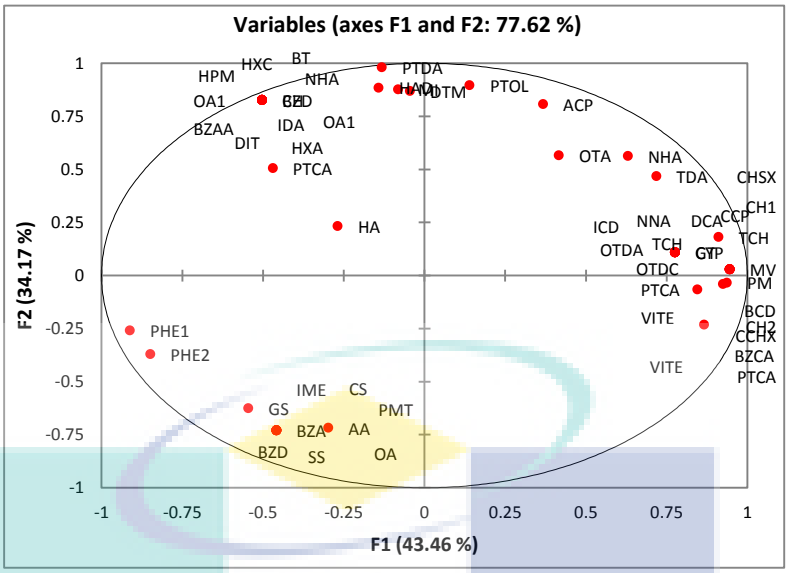




Correlations between variables and factors:

	F1	F2	F3	F4
PM	0.936	-0.034	-0.098	0.336
MV	0.946	0.028	-0.052	0.320
CCHX	0.946	0.028	-0.052	0.320
PHE1	-0.912	-0.260	-0.281	-0.145
ACP	0.367	0.807	-0.265	0.380
CHSX	0.946	0.028	-0.052	0.320
CH1	0.946	0.028	-0.052	0.320

PHE2	-0.849	-0.371	0.095	-0.364
CH2	0.946	0.028	-0.052	0.320
TDA	0.718	0.467	-0.123	0.501
BCD	0.846	-0.066	0.526	-0.066
PTDA	-0.132	0.980	0.148	0.009
NHA	0.630	0.562	0.533	-0.048
HA	-0.269	0.233	0.782	-0.512
PTOL	0.140	0.895	0.415	-0.079
OTA	0.416	0.565	-0.504	0.503
PTCA	-0.470	0.505	0.589	-0.421
PTCA	0.946	0.028	-0.052	0.320
BZCA	0.867	-0.233	-0.240	0.371
TCH	0.911	0.180	-0.097	0.358
VITE	0.925	-0.041	0.374	0.052
VITE	0.946	0.028	-0.052	0.320
GT	0.776	0.108	-0.420	-0.458
MI	-0.081	0.876	-0.453	-0.147
CYP	0.776	0.108	-0.420	-0.458
DTM	-0.045	0.868	-0.466	-0.165
PTCA	0.776	0.108	-0.420	-0.458
CCP	0.776	0.108	-0.420	-0.458
ICD	0.776	0.108	-0.420	-0.458
HAD	-0.142	0.884	-0.430	-0.115
OTDC	0.776	0.108	-0.420	-0.458
OTDA	0.776	0.108	-0.420	-0.458
NNA	0.776	0.108	-0.420	-0.458
DCA	0.776	0.108	-0.420	-0.458
TCH	0.776	0.108	-0.420	-0.458
BZD	-0.503	0.827	-0.231	0.100
CH	-0.503	0.827	-0.231	0.100
BZAA	-0.503	0.827	-0.231	0.100
IDA	-0.503	0.827	-0.231	0.100
NHA	-0.503	0.827	-0.231	0.100
OA1	-0.503	0.827	-0.231	0.100
HPM	-0.503	0.827	-0.231	0.100
HXC	-0.503	0.827	-0.231	0.100
HXA	-0.503	0.827	-0.231	0.100
GS	-0.545	-0.626	-0.553	0.076
AA	-0.298	-0.718	-0.338	0.530
BT	-0.503	0.827	-0.231	0.100
DIT	-0.503	0.827	0.231	0.100
BZA	-0.458	-0.729	-0.505	0.060
BZD	-0.458	-0.729	-0.505	0.060
PMT	-0.458	-0.729	-0.505	0.060
SS	-0.458	-0.729	-0.505	0.060
OA	-0.458	-0.729	-0.505	0.060
IME	-0.458	-0.729	-0.505	0.060
CS	-0.458	-0.729	-0.505	0.060



The compounds were phytochemical (phenol/PHE and isocholesterylmethyl ether/IME), phytoseroid (gammasitosterol/GS; campesterol/CS and stigmasterol/ SS), octadecanoic acid (OA), benzoicacid (BZA), 1,2-benzenediol (BZD), phenol, 3-methoxy-2,4,5-trimethyl (PMT) and alpha-amyrin(AA).

4.2.3 Result of Compounds in Torbangun Leave Using Infrared

The infrared spectrum result of Torbangun leaves were 1019, 1587 and 2877 (Figure 4.1). The infrared spectrum Torbangun leaves included functional group of alcohol, ether, carboxylic acids and esters (CO); aromatic rings (C=C) and alkenes strong (C-H).

Simple aromatic rings are aromatic organic compounds that consist only of a conjugated planar ring system with delocalized electron clouds. They usually are found as substructures of more complex molecules. Typical simple aromatic compounds are benzene, indole, and cyclotetradeca heptanes.

Alkenes is paraffin so waxes. A number of waxes are produced commercially in large amounts for use in cosmetics, lubricants, polishes, surface coatings, inks and many other applications. Some of these are of mineral origin (Christi, 2011).

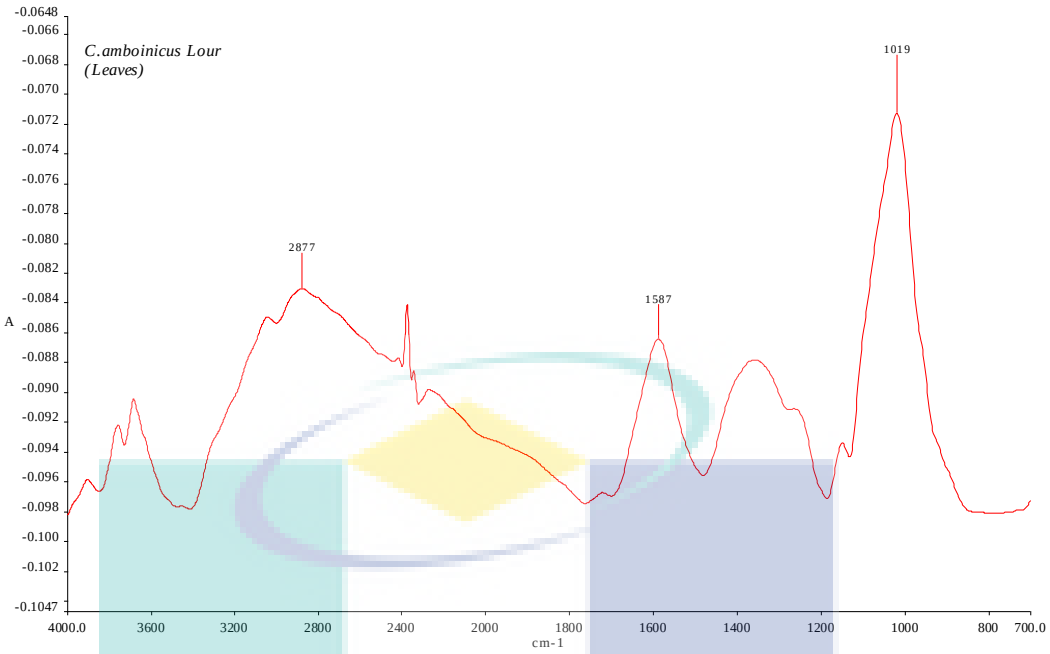


Figure 4.2. Infrared spectrum of Torbangun leave extract

4.2.4 Result of Antibiotic Screening Test (Bioassay) in Torbangun Leaves

The result of antibiotic activity analysis in Torbangun plant, mainly in fresh leaves extract with distilled water was not found penicillin, tetracycline, aminoglycoside and macrolide groups (Table 4.2a).

Table 4.2a. Antibiotics in Torbangun plant (*Coleus amboinicusLour*) extract with distilled water solution.

No	Name of Sample	Result of Antibiotics Analysis			
		PC's	ML's	AG's	TC's
1	Freeze dry Torbangun stem with distil water solution	Negative	Negative	Negative	Negative
2	Freeze dry Torbangun leaf with distil water solution	Negative	Negative	Negative	Negative
3	Fresh Torbangun stem with distil water solution	Negative	Negative	Negative	Negative
4	Fresh Torbangun leaf with distil water solution	Negative	Negative	Negative	Negative

The result of antibiotic activity analysis in Torbangun plant, mainly in fresh leaves extract with methanol solution were found penicillin, tetracycline,

aminoglycoside and macrolide groups. The extract of Torbangun with methanol solution shown positive result in antibiotics analysis and the results were differences between leaves and stems; (Table 4.2b).

Table 4.2b. Antibiotics in Torbangun plant (*Coleus amboinicusLor*) extract with methanol solution.

N	Name of Sample	Result of Antibiotics Analysis			
		PC's	ML's	AG's	TC's
1	Freeze dry Torbangun stem with methanol solution	Positive	Negative	Negative	Positive
2	Freeze dry Torbangun leaf with methanol solution	Positive	Negative	Positive	Positive
3	Fresh Torbangun stem with methanol solution	Negative	Negative	Negative	Positive
4	Fresh Torbangun leaf with methanol solution	Positive	Positive	Positive	Positive

PC's = Penicillin group **ML's = Macrolide group**
AG's = Amino glycoside group **TC's = Tetracycline group**

4.2.5 Result of Total Phenolic Compound in Torbangun Leaves

Optimization of total phenolic compound in Torbangun leaves being derived from expression and expressed as mg gallic acid equivalent (GAE) in g of dry weight of lyophilized plant extracts (R2=0.999). The Gallic acid standard curve is being shown Figure.4.3.

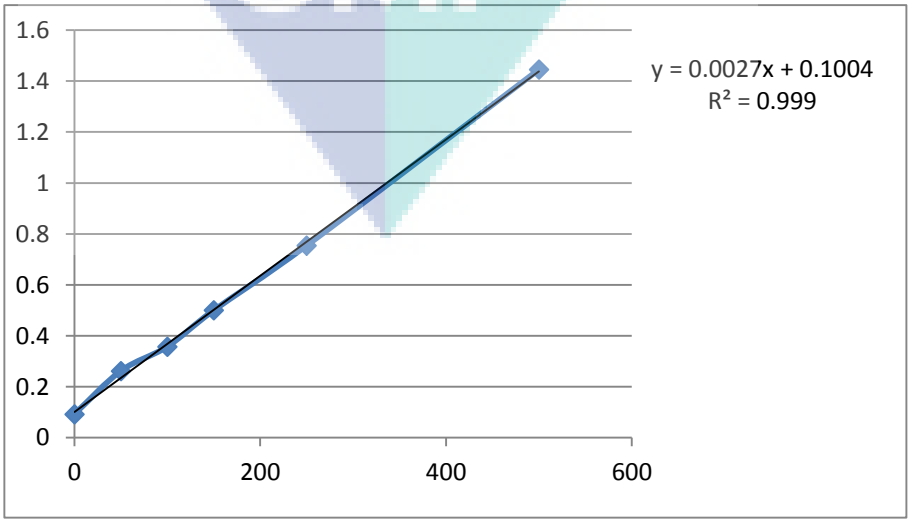


Figure 4.3. Gallic acid Standard Curve

The optimization result of total phenolic compound in Torbangun leaves is 636.52 optimum point at 50^{O}C temperature for 10 minutes and the lower point 158.37 at 80^{O}C for 60 minutes, see Table 4.3.

Table 4.3. The optimization result of total phenolic compound in Torbangun leaves with parameter temperature (T) and time (t).

Temprature (Celcius)	**Time (Minutes)**					
	t 10	**t 20**	**t 30**	**t 40**	**t 50**	**t 60**
T 50	636.52	574.67	558	506.52	495.41	461.7
T 60	461.7	417.63	382.81	413.55	349.11	410.59
T 70	425.4	414.3	456.15	456.89	376.15	391.7
T 80	276.89	233.55	104.67	226.52	316.52	158.37
T 90	289.85	209.11	225.04	222.81	233.19	292.44
T 100	388	414.67	313.19	294.67	305.04	281.7

Figure 4.4 is the optimization result of total phenolic compound in Torbangun leaves and the blue colour is the optimum point which is at 50^{O}C for 10 minutes of extraction time (636.52).

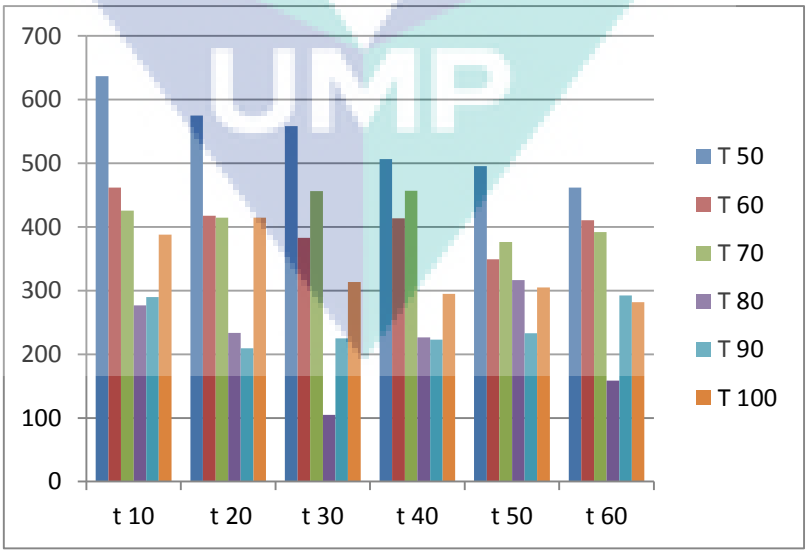


Figure 4.4. Result optimization of total phenolic compound in Torbangun leaves with parameter temperature (T) and time (t).

4.2.6 Result of Total Flavonoid Compound in Torbangun Leaves

Total flavonoid compound derived from expression and it is being expressed as mg catechin equivalents (CE)/g dry weigh of lyophilized plant extract (R^2 = 0.9874) in catechin standard curve (Figure. 4.5).

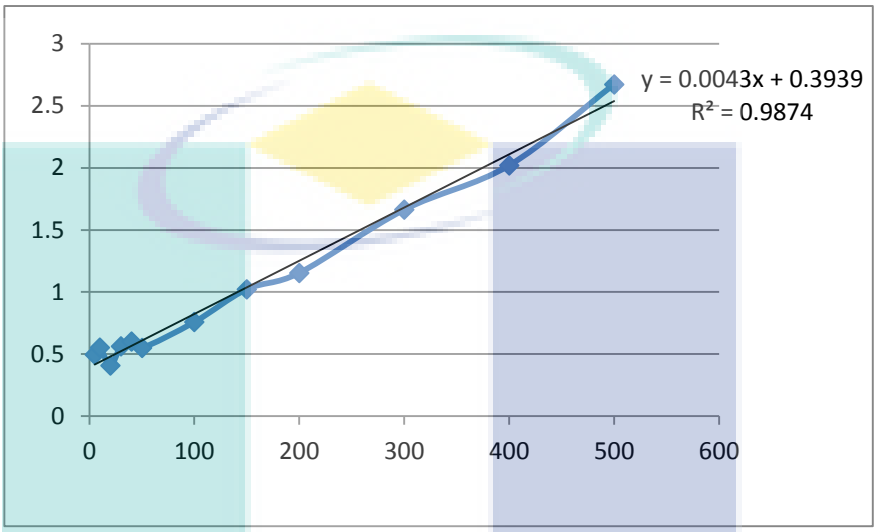


Figure 4.5.Catechin Standard Curve

The optimization result of total flavonoid compound in Torbangun leaves is 186.53 optimum point at 50^{O}C temperature for 10 minutes and the lower point 4.39 at 100^{O}C for 60 minutes, see Table 4.4.

Table 4.4. Result optimization of total flavonoid compound in Torbangun leaves with parameter temperature (T) and time (t).

Temprature (Celcius)	**Time (Minutes)**					
	t 10	**t 20**	**t 30**	**t 40**	**t 50**	**t 60**
T 50	186.53	123.51	114.21	107.47	103.28	95.84
T 60	93.74	95.14	93.28	92.35	87.69	84.21
T 70	87.00	70.72	62.58	44.44	47.23	49.79
T 80	36.07	2.58	1.88	20.72	33.97	4.91
T 90	18.58	34.91	3.00	6.30	1.37	64.44
T 100	31.88	11.18	9.32	58.39	19.55	4.39

Figure 4.6 is an optimization result of total flavonoid compound in Torbangun leaves and the blue colour is an optimum point at 50^{O}C for 10 minutes of extraction time (186.53).

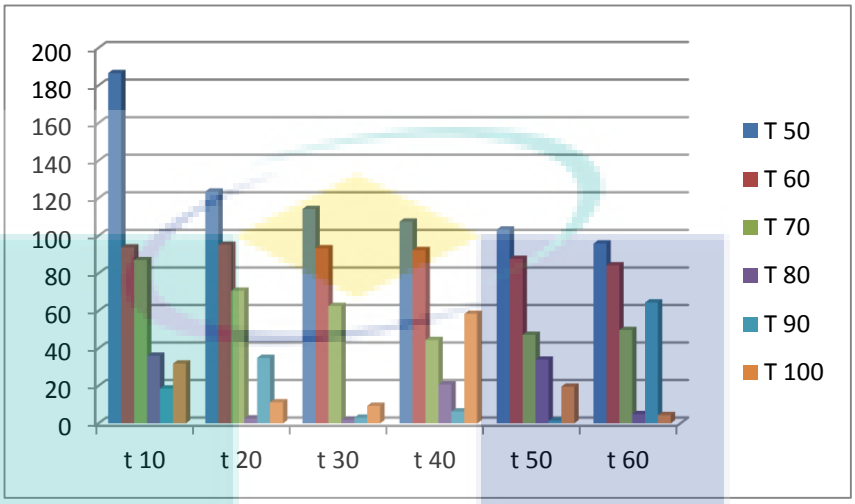


Figure 4.6. Result optimization of total flavonoid compound in Torbangun leaves with parameter temperature (T) and time (t).

4.2.7 Result of Antioxidant Activity in Torbangun Leaves

Antioxidant activity was being derived from expression and being expressed as mg vitamin C equivalents (Vit.CE)/g dry weigh of lyophilized plantextract (R2=0.9993). The vitamin C standard curve is shown in Figure. 4.7.

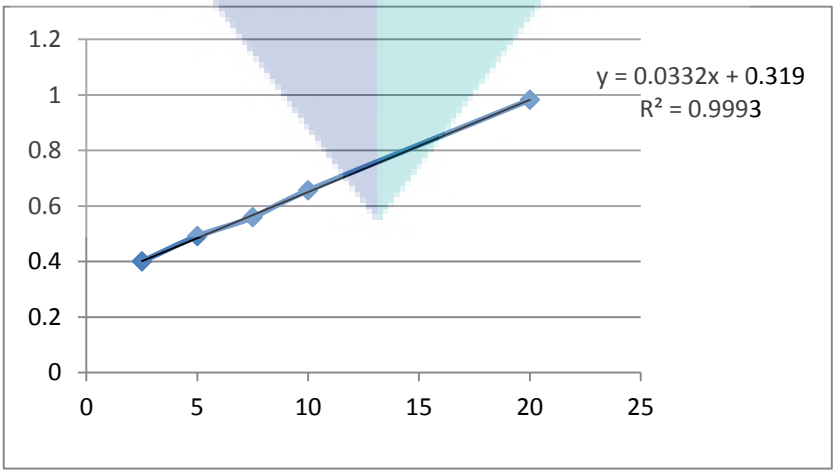


Figure 4.7.Vitamin C standard curve

The optimization result of antioxidant activity in Torbangun leaves value is 38.68 at 50^{O}C temperature for 10 minutes extraction time and the lower value is 2.09 at 80^{O}C for 60 minutes, see Table 4.5.

Table 4.5. Result optimization of antioxidant activity compound in Torbangun leaves with parameter temperature (T) and time (t).

Temprature (Celcius)	**Time (Minutes)**					
	t 10	**t 20**	**t 30**	**t 40**	**t 50**	**t 60**
T 50	38.69	29.25	25.23	18.97	16.82	9.15
T 60	36.16	28.13	20.65	18.5	15.23	6.26
T 70	35.61	26.72	20.74	17.8	16.26	5.05
T 80	34.11	26.17	24.67	17.85	10.09	1.77
T 90	31.77	25.79	24.11	18.76	15.23	2.06
T 100	29.53	25.79	20.18	19.91	9.15	5.88

Figure 4.8 is an optimization result of the antioxidation activity in Torbangun leaves and the blue colour is the optimum point which is at 50^{O}C for 10 minutes extraction (38.69).

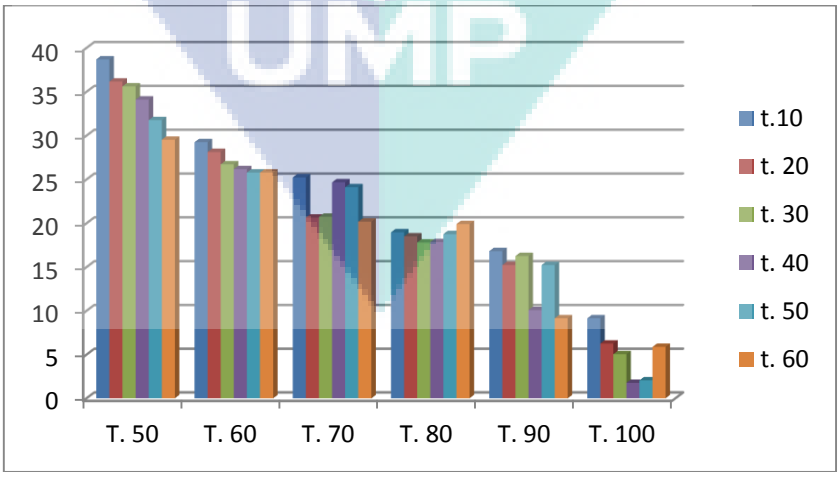


Figure 4.8. Result optimization of antioxidant activity in Torbangun leaves with parameter temperature (T) and time (t).

4.2.8 Result of Heavy Metals

Heavy metals in the leaves of Torbangun plant (*Coleus amboinicus* Lour) from being cultivated in poly-bags presented six parameters such as magnesium, copper, arsenic, selenium, cadmium and lead. Arsenic was very low and magnesium was high in Torbangunplant (Table 4.6).

Table 4.6. Result of heavy metals analysis in Torbangun leaves

No	Parameters	Amount (ppm)
1	Arsenic (As)	0.10
2	Lead (Pb)	2.39
3	Cadmium (Cd)	0.07
4	Selenium (Se)	0.00
5	Magnesium (Mg)	23.38
6	Copper (Cu)	15.42

Concentrations of arsenic in plant tissues of Torbangun extract was 0.01ppm (mg/kg). Whereas, arsenic concentration in food being prepared for human consumption is commonly < 0.02mg/kg (Ministry of Agriculture, Fisheries and Food, 1982).

4.2.9 Result of Clinical Trial

1. The Result of Clinical Trial in Lactating Mice

The raw data of clinical trial result using six control mice and thirty treatment mice (six mice for every 1%, 5%, 10%, 15% and 20% Torbangun leaves extract treatment). Each mouse has eight pups and all pups allowed to milk from 08.00 am and 13.00 pm. The data is result of pup weighed before start milking at 08.00 am and after milking at 13.00 pm, see Table 4.7a to Table 4.7aj.

Table 4.7a. Pups and mouse mother weight (gram) before start milking at 08.00 am and after milking at 13.00 pm (Mice Control-1).

No / Day	Pups weight (gram) - Weighed at 08 am								Mother (gram)	Pups weigh (gram) - Weighed at 13 pm								Mother (gram)
	1	2	3	4	5	6	7	8		1	2	3	4	5	6	7	8	
1st	1.58	1.58	1.6	1.6	1.6	1.6	1.6	1.65	37.6	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	36.6
2nd	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	37.5	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	36.5
3rd	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	37.7	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	36.6
4th	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	37.6	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	36.6
5th	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	37.6	3.35	3.35	3.35	3.35	3.35	3.35	3.35	3.35	36.6
6th	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75	37.8	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	36.8
7th	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3	37.6	4.45	4.45	4.45	4.45	4.45	4.45	4.45	4.45	36.6
8th	4.85	4.85	4.85	4.85	4.85	4.85	4.85	4.85	37.8	5.05	5.05	5.05	5.05	5.05	5.05	5.05	5.05	36.2
9th	5.55	5.55	5.55	5.55	5.55	5.55	5.55	5.55	37.9	5.75	5.75	5.75	5.75	5.75	5.75	5.75	5.75	36.4
10th	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25	37.6	6.45	6.45	6.45	6.45	6.45	6.45	6.45	6.45	35.6
11th	6.95	6.95	6.95	6.95	6.95	6.95	6.95	6.95	37.5	7.15	7.15	7.15	7.15	7.15	7.15	7.15	7.15	35.5
12th	7.65	7.65	7.65	7.65	7.65	7.65	7.65	7.65	37.6	7.9	7.9	7.9	7.9	7.9	7.9	7.9	7.9	34.6
13th	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	37.7	8.75	7.65	7.65	7.65	7.65	7.65	7.65	7.65	34.7
14th	9.35	9.35	9.35	9.35	9.35	9.35	9.35	9.35	37.8	9.6	9.6	9.6	9.6	9.6	9.6	9.6	9.6	34.8
15th	10.3	10.3	10.3	10.3	10.3	10.3	10.3	10.3	37.5	10.6	10.6	10.6	10.6	10.6	10.6	10.6	10.6	34.6

Table 4.7b. Pups and mouse mother weight (gram) before start milking at 08.00 am and after milking at 13.00 pm (Mice Control-2).

No / Day	Pups weight (gram) - Weighed at 08 am								Mother (gram)	Pups weigh (gram) - Weighed at 13 pm								Mother (gram)
	1	2	3	4	5	6	7	8		1	2	3	4	5	6	7	8	
1st	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	38.0	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	37.0
2nd	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9	38.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	37.0
3rd	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3	38.0	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	36.6
4th	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	37.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	36.3
5th	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	37.8	3.25	3.25	3.25	3.25	3.25	3.25	3.25	3.25	36.3
6th	3.65	3.65	3.65	3.265	3.65	3.65	3.65	3.65	37.9	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	37.4
7th	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	37.9	4.35	4.35	4.35	4.35	4.35	4.35	4.35	4.35	37.4
8th	4.75	4.75	4.75	4.75	4.75	4.75	4.75	4.75	37.8	4.95	4.95	4.95	4.95	4.95	4.95	4.95	4.95	35.8
9th	5.45	5.45	5.45	5.45	5.45	5.45	5.45	5.45	37.8	5.65	5.65	5.65	5.65	5.65	5.65	5.65	5.65	35.8
10th	6.15	6.15	6.15	6.15	6.15	6.15	6.15	6.15	37.8	6.35	6.35	6.35	6.35	6.35	6.35	6.35	6.35	35.7
11th	6.65	6.65	6.65	6.65	6.65	6.65	6.65	6.65	37.8	6.85	6.85	6.85	6.85	6.85	6.85	6.85	6.85	35.8
12th	7.35	7.35	7.35	7.35	7.35	7.35	7.35	7.35	37.9	7.6	7.6	7.6	7.6	7.6	7.6	7.6	7.6	35.9
13th	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2	38.0	8.45	8.45	8.45	8.45	8.45	8.45	8.45	8.45	35.0
14th	9.05	9.05	9.05	9.05	9.05	9.05	9.05	9.05	38.0	9.35	9.35	9.35	9.35	9.35	9.35	9.35	9.35	35.0
15th	10.05	10.05	10.05	10.05	10.05	10.05	10.05	10.05	38.0	10.35	10.35	10.35	10.35	10.35	10.35	10.35	10.35	34.1

Table 4.7c. Pups and mouse mother weight (gram) before start milking at 08.00 am and after milking at 13.00 pm (Mice Control-3).

No / Day	Pups weight (gram) - Weighed at 08 am								Mother (gram)	Pups weigh (gram) - Weighed at 13 pm								Mother (gram)
	1	2	3	4	5	6	7	8		1	2	3	4	5	6	7	8	
1st	1.6	1.58	1.6	1.6	1.6	1.6	1.6	1.6	37.9	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	37.1
2nd	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	37.8	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	37.0
3rd	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	37.8	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	37.0
4th	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	37.9	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	37.1
5th	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	37.7	3.55	3.55	3.55	3.55	3.55	3.55	3.55	3.55	36.7
6th	3.95	3.95	3.95	3.95	3.95	3.95	3.95	3.95	37.8	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	36.8
7th	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	37.6	4.65	4.65	4.65	4.65	4.65	4.65	4.65	4.65	36.6
8th	5.05	5.05	5.05	5.05	5.05	5.05	5.05	5.05	37.7	5.25	5.25	5.25	5.25	5.25	5.25	5.25	5.25	36.1
9th	5.75	5.75	5.75	5.75	5.75	5.75	5.75	5.75	37.8	5.95	5.95	5.95	5.95	5.95	5.95	5.95	5.95	36.2
10th	6.45	6.45	6.45	6.45	6.45	6.45	6.45	6.45	37.9	6.65	6.65	6.65	6.65	6.65	6.65	6.65	6.65	36.3
11th	7.05	7.05	7.05	7.05	7.05	7.05	7.05	7.05	37.9	7.25	7.25	7.25	7.25	7.25	7.25	7.25	7.25	36.3
12th	7.75	7.75	7.75	7.75	7.75	7.75	7.75	7.75	37.7	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	35.7
13th	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	37.8	8.75	8.75	8.75	8.75	8.75	8.75	8.75	8.75	35.8
14th	9.25	9.25	9.25	9.25	9.25	9.25	9.25	9.25	37.7	9.55	9.55	9.55	9.55	9.55	9.55	9.55	9.55	35.3
15th	10.25	10.25	10.25	10.25	10.25	10.25	10.25	10.25	37.7	10.55	10.55	10.55	10.55	10.55	10.55	10.55	10.55	35.3

Table 4.7d. Pups and mouse mother weight (gram) before start milking at 08.00 am and after milking at 13.00 pm (Mice Control-4).

No / Day	Pups weight (gram) - Weighed at 08 am								Mother (gram)	Pups weigh (gram) - Weighed at 13 pm								Mother (gram)
	1	2	3	4	5	6	7	8		1	2	3	4	5	6	7	8	
1st	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	37.9	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	37.1
2nd	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	37.9	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	37.1
3rd	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	37.8	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	37.0
4th	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	37.9	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	37.1
5th	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	37.7	3.45	3.45	3.45	3.45	3.45	3.45	3.45	3.45	36.7
6th	3.95	3.95	3.95	3.95	3.95	3.95	3.95	3.95	37.7	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	36.7
7th	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	37.9	4.65	4.65	4.65	4.65	4.65	4.65	4.65	4.65	36.9
8th	5.05	5.05	5.05	5.05	5.05	5.05	5.05	5.05	37.8	5.25	5.25	5.25	5.25	5.25	5.25	5.25	5.25	36.2
9th	5.75	5.75	5.75	5.75	5.75	5.75	5.75	5.75	37.8	5.95	5.95	5.95	5.95	5.95	5.95	5.95	5.95	36.2
10th	6.45	6.45	6.45	6.45	6.45	6.45	6.45	6.45	37.8	6.65	6.65	6.65	6.65	6.65	6.65	6.65	6.65	36.2
11th	7.15	7.15	7.15	7.15	7.15	7.15	7.15	7.15	37.7	7.25	7.25	7.25	7.25	7.25	7.25	7.25	7.25	36.1
12th	7.75	7.75	7.75	7.75	7.75	7.75	7.75	7.75	37.8	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	35.8
13th	8.6	8.6	8.6	8.6	8.6	8.6	8.6	8.6	37.7	8.85	8.85	8.85	8.85	8.85	8.85	8.85	8.85	35.7
14th	9.45	9.45	9.45	9.45	9.45	9.45	9.45	9.45	37.6	9.75	9.75	9.75	9.75	9.75	9.75	9.75	9.75	35.2
15th	10.45	10.45	10.45	10.45	10.45	10.45	10.45	10.45	37.6	10.75	10.75	10.75	10.75	10.75	10.75	10.75	10.75	35.2

Table 4.7e. Pups and mouse mother weight (gram) before start milking at 08.00 am and after milking at 13.00 pm (Mice Control-5).

No / Day	Pups weight (gram) - Weighed at 08 am								Mother (gram)	Pups weigh (gram) - Weighed at 13 pm								Mother (gram)
	1	2	3	4	5	6	7	8		1	2	3	4	5	6	7	8	
1st	1.65	1.65	1.65	1.65	1.65	1.65	1.65	1.65	37.7	1.75	1.75	1.75	1.75	1.75	1.75	1.75	1.75	36.9
2nd	2.05	2.05	2.05	2.05	2.05	2.05	2.05	2.05	37.7	2.15	2.15	2.15	2.15	2.15	2.15	2.15	2.15	36.9
3rd	2.45	2.45	2.45	2.45	2.45	2.45	2.45	2.45	37.8	2.75	2.75	2.75	2.75	2.75	2.75	2.75	2.75	37.0
4th	3.05	3.05	3.05	3.05	3.05	3.05	3.05	3.05	37.9	3.15	3.15	3.15	3.15	3.15	3.15	3.15	2.7	37.1
5th	3.45	3.45	3.45	3.45	3.45	3.45	3.45	3.45	37.8	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	36.8
6th	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	37.8	4.15	4.15	4.15	4.15	4.15	4.15	4.15	4.15	36.8
7th	4.55	4.55	4.55	4.55	4.55	4.55	4.55	4.55	37.7	4.70	4.70	4.70	4.70	4.70	4.70	4.70	4.70	36.7
8th	5.1	5.1	5.1	5.1	5.1	5.1	5.1	5.1	37.6	5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3	36.0
9th	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	37.7	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	36.1
10th	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	37.8	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7	36.2
11th	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2	37.8	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4	36.2
12th	7.9	7.9	7.9	7.9	7.9	7.9	7.9	7.9	37.9	8.15	8.15	8.15	8.15	8.15	8.15	8.15	8.15	35.9
13th	8.75	8.75	8.75	8.75	8.75	8.75	8.75	8.75	37.7	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	35.7
14th	9.6	9.6	9.6	9.6	9.6	9.6	9.6	9.6	37.6	9.9	9.9	9.9	9.9	9.9	9.9	9.9	9.9	35.2
15th	10.6	10.6	10.6	10.6	10.6	10.6	10.6	10.6	37.6	10.9	10.9	10.9	10.9	10.9	10.9	10.9	10.9	35.2

Table 4.7f. Pups and mouse mother weight (gram) before start milking at 08.00 am and after milking at 13.00 pm (Mice Control-6).

No / Day	Pups weight (gram) - Weighed at 08 am								Mother (gram)	Pups weigh (gram) - Weighed at 13 pm								Mother (gram)
	1	2	3	4	5	6	7	8		1	2	3	4	5	6	7	8	
1st	1.7	1.7	1.73	1.7	1.7	1.71	1.7	1.7	37.8	1.8	1.8	1.83	1.8	1.8	1.81	1.8	1.8	37.0
2nd	2.1	2.12	2.1	2.1	2.11	2.13	2.1	2.1	37.8	2.2	2.22	2.2	2.2	2.21	2.23	2.2	2.2	37.0
3rd	2.5	2.5	2.52	2.53	2.5	2.5	2.5	2.54	37.9	2.6	2.6	2.62	2.63	2.6	2.6	2.6	2.64	37.1
4th	2.91	2.9	2.93	2.91	2.9	2.9	2.92	2.93	37.8	3.21	3.23	3.21	3.2	3.2	3.2	3.22	3.23	37.0
5th	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	37.7	3.65	3.65	3.65	3.65	3.65	3.65	3.65	3.65	36.7
6th	4.05	4.05	4.05	4.05	4.05	4.05	4.05	4.05	37.7	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	36.7
7th	4.6	4.62	4.6	4.64	4.6	4.6	4.6	4.6	37.7	4.75	4.752	4.75	4.754	4.75	4.75	4.75	4.75	36.7
8th	5.15	5.15	5.15	5.15	5.15	5.15	5.15	5.15	37.8	5.35	5.35	5.35	5.35	5.35	5.35	5.35	5.35	36.2
9th	5.85	5.85	5.85	5.85	5.85	5.85	5.85	5.85	37.6	6.05	6.05	6.05	6.05	6.05	6.05	6.05	6.05	36.0
10th	6.55	6.55	6.55	6.55	6.55	6.55	6.55	6.55	37.6	6.75	6.75	6.75	6.75	6.75	6.75	6.75	6.75	36.0
11th	7.25	7.25	7.25	7.25	7.25	7.25	7.25	7.25	37.7	7.45	7.45	7.45	7.45	7.45	7.45	7.45	7.45	36.1
12th	7.95	7.95	7.95	7.95	7.95	7.95	7.95	7.95	37.8	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2	35.8
13th	8.8	8.8	8.8	8.8	8.8	8.8	8.8	8.8	37.8	9.15	9.15	9.15	9.15	9.15	9.15	9.15	9.15	35.8
14th	9.75	9.75	9.75	9.75	9.75	9.75	9.75	9.75	37.7	10.05	10.05	10.05	10.05	10.05	10.05	10.05	10.05	35.3
15th	10.75	10.75	10.75	10.75	10.75	10.75	10.75	10.75	37.7	11.05	11.05	11.05	11.05	11.05	11.05	11.05	11.05	35.3

Table 4.7g. Pups and mouse mother weight (gram) before start milking at 08.00 am and after milking at 13.00 pm (1% Torbangun leaves extracts-1).

No Day	Pups weight (gram) - Weighed at 08 am								Mother (gram)	Pups weigh (gram) - Weighed at 13 pm								Mother (gram)
	1	2	3	4	5	6	7	8		1	2	3	4	5	6	7	8	
1st	1.55	1.55	1.55	1.55	1.55	1.55	1.55	1.55	37.8	1.65	1.65	1.65	1.65	1.65	1.65	1.65	1.65	37.0
2nd	1.95	1.95	1.95	1.95	1.95	1.95	1.95	1.95	37.8	2.05	2.05	2.05	2.05	2.05	2.05	2.05	2.05	37.0
3rd	2.35	2.35	2.35	2.35	2.35	2.35	2.35	2.35	37.8	2.45	2.45	2.45	2.45	2.45	2.45	2.45	2.45	37.0
4th	2.75	2.75	2.75	2.75	2.75	2.75	2.75	2.75	37.7	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	36.7
5th	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	37.7	3.45	3.45	3.45	3.45	3.45	3.45	3.45	3.45	36.7
6th	3.95	3.95	3.95	3.95	3.95	3.95	3.95	3.95	37.8	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	36.8
7th	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	37.8	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	36.2
8th	5.2	5.2	5.2	5.2	5.2	5.2	5.2	5.2	37.7	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	36.1
9th	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	37.9	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	36.3
10th	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	37.9	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8	36.3
11th	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	37.8	7.55	7.55	7.55	7.55	7.55	7.55	7.55	7.55	35.8
12th	8.15	8.15	8.15	8.15	8.15	8.15	8.15	8.15	37.8	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	35.8
13th	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	37.9	9.3	9.3	9.3	9.3	9.3	9.3	9.3	9.3	35.5
14th	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	37.8	10.3	10.3	10.3	10.3	10.3	10.3	10.3	10.3	35.4
15th	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	37.7	11.3	11.3	11.3	11.3	11.3	11.3	11.3	11.3	35.3

Table 4.7h. Pups and mouse mother weight (gram) before start milking at 08.00 am and after milking at 13.00 pm (1% Torbangun leaves extracts-2)

No Day	Pups weight (gram) - Weighed at 08 am								Mother (gram)	Pups weigh (gram) - Weighed at 13 pm								Mother (gram)
	1	2	3	4	5	6	7	8		1	2	3	4	5	6	7	8	
1st	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	37.8	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	37.0
2nd	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	37.8	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	37.0
3rd	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	37.7	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	36.9
4th	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	37.8	2.95	2.95	2.95	2.95	2.95	2.95	2.95	2.95	36.8
5th	3.35	3.35	3.35	3.35	3.35	3.35	3.35	3.35	37.9	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	36.9
6th	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	37.9	4.05	4.05	4.05	4.05	4.05	4.05	4.05	4.05	36.9
7th	4.45	4.45	4.45	4.45	4.45	4.45	4.45	4.45	37.7	4.65	4.65	4.65	4.65	4.65	4.65	4.65	4.65	36.1
8th	5.15	5.15	5.15	5.15	5.15	5.15	5.15	5.15	37.8	5.35	5.35	5.35	5.35	5.35	5.35	5.35	5.35	36.2
9th	5.85	5.85	5.85	5.85	5.85	5.85	5.85	5.85	37.8	6.05	6.05	6.05	6.05	6.05	6.05	6.05	6.05	36.2
10th	6.55	6.55	6.55	6.55	6.55	6.55	6.55	6.55	37.9	6.75	6.75	6.75	6.75	6.75	6.75	6.75	6.75	36.3
11th	7.25	7.25	7.25	7.25	7.25	7.25	7.25	7.25	37.9	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	35.9
12th	8.1	8.1	8.1	8.1	8.1	8.1	8.1	8.1	37.7	8.35	8.35	8.35	8.35	8.35	8.35	8.35	8.35	35.9
13th	8.95	8.95	8.95	8.95	8.95	8.95	8.95	8.95	37.7	9.25	9.25	9.25	9.25	9.25	9.25	9.25	9.25	35.3
14th	9.95	9.95	9.95	9.95	9.95	9.95	9.95	9.95	37.8	10.25	10.25	10.25	10.25	10.25	10.25	10.25	10.25	35.4
15th	10.95	10.95	10.95	10.95	10.95	10.95	10.95	10.95	37.7	11.25	11.25	11.25	11.25	11.25	11.25	11.25	11.25	35.3

Table 4.7i. Pups and mouse mother weight (gram) before start milking at 08.00 am and after milking at 13.00 pm (1% Torbangun leaves extracts-3)

No / Day	Pups weight (gram) - Weighed at 08 am								Mother (gram)	Pups weigh (gram) - Weighed at 13 pm								Mother (gram)
	1	2	3	4	5	6	7	8		1	2	3	4	5	6	7	8	
1st	1.6	1.62	1.61	1.6	1.6	1.62	1.6	1.63	37.9	1.7	1.72	1.71	1.7	1.7	1.72	1.7	1.73	37.1
2nd	2.0	2.02	2.02	2.01	2.02	2.03	2.02	2.02	37.9	2.1	2.12	2.12	2.11	2.12	2.13	2.12	2.12	37.1
3rd	2.4	2.42	2.42	2.41	2.43	2.42	2.43	2.43	37.8	2.5	2.52	2.52	2.52	2.53	2.52	2.53	2.53	37.0
4th	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	37.8	2.95	2.95	2.95	2.95	2.95	2.95	2.95	2.95	36.8
5th	3.35	3.35	3.35	3.35	3.35	3.35	3.35	3.35	37.9	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	36.9
6th	3.91	3.9	3.92	3.92	3.9	3.9	3.9	3.9	37.9	4.06	4.05	4.07	4.05	4.07	4.05	4.05	4.05	36.9
7th	4.45	4.46	4.45	4.45	4.47	4.45	4.45	4.45	37.8	4.65	4.66	4.65	4.65	4.65	4.65	4.65	4.65	36.2
8th	5.15	5.15	5.15	5.15	5.15	5.15	5.15	5.15	37.7	5.35	5.35	5.35	5.35	5.35	5.35	5.35	5.35	36.1
9th	5.85	5.85	5.85	5.85	5.85	5.85	5.85	5.85	37.7	6.05	6.05	6.05	6.05	6.05	6.05	6.05	6.05	36.1
10th	6.55	6.55	6.55	6.55	6.55	6.55	6.55	6.55	37.8	6.75	6.75	6.75	6.75	6.75	6.75	6.75	6.75	36.2
11th	7.26	7.25	7.27	7.25	7.25	7.28	7.25	7.29	37.7	7.51	7.5	7.52	7.5	7.5	7.53	7.5	7.54	35.7
12th	8.15	8.12	8.11	8.12	8.13	8.12	8.12	8.14	37.8	8.40	8.37	8.36	8.37	8.38	8.37	8.37	8.39	35.8
13th	9.05	9.06	9.05	9.06	9.05	9.05	9.05	9.07	37.8	9.30	9.31	9.30	9.31	9.30	9.30	9.30	9.32	35.4
14th	9.96	9.96	9.95	9.97	9.95	9.95	9.95	9.95	37.7	10.26	10.26	10.25	10.27	10.25	10.25	10.25	10.25	35.3
15th	10.96	10.96	10.95	10.97	10.95	10.96	10.95	10.97	37.7	11.26	11.26	11.25	11.27	11.25	11.26	11.25	11.27	35.3

Table 4.7j. Pups and mouse mother weight (gram) before start milking at 08.00 am and after milking at 13.00 pm (1% Torbangun leaves extracts-4)

No Day	Pups weight (gram) - Weighed at 08 am								Mother (gram)	Pups weigh (gram) - Weighed at 13 pm								Mother (gram)
	1	2	3	4	5	6	7	8		1	2	3	4	5	6	7	8	
1st	1.65	1.65	1.65	1.65	1.65	1.65	1.65	1.65	37.7	1.75	1.75	1.75	1.75	1.75	1.75	1.75	1.75	36.9
2nd	2.05	2.05	2.05	2.05	2.05	2.05	2.05	2.05	37.7	2.15	2.15	2.15	2.15	2.15	2.15	2.15	2.15	36.9
3rd	2.45	2.45	2.45	2.45	2.45	2.45	2.45	2.45	37.8	2.55	2.55	2.55	2.55	2.55	2.55	2.55	2.55	37.0
4th	2.85	2.85	2.85	2.85	2.85	2.85	2.85	2.85	37.7	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	36.7
5th	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	37.8	3.55	3.55	3.55	3.55	3.55	3.55	3.55	3.55	36.8
6th	3.95	3.95	3.95	3.95	3.95	3.95	3.95	3.95	37.8	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	36.8
7th	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	37.9	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	36.3
8th	5.2	5.2	5.2	5.2	5.2	5.2	5.2	5.2	37.9	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	36.3
9th	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	37.8	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	36.2
10th	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	37.7	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8	36.1
11th	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	37.8	7.55	7.55	7.55	7.55	7.55	7.55	7.55	7.55	35.8
12th	8.15	8.15	8.15	8.15	8.15	8.15	8.15	8.15	37.8	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	35.8
13th	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	37.9	9.3	9.3	9.3	9.3	9.3	9.3	9.3	9.3	35.5
14th	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	37.7	10.3	10.3	10.3	10.3	10.3	10.3	10.3	10.3	35.3
15th	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	37.7	11.3	11.3	11.3	11.3	11.3	11.3	11.3	11.3	35.3

Table 4.7k. Pups and mouse mother weight (gram) before start milking at 08.00 am and after milking at 13.00 pm (1% Torbangun leaves extracts-5)

No Day	Pups weight (gram) - Weighed at 08 am								Mother (gram)	Pups weigh (gram) - Weighed at 13 pm								Mother (gram)
	1	2	3	4	5	6	7	8		1	2	3	4	5	6	7	8	
1st	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	37.6	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	36.8
2nd	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	37.6	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	36.8
3rd	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	37.7	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	36.9
4th	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	37.7	3.05	3.05	3.05	3.05	3.05	3.05	3.05	3.05	36.7
5th	3.45	3.45	3.45	3.45	3.45	3.45	3.45	3.45	37.7	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	36.7
6th	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	37.8	4.15	4.15	4.15	4.15	4.15	4.15	4.15	4.15	36.8
7th	4.55	4.55	4.55	4.55	4.55	4.55	4.55	4.55	37.8	4.75	4.75	4.75	4.75	4.75	4.75	4.75	4.75	36.2
8th	5.25	5.25	5.25	5.25	5.25	5.25	5.25	5.25	37.7	5.45	5.45	5.45	5.45	5.45	5.45	5.45	5.45	36.1
9th	5.95	5.95	5.95	5.95	5.95	5.95	5.95	5.95	37.7	6.15	6.15	6.15	6.15	6.15	6.15	6.15	6.15	36.1
10th	6.65	6.65	6.65	6.65	6.65	6.65	6.65	6.65	37.9	6.85	6.85	6.85	6.85	6.85	6.85	6.85	6.85	36.3
11th	7.35	7.35	7.35	7.35	7.35	7.35	7.35	7.35	37.8	7.6	7.6	7.6	7.6	7.6	7.6	7.6	7.6	35.8
12th	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2	37.8	8.45	8.45	8.45	8.45	8.45	8.45	8.45	8.45	35.8
13th	9.05	9.05	9.05	9.05	9.05	9.05	9.05	9.05	37.7	9.35	9.35	9.35	9.35	9.35	9.35	9.35	9.35	35.3
14th	10.05	10.05	10.05	10.05	10.05	10.05	10.05	10.05	37.6	10.35	10.35	10.35	10.35	10.35	10.35	10.35	10.35	35.2
15th	11.5	11.05	11.05	11.05	11.05	11.05	11.05	11.05	37.6	11.35	11.35	11.35	11.35	11.35	11.35	11.35	11.35	35.2

Table 4.7l. Pups and mouse mother weight (gram) before start milking at 08.00 am and after milking at 13.00 pm (1% Torbangun leaves extracts-6)

No / Day	Pups weight (gram) - Weighed at 08 am								Mother (gram)	Pups weigh (gram) - Weighed at 13 pm								Mother (gram)
	1	2	3	4	5	6	7	8		1	2	3	4	5	6	7	8	
1st	1.68	1.7	1.7	1.71	1.71	1.7	1.7	1.72	37.6	1.79	1.8	1.8	1.81	1.81	1.8	1.8	1.82	36.8
2nd	2.1	2.11	2.12	2.11	2.1	2.13	2.13	2.13	37.6	2.2	2.21	2.22	2.21	2.2	2.23	2.23	2.23	36.8
3rd	2.51	2.51	2.53	2.52	2.52	2.53	2.52	2.52	37.7	2.61	2.61	2.63	2.62	2.62	2.63	2.62	2.62	36.9
4th	2.92	2.91	2.93	2.93	2.92	2.93	2.94	2.94	37.7	3.07	3.06	3.08	3.08	3.07	3.08	3.09	3.09	36.7
5th	3.45	3.45	3.45	3.45	3.45	3.45	3.45	3.45	37.8	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	36.8
6th	4.02	4.02	4.03	4.03	4.01	4.03	4.02	4.03	37.7	4.17	4.17	4.18	4.18	4.16	4.18	4.17	4.18	36.7
7th	4.55	4.57	4.57	4.56	4.58	4.56	4.58	4.58	37.9	4.75	4.77	4.77	4.76	4.78	4.76	4.78	4.78	36.3
8th	5.26	5.26	5.28	5.27	5.27	5.28	5.26	5.28	37.9	5.46	5.46	5.48	5.47	5.45	5.45	5.45	5.45	36.3
9th	5.95	5.95	5.95	5.95	5.95	5.95	5.95	5.95	37.8	6.15	6.15	6.15	6.15	6.15	6.15	6.15	6.15	36.2
10th	6.65	6.65	6.65	6.65	6.65	6.65	6.65	6.65	37.8	6.85	6.85	6.85	6.85	6.85	6.85	6.85	6.85	36.2
11th	7.35	7.35	7.35	7.35	7.35	7.35	7.35	7.35	37.9	7.6	7.6	7.6	7.6	7.6	7.6	7.6	7.6	35.9
12th	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2	37.9	8.45	8.45	8.45	8.45	8.45	8.45	8.45	8.45	35.9
13th	9.05	9.05	9.05	9.05	9.05	9.05	9.05	9.05	37.7	9.35	9.35	9.35	9.35	9.35	9.35	9.35	9.35	35.3
14th	10.05	10.05	10.05	10.05	10.05	10.05	10.05	10.05	37.6	10.35	10.35	10.35	10.35	10.35	10.35	10.35	10.35	35.2
15th	11.5	11.05	11.05	11.05	11.05	11.05	11.05	11.05	37.6	11.35	11.35	11.35	11.35	11.35	11.35	11.35	11.35	35.2

Table 4.7m. Pups and mouse mother weight (gram) before start milking at 08.00 am and after milking at 13.00 pm (5% Torbangun leaves extracts-1)

No / Day	Pups weight (gram) - Weighed at 08 am								Mother (gram)	Pups weigh (gram) - Weighed at 13 pm								Mother (gram)
	1	2	3	4	5	6	7	8		1	2	3	4	5	6	7	8	
1st	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	37.9	1.75	1.75	1.75	1.75	1.75	1.75	1.75	1.75	36.9
2nd	2.15	2.15	2.15	2.15	2.15	2.15	2.15	2.15	37.9	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3	36.9
3rd	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	37.8	2.85	2.85	2.85	2.85	2.85	2.85	2.85	2.85	36.8
4th	3.25	3.25	3.25	3.25	3.25	3.25	3.25	3.25	37.7	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	36.7
5th	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	37.9	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	36.3
6th	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	37.8	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	36.2
7th	5.2	5.2	5.2	5.2	5.2	5.2	5.2	5.2	37.7	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	36.1
8th	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	37.7	6.15	6.15	6.15	6.15	6.15	6.15	6.15	6.15	35.7
9th	6.75	6.75	6.75	6.75	6.75	6.75	6.75	6.75	37.8	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	35.8
10th	7.6	7.6	7.6	7.6	7.6	7.6	7.6	7.6	37.9	7.85	7.85	7.85	7.85	7.85	7.85	7.85	7.85	35.9
11th	8.45	8.45	8.45	8.45	8.45	8.45	8.45	8.45	38.0	8.75	8.75	8.75	8.75	8.75	8.75	8.75	8.75	35.6
12th	9.45	9.45	9.45	9.45	9.45	9.45	9.45	9.45	38.0	9.75	9.75	9.75	9.75	9.75	9.75	9.75	9.75	35.6
13th	10.45	10.45	10.45	10.45	10.45	10.45	10.45	10.45	37.9	10.75	10.75	10.75	10.75	10.75	10.75	10.75	10.75	35.5
14th	11.45	11.45	11.45	11.45	11.45	11.45	11.45	11.45	37.9	11.8	11.8	11.8	11.8	11.8	11.8	11.8	11.8	35.1
15th	12.6	12.6	12.6	12.6	12.6	12.6	12.6	12.6	37.9	12.95	12.95	12.95	12.95	12.95	12.95	12.95	12.95	35.1

Table 4.7n. Pups and mouse mother weight (gram) before start milking at 08.00 am and after milking at 13.00 pm (5% Torbangun leaves extracts-2)

No / Day	Pups weight (gram) - Weighed at 08 am								Mother (gram)	Pups weigh (gram) - Weighed at 13 pm								Mother (gram)
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**		1	2	3	4	5	6	7	8	
1st	1.63	1.62	1.62	1.6	1.61	1.6	1.6	1.6	37.9	1.78	1.77	1.75	1.75	1.76	1.75	1.75	1.75	36.9
2nd	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	37.8	2.35	2.35	2.35	2.35	2.35	2.35	2.35	2.35	36.8
3rd	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	37.9	2.95	2.95	2.95	2.95	2.95	2.95	2.95	2.95	36.9
4th	3.25	3.25	3.25	3.25	3.25	3.25	3.25	3.25	37.9	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	36.9
5th	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	38.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	36.4
6th	4.51	4.53	4.53	4.5	4.5	4.5	4.5	4.5	37.9	4.71	4.73	4.73	4.7	4.7	4.7	4.7	4.7	36.3
7th	5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3	37.8	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	36.2
8th	5.92	5.92	5.91	5.91	5.92	5.9	5.9	5.91	38.0	6.152	6.152	6.151	6.151	6.15	6.152	6.15	6.151	36.0
9th	6.85	6.85	6.85	6.85	6.85	6.85	6.85	6.85	38.0	7.10	7.10	7.10	7.10	7.10	7.10	7.10	7.10	36.0
10th	7.62	7.62	7.61	7.63	7.63	7.62	7.61	7.61	37.9	7.87	7.87	7.86	7.88	7.88	7.87	7.86	7.86	35.9
11th	8.55	8.56	8.55	8.55	8.56	8.55	8.55	8.56	37.9	8.85	8.86	8.85	8.85	8.86	8.85	8.85	8.86	35.5
12th	9.56	9.56	9.55	9.55	9.55	9.55	9.55	9.55	37.8	9.86	9.86	9.85	9.85	9.85	9.85	9.85	9.85	35.4
13th	10.55	10.55	10.55	10.55	10.55	10.55	10.55	10.55	37.8	10.85	10.85	10.85	10.85	10.85	10.85	10.85	10.85	35.0
14th	11.45	11.45	11.45	11.45	11.45	11.45	11.45	11.45	37.9	11.8	11.8	11.8	11.8	11.8	11.8	11.8	11.8	35.1
15th	12.61	12.61	12.63	12.62	12.6	12.6	12.62	12.61	37.9	12.96	12.96	12.98	12.97	12.95	12.95	12.95	12.96	35.1

Table 4.7o. Pups and mouse mother weight (gram) before start milking at 08.00 am and after milking at 13.00 pm (5% Torbangun leaves extracts-3)

No / Day	Pups weight (gram) - Weighed at 08 am								Mother (gram)	Pups weigh (gram) - Weighed at 13 pm								Mother (gram)
	1	2	3	4	5	6	7	8		1	2	3	4	5	6	7	8	
1st	1.65	1.65	1.65	1.65	1.65	1.65	1.65	1.65	38.0	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	37.0
2nd	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	38.0	2.35	2.35	2.35	2.35	2.35	2.35	2.35	2.35	37.0
3rd	2.75	2.75	2.75	2.75	2.75	2.75	2.75	2.75	37.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	36.9
4th	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	37.8	3.15	3.15	3.15	3.15	3.15	3.15	3.15	3.15	36.8
5th	3.55	3.55	3.55	3.55	3.55	3.55	3.55	3.55	38.0	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75	36.4
6th	4.25	4.25	4.25	4.25	4.25	4.25	4.25	4.25	37.9	4.45	4.45	4.45	4.45	4.45	4.45	4.45	4.45	36.3
7th	4.95	4.95	4.95	4.95	4.95	4.95	4.95	4.95	37.8	5.15	5.15	5.15	5.15	5.15	5.15	5.15	5.15	36.2
8th	5.65	5.65	5.65	5.65	5.65	5.65	5.65	5.65	37.8	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	35.8
9th	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	37.9	6.75	6.75	6.75	6.75	6.75	6.75	6.75	6.75	35.9
10th	7.35	7.35	7.35	7.35	7.35	7.35	7.35	7.35	37.9	7.6	7.6	7.6	7.6	7.6	7.6	7.6	7.6	35.9
11th	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2	38.0	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	35.6
12th	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2	37.9	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	35.5
13th	10.2	10.2	10.2	10.2	10.2	10.2	10.2	10.2	37.9	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	35.5
14th	11.2	11.2	11.2	11.2	11.2	11.2	11.2	11.2	38.0	11.55	11.55	11.55	11.55	11.55	11.55	11.55	11.55	35.2
15th	12.35	12.35	12.35	12.35	12.35	12.35	12.35	12.35	38.0	12.7	12.7	12.7	12.7	12.7	12.7	12.7	12.7	35.2

Table 4.7p. Pups and mouse mother weight (gram) before start milking at 08.00 am and after milking at 13.00 pm (5% Torbangun leaves extracts-4)

No / Day	Pups weight (gram) - Weighed at 08 am								Mother (gram)	Pups weigh (gram) - Weighed at 13 pm								Mother (gram)
	1	2	3	4	5	6	7	8		1	2	3	4	5	6	7	8	
1st	1.66	1.65	1.65	1.67	1.65	1.66	1.65	1.67	37.9	1.81	1.8	1.8	1.82	1.8	1.81	1.8	1.82	36.9
2nd	2.25	2.25	2.25	2.25	2.25	2.25	2.25	2.25	37.8	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	36.8
3rd	2.76	2.75	2.75	2.75	2.75	2.75	2.75	2.75	37.9	2.91	2.9	2.9	2.9	2.9	2.9	2.9	2.9	36.9
4th	3.32	3.3	3.3	3.3	3.3	3.3	3.3	3.3	38.0	3.17	3.15	3.15	3.15	3.15	3.15	3.15	3.15	37.0
5th	3.65	3.65	3.65	3.65	3.65	3.65	3.65	3.65	38.0	3.85	3.85	3.85	3.85	3.85	3.85	3.85	3.85	36.4
6th	4.25	4.25	4.25	4.25	4.25	4.25	4.25	4.25	37.9	4.45	4.45	4.45	4.45	4.45	4.45	4.45	4.45	36.4
7th	4.95	4.95	4.95	4.95	4.95	4.95	4.95	4.95	37.9	5.15	5.15	5.15	5.15	5.15	5.15	5.15	5.15	36.3
8th	5.65	5.65	5.65	5.65	5.65	5.65	5.65	5.65	37.8	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	35.8
9th	6.51	6.52	6.52	6.53	6.52	6.51	6.51	6.51	37.9	6.76	6.76	6.76	6.78	6.77	6.76	6.76	6.76	35.9
10th	7.35	7.35	7.35	7.35	7.35	7.35	7.35	7.35	37.8	7.6	7.6	7.6	7.6	7.6	7.6	7.6	7.6	35.8
11th	8.3	8.3	8.3	8.3	8.3	8.3	8.3	8.3	37.7	8.6	8.6	8.6	8.6	8.6	8.6	8.6	8.6	35.3
12th	9.4	9.4	9.4	9.4	9.4	9.4	9.4	9.4	37.7	9.7	9.7	9.7	9.7	9.7	9.7	9.7	9.7	35.3
13th	10.3	10.3	10.3	10.3	10.3	10.3	10.3	10.3	37.8	10.6	10.6	10.6	10.6	10.6	10.6	10.6	10.6	35.4
14th	11.21	11.21	11.22	11.2	11.2	11.2	11.23	11.23	37.9	11.56	11.56	11.57	11.55	11.55	11.55	11.58	11.58	35.1
15th	12.4	12.4	12.4	12.4	12.4	12.4	12.4	12.4	37.9	12.75	12.75	12.75	12.75	12.75	12.75	12.75	12.75	35.1

Table 4.7q. Pups and mouse mother weight (gram) before start milking at 08.00 am and after milking at 13.00 pm (5% Torbangun leaves extracts-5)

No / Day	Pups weight (gram) - Weighed at 08 am								Mother (gram)	Pups weigh (gram) - Weighed at 13 pm								Mother (gram)
	1	2	3	4	5	6	7	8		1	2	3	4	5	6	7	8	
1st	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	38.0	1.85	1.85	1.85	1.85	1.85	1.85	1.85	1.85	37.0
2nd	2.25	2.25	2.25	2.25	2.25	2.25	2.25	2.25	37.9	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	36.9
3rd	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	38.0	2.95	2.95	2.95	2.95	2.95	2.95	2.95	2.95	37.0
4th	3.35	3.35	3.35	3.35	3.35	3.35	3.35	3.35	38.0	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	37.0
5th	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	37.8	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	36.2
6th	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6	37.9	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8	36.3
7th	5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3	37.9	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	36.3
8th	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	37.8	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25	35.8
9th	6.85	6.85	6.85	6.85	6.85	6.85	6.85	6.85	37.8	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	35.8
10th	7.7	7.7	7.7	7.7	7.7	7.7	7.7	7.7	37.7	7.95	7.95	7.95	7.95	7.95	7.95	7.95	7.95	35.7
11th	8.55	8.55	8.55	8.55	8.55	8.55	8.55	8.55	37.7	8.85	8.85	8.85	8.85	8.85	8.85	8.85	8.85	35.3
12th	9.55	9.55	9.55	9.55	9.55	9.55	9.55	9.55	37.9	9.85	9.85	9.85	9.85	9.85	9.85	9.85	9.85	35.5
13th	10.55	10.55	10.55	10.55	10.55	10.55	10.55	10.55	37.8	10.85	10.85	10.85	10.85	10.85	10.85	10.85	10.85	35.4
14th	11.55	11.55	11.55	11.55	11.55	11.55	11.55	11.55	38.0	11.9	11.9	11.9	11.9	11.9	11.9	11.9	11.9	35.2
15th	12.7	12.7	12.7	12.7	12.7	12.7	12.7	12.7	38.0	13.05	13.05	13.05	13.05	13.05	13.05	13.05	13.05	35.2

Table 4.7r. Pups and mouse mother weight (gram) before start milking at 08.00 am and after milking at 13.00 pm (5% Torbangun leaves extracts-6)

No / Day	Pups weight (gram) - Weighed at 08 am								Mother (gram)	Pups weigh (gram) - Weighed at 13 pm								Mother (gram)
	1	2	3	4	5	6	7	8		1	2	3	4	5	6	7	8	
1st	1.72	1.72	1.72	1.72	1.72	1.72	1.72	1.72	37.9	1.87	1.87	1.87	1.87	1.87	1.87	1.87	1.87	36.9
2nd	2.25	2.25	2.25	2.25	2.25	2.25	2.25	2.25	37.9	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	36.9
3rd	2.85	2.85	2.85	2.85	2.85	2.85	2.85	2.85	37.8	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	36.8
4th	3.36	3.35	3.37	3.36	3.36	3.35	3.35	3.37	37.9	3.51	3.5	3.52	3.51	3.51	3.5	3.5	3.52	36.9
5th	3.93	3.93	3.91	3.92	3.92	3.91	3.93	3.93	37.8	4.13	4.13	4.13	4.13	4.13	4.13	4.13	4.13	36.2
6th	4.74	4.74	4.75	4.73	4.72	4.74	4.73	4.74	37.8	4.94	4.94	4.95	4.93	4.92	4.94	4.93	4.94	36.2
7th	5.32	5.31	5.32	5.32	5.33	5.32	5.32	5.32	37.7	5.52	5.51	5.52	5.52	5.53	5.52	5.52	5.52	36.1
8th	6.05	6.05	6.06	6.06	6.05	6.05	6.07	6.07	37.9	6.3	6.3	6.31	6.31	6.3	6.3	6.32	6.32	35.9
9th	6.95	6.95	6.95	6.95	6.95	6.95	6.95	6.95	37.9	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2	35.9
10th	7.71	7.72	7.72	7.71	7.73	7.73	7.72	7.73	38.0	7.96	7.97	7.97	7.96	7.98	7.98	7.97	7.98	36.0
11th	8.6	8.6	8.6	8.6	8.6	8.6	8.6	8.6	38.1	8.9	8.9	8.9	8.9	8.9	8.9	8.9	8.9	35.7
12th	9.57	9.56	9.55	9.55	9.55	9.55	9.55	9.55	38.0	9.87	9.86	9.85	9.85	9.85	9.85	9.85	9.85	35.6
13th	10.6	10.6	10.6	10.6	10.6	10.6	10.6	10.6	37.9	10.9	10.9	10.9	10.9	10.9	10.9	10.9	10.9	35.5
14th	11.57	11.57	11.55	11.55	11.56	11.57	11.55	11.55	37.8	11.92	11.92	11.9	11.9	11.91	11.92	11.9	11.9	35.0
15th	12.8	12.82	12.8	12.81	12.81	12.8	12.82	12.82	37.9	13.15	13.17	13.15	13.16	13.16	13.15	13.15	13.17	35.1

Table 4.7s. Pups and mouse mother weight (gram) before start milking at 08.00 am and after milking at 13.00 pm (10% Torbangun leaves extracts-1)

No / Day	Pups weight (gram) - Weighed at 08 am								Mother (gram)	Pups weigh (gram) - Weighed at 13 pm								Mother (gram)
	1	2	3	4	5	6	7	8		1	2	3	4	5	6	7	8	
1st	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	38.0	1.75	1.75	1.75	1.75	1.75	1.75	1.75	1.75	37.0
2nd	2.15	2.15	2.15	2.15	2.15	2.15	2.15	2.15	38.0	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3	37.0
3rd	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	38.2	2.85	2.85	2.85	2.85	2.85	2.85	2.85	2.85	37.2
4th	3.25	3.25	3.25	3.25	3.25	3.25	3.25	3.25	38.1	3.45	3.45	3.45	3.45	3.45	3.45	3.45	3.45	36.5
5th	3.95	3.95	3.95	3.95	3.95	3.95	3.95	3.95	38.1	4.15	4.15	4.15	4.15	4.15	4.15	4.15	4.15	36.5
6th	4.65	4.65	4.65	4.65	4.65	4.65	4.65	4.65	38.0	4.85	4.85	4.85	4.85	4.85	4.85	4.85	4.85	36.4
7th	5.35	5.35	5.35	5.35	5.35	5.35	5.35	5.35	38.0	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	36.0
8th	6.2	6.2	6.2	6.2	6.2	6.2	6.2	6.2	37.9	6.45	6.45	6.45	6.45	6.45	6.45	6.45	6.45	36.0
9th	7.05	7.05	7.05	7.05	7.05	7.05	7.05	7.05	37.9	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	35.9
10th	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	38.0	8.3	8.3	8.3	8.3	8.3	8.3	8.3	8.3	35.6
11th	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	38.2	9.3	9.3	9.3	9.3	9.3	9.3	9.3	9.3	35.8
12th	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	38.2	10.3	10.3	10.3	10.3	10.3	10.3	10.3	10.3	35.8
13th	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	38.3	11.3	11.3	11.3	11.3	11.3	11.3	11.3	11.3	35.9
14th	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0	38.4	12.4	12.4	12.4	12.4	12.4	12.4	12.4	12.4	35.2
15th	13.3	13.3	13.3	13.3	13.3	13.3	13.3	13.3	38.4	13.7	13.7	13.7	13.7	13.7	13.7	13.7	13.7	35.2

Table 4.7t. Pups and mouse mother weight (gram) before start milking at 08.00 am and after milking at 13.00 pm (10% Torbangun leaves extracts-2)

No / Day	Pups weight (gram) - Weighed at 08 am								Mother (gram)	Pups weigh (gram) - Weighed at 13 pm								Mother (gram)
	1	2	3	4	5	6	7	8		1	2	3	4	5	6	7	8	
1st	1.62	1.62	1.62	1.62	1.62	1.62	1.62	1.62	38.1	1.77	1.77	1.77	1.77	1.77	1.77	1.77	1.77	37.1
2nd	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	38.0	2.35	2.35	2.35	2.35	2.35	2.35	2.35	2.35	37.0
3rd	2.72	2.72	2.72	2.72	2.72	2.72	2.72	2.72	38.0	2.87	2.87	2.87	2.87	2.87	2.87	2.87	2.87	37.0
4th	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	38.1	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	36.5
5th	3.97	3.97	3.97	3.97	3.97	3.97	3.97	3.97	38.1	4.17	4.17	4.17	4.17	4.17	4.17	4.17	4.17	36.5
6th	4.67	4.67	4.67	4.67	4.67	4.67	4.67	4.67	37.9	4.87	4.87	4.87	4.87	4.87	4.87	4.87	4.87	36.3
7th	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	38.0	5.65	5.65	5.65	5.65	5.65	5.65	5.65	5.65	36.0
8th	6.23	6.23	6.21	6.22	6.21	6.22	6.21	6.21	38.2	6.48	6.48	6.46	6.47	6.46	6.47	6.46	6.46	36.2
9th	7.25	7.25	7.25	7.25	7.25	7.25	7.25	7.25	38.2	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	36.2
10th	8.02	8.02	8.02	8.02	8.02	8.02	8.02	8.02	38.3	8.32	8.32	8.32	8.32	8.32	8.32	8.32	8.32	35.9
11th	9.03	9.03	9.03	9.03	9.03	9.03	9.03	9.03	38.3	9.33	9.33	9.33	9.33	9.33	9.33	9.33	9.33	35.9
12th	10.1	10.1	10.1	10.1	10.1	10.1	10.1	10.1	38.4	10.4	10.4	10.4	10.4	10.4	10.4	10.4	10.4	36.0
13th	11.2	11.1	11.2	11.2	11.2	11.4	11.2	11.3	38.4	11.5	11.4	11.5	11.5	11.5	11.6	11.5	116	36.0
14th	12.1	12.1	12.2	12.1	12.2	12.1	12.1	12.1	38.3	12.5	12.5	12.6	12.5	12.6	12.5	12.5	12.5	35.1
15th	13.4	13.3	13.3	13.4	13.4	13.4	13.4	13.5	38.3	13.8	13.7	13.7	13.8	13.8	13.8	13.8	13.9	35.1

Table 4.7u. Pups and mouse mother weight (gram) before start milking at 08.00 am and after milking at 13.00 pm (10% Torbangun leaves extracts-3)

No / Day	Pups weight (gram) - Weighed at 08 am								Mother (gram)	Pups weigh (gram) - Weighed at 13 pm								Mother (gram)
	1	2	3	4	5	6	7	8		1	2	3	4	5	6	7	8	
1st	1.65	1.65	1.65	1.65	1.65	1.65	1.65	1.65	38.3	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	37.3
2nd	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	38.3	2.35	2.35	2.35	2.35	2.35	2.35	2.35	2.35	37.3
3rd	2.75	2.75	2.75	2.75	2.75	2.75	2.75	2.75	38.2	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	37.2
4th	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	38.1	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	36.5
5th	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	38.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	36.6
6th	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	38.3	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9	36.7
7th	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	38.1	5.65	5.65	5.65	5.65	5.65	5.65	5.65	5.65	36.1
8th	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25	38.0	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	36.0
9th	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	38.0	7.35	7.35	7.35	7.35	7.35	7.35	7.35	7.35	36.0
10th	7.95	7.95	7.95	7.95	7.95	7.95	7.95	7.95	38.1	8.25	8.25	8.25	8.25	8.25	8.25	8.25	8.25	35.7
11th	8.95	8.95	8.95	8.95	8.95	8.95	8.95	8.95	38.1	9.25	9.25	9.25	9.25	9.25	9.25	9.25	9.25	35.7
12th	9.95	9.95	9.95	9.95	9.95	9.95	9.95	9.95	38.2	10.25	10.25	10.25	10.25	10.25	10.25	10.25	10.25	35.8
13th	10.95	10.95	10.95	10.95	10.95	10.95	10.95	10.95	38.1	11.25	11.25	11.25	11.25	11.25	11.25	11.25	11.25	35.7
14th	11.95	11.95	11.95	11.95	11.95	11.95	11.95	11.95	38.2	12.35	12.35	12.35	12.35	12.35	12.35	12.35	12.35	35.0
15th	13.25	13.25	13.25	13.25	13.25	13.25	13.25	13.25	38.3	13.65	13.65	13.65	13.65	13.65	13.65	13.65	13.65	35.1

Table 4.7v. Pups and mouse mother weight (gram) before start milking at 08.00 am and after milking at 13.00 pm (10% Torbangun leaves extracts-4)

No / Day	Pups weight (gram) - Weighed at 08 am								Mother (gram)	Pups weigh (gram) - Weighed at 13 pm								Mother (gram)
	1	2	3	4	5	6	7	8		1	2	3	4	5	6	7	8	
1st	1.68	1.68	1.69	1.69	1.67	1.68	1.68	1.69	38.3	1.83	1.83	1.84	1.84	1.82	1.83	1.83	1.84	37.3
2nd	2.24	2.23	2.22	2.24	2.24	2.23	2.24	2.24	38.2	2.39	2.38	2.37	2.39	2.39	2.38	2.39	2.39	37.2
3rd	2.85	2.85	2.85	2.86	2.85	2.87	2.85	2.85	38.3	3.0	3.0	3.0	3.01	3.0	3.0	3.0	3.0	37.3
4th	3.32	3.32	3.32	3.32	3.32	3.32	3.32	3.32	38.3	3.52	3.52	3.52	3.52	3.52	3.52	3.52	3.52	36.7
5th	4.14	4.14	4.13	4.12	4.13	4.13	4.12	4.11	38.1	4.34	4.34	4.33	4.32	4.33	4.33	4.32	4.31	36.5
6th	4.73	4.72	4.73	4.74	4.73	4.73	4.73	4.73	38.2	4.93	4.92	4.93	4.94	4.93	4.93	4.93	4.93	36.6
7th	5.45	5.46	5.46	5.45	5.46	5.45	5.45	5.47	38.2	5.7	5.71	5.71	5.7	5.71	5.7	5.7	5.72	36.2
8th	6.27	6.27	6.27	6.28	6.27	6.278	6.27	6.28	38.1	6.52	6.52	6.52	6.53	6.52	6.53	6.52	6.53	36.1
9th	7.14	7.14	7.14	7.14	7.14	7.14	7.14	7.14	38.0	7.39	7.39	7.39	7.39	7.39	7.39	7.39	7.39	36.0
10th	7.96	7.96	7.96	7.96	7.96	7.96	7.96	7.96	38.1	8.26	8.26	8.26	8.26	8.26	8.26	8.26	8.26	35.7
11th	8.97	8.97	8.97	8.97	8.97	8.97	8.97	8.97	38.1	9.27	9.27	9.27	9.27	9.27	9.27	9.27	9.27	35.7
12th	9.96	9.95	9.95	9.95	9.95	9.95	9.95	9.95	38.2	10.26	10.26	10.26	10.26	10.26	10.26	10.26	10.26	35.8
13th	10.94	10.94	10.94	10.94	10.94	10.94	10.94	10.94	38.1	11.24	11.24	11.24	11.24	11.24	11.24	11.24	11.24	35.7
14th	11.93	11.93	11.93	11.93	11.93	11.93	11.93	11.93	38.2	12.33	12.33	12.33	12.33	12.33	12.33	12.33	12.33	35.0
15th	13.3	13.3	13.3	13.3	13.3	13.3	13.3	13.3	38.2	13.7	13.7	13.7	13.7	13.7	13.7	13.7	13.7	35.0

Table 4.7w. Pups and mouse mother weight (gram) before start milking at 08.00 am and after milking at 13.00 pm (10% Torbangun leaves extracts-5)

No / Day	Pups weight (gram) - Weighed at 08 am								Mother (gram)	Pups weigh (gram) - Weighed at 13 pm								Mother (gram)
	1	2	3	4	5	6	7	8		1	2	3	4	5	6	7	8	
1st	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	38.2	1.85	1.85	1.85	1.85	1.85	1.85	1.85	1.85	37.2
2nd	2.25	2.25	2.25	2.25	2.25	2.25	2.25	2.25	38.2	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	38.2
3rd	3.35	3.35	3.35	3.35	3.35	3.35	3.35	3.35	38.1	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	37.1
4th	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	38.0	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	36.4
5th	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6	38.0	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8	36.4
6th	5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3	38.1	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	36.5
7th	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	38.2	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25	36.2
8th	6.85	6.85	6.85	6.85	6.85	6.85	6.85	6.85	38.3	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	36.3
9th	7.7	7.7	7.7	7.7	7.7	7.7	7.7	7.7	38.2	7.95	7.95	7.95	7.95	7.95	7.95	7.95	7.95	36.2
10th	8.55	8.55	8.55	8.55	8.55	8.55	8.55	8.55	38.3	8.85	8.85	8.85	8.85	8.85	8.85	8.85	8.85	35.9
11th	9.55	9.55	9.55	9.55	9.55	9.55	9.55	9.55	38.2	9.85	9.85	9.85	9.85	9.85	9.85	9.85	9.85	35.8
12th	10.55	10.55	10.55	10.55	10.55	10.55	10.55	10.55	38.1	10.85	10.85	10.85	10.85	10.85	10.85	10.85	10.85	35.7
13th	11.55	11.55	11.55	11.55	11.55	11.55	11.55	11.55	38.0	11.85	11.85	11.85	11.85	11.85	11.85	11.85	11.85	35.6
14th	12.55	12.55	12.55	12.55	12.55	12.55	12.55	12.55	38.2	12.95	12.95	12.95	12.95	12.95	12.95	12.95	12.95	35.0
15th	13.85	13.85	13.85	13.85	13.85	13.85	13.85	13.85	38.2	14.25	14.25	14.25	14.25	14.25	14.25	14.25	14.25	35.0

Table 4.7x. Pups and mouse mother weight (gram) before start milking at 08.00 am and after milking at 13.00 pm (10% Torbangun leaves extracts-6)

No / Day	Pups weight (gram) - Weighed at 08 am								Mother (gram)	Pups weigh (gram) - Weighed at 13 pm								Mother (gram)
	1	2	3	4	5	6	7	8		1	2	3	4	5	6	7	8	
1st	1.72	1.72	1.72	1.72	1.72	1.72	1.72	1.72	38.1	1.87	1.87	1.87	1.87	1.87	1.87	1.87	1.87	37.1
2nd	2.27	2.27	2.27	2.27	2.27	2.27	2.27	2.27	38.1	2.42	2.42	2.42	2.42	2.42	2.42	2.42	2.42	37.1
3rd	3.37	3.37	3.37	3.37	3.37	3.37	3.37	3.37	38.2	3.52	3.52	3.52	3.52	3.52	3.52	3.52	3.52	37.2
4th	4.05	4.05	4.05	4.05	4.05	4.05	4.05	4.05	38.0	4.25	4.25	4.25	4.25	4.25	4.25	4.25	4.25	36.4
5th	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6	38.0	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8	36.4
6th	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	37.9	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	36.3
7th	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	38.0	6.35	6.35	6.35	6.35	6.35	6.35	6.35	6.35	36.0
8th	6.95	6.95	6.95	6.95	6.95	6.95	6.95	6.95	37.9	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2	35.9
9th	7.71	7.71	7.71	7.71	7.71	7.71	7.71	7.71	38.1	7.96	7.96	7.96	7.96	7.96	7.96	7.96	7.96	36.1
10th	8.57	8.57	8.57	8.57	8.57	8.57	8.57	8.57	38.1	8.87	8.87	8.87	8.87	8.87	8.87	8.87	8.87	35.7
11th	9.56	9.56	9.56	9.56	9.56	9.56	9.56	9.56	38.2	9.86	9.86	9.86	9.86	9.86	9.86	9.86	9.86	35.8
12th	10.56	10.56	10.56	10.56	10.56	10.56	10.56	10.56	38.2	10.86	10.86	10.86	10.86	10.86	10.86	10.86	10.86	35.8
13th	11.58	11.58	11.58	11.58	11.58	11.58	11.58	11.58	38.0	11.88	11.88	11.88	11.88	11.88	11.88	11.88	11.88	35.6
14th	12.55	12.55	12.55	12.55	12.55	12.55	12.55	12.55	38.3	12.95	12.95	12.95	12.95	12.95	12.95	12.95	12.95	35.1
15th	13.9	13.9	13.9	13.9	13.9	13.9	13.9	13.9	38.4	14.3	14.3	14.3	14.3	14.3	14.3	14.3	14.3	35.3

Table 4.7y. Pups and mouse mother weight (gram) before start milking at 08.00 am and after milking at 13.00 pm (15% Torbangun leaves extracts-1)

No / Day	Pups weight (gram) - Weighed at 08 am								Mother (gram)	Pups weigh (gram) - Weighed at 13 pm								Mother (gram)
	1	2	3	4	5	6	7	8		1	2	3	4	5	6	7	8	
1st	1.65	1.65	1.65	1.65	1.65	1.65	1.65	1.65	39.1	1.85	1.85	1.85	1.85	1.85	1.85	1.85	1.85	37.5
2nd	2.35	2.35	2.35	2.35	2.35	2.35	2.35	2.35	39.0	2.55	2.55	2.55	2.55	2.55	2.55	2.55	2.55	37.4
3rd	3.05	3.05	3.05	3.05	3.05	3.05	3.05	3.05	39.1	3.25	3.25	3.25	3.25	3.25	3.25	3.25	3.25	37.5
4th	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75	39.2	3.95	3.95	3.95	3.95	3.95	3.95	3.95	3.95	37.6
5th	4.45	4.45	4.45	4.45	4.45	4.45	4.45	4.45	39.0	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	37.0
6th	5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3	39.0	5.55	5.55	5.55	5.55	5.55	5.55	5.55	5.55	37.0
7th	6.15	6.15	6.15	6.15	6.15	6.15	6.15	6.15	38.9	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4	36.9
8th	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	38.9	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	36.4
9th	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	39.1	8.3	8.3	8.3	8.3	8.3	8.3	8.3	8.3	36.7
10th	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	39.2	9.3	9.3	9.3	9.3	9.3	9.3	9.3	9.3	36.8
11th	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	39.2	10.4	10.4	10.4	10.4	10.4	10.4	10.4	10.4	36.0
12th	11.3	11.3	11.3	11.3	11.3	11.3	11.3	11.3	39.3	11.7	11.7	11.7	11.7	11.7	11.7	11.7	11.7	36.1
13th	12.6	12.6	12.6	12.6	12.6	12.6	12.6	12.6	39.3	13.0	13.0	13.0	13.0	13.0	13.0	13.0	13.0	36.1
14th	13.9	13.9	13.9	13.9	13.9	13.9	13.9	13.9	39.1	14.4	14.4	14.4	14.4	14.4	14.4	14.4	14.4	35.1
15th	15.5	15.5	15.5	15.5	15.5	15.5	15.5	15.5	39.2	16.0	15.5	15.5	15.5	15.5	15.5	15.5	15.5	35.1

Table 4.7z. Pups and mouse mother weight (gram) before start milking at 08.00 am and after milking at 13.00 pm (15% Torbangun leaves extracts-2)

No Day	Pups weight (gram) - Weighed at 08 am								Mother (gram)	Pups weigh (gram) - Weighed at 13 pm								Mother (gram)
	1	2	3	4	5	6	7	8		1	2	3	4	5	6	7	8	
1st	1.66	1.66	1.66	1.66	1.66	1.66	1.66	1.66	38.9	1.86	1.86	1.86	1.86	1.86	1.86	1.86	1.86	37.3
2nd	2.45	2.45	2.45	2.45	2.45	2.45	2.45	2.45	38.8	2.65	2.65	2.65	2.65	2.65	2.65	2.65	2.65	37.2
3rd	3.07	3.07	3.07	3.07	3.07	3.07	3.07	3.07	38.9	3.27	3.27	3.27	3.27	3.27	3.27	3.27	3.27	37.3
4th	3.76	3.76	3.76	3.76	3.76	3.76	3.76	3.76	39.0	3.96	3.96	3.96	3.96	3.96	3.96	3.96	3.96	37.4
5th	4.55	4.55	4.55	4.55	4.55	4.55	4.55	4.55	39.0	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8	37.0
6th	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	38.9	5.65	5.65	5.65	5.65	5.65	5.65	5.65	5.65	37.0
7th	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25	38.8	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	36.8
8th	7.15	7.15	7.15	7.15	7.15	7.15	7.15	7.15	39.0	7.45	7.45	7.45	7.45	7.45	7.45	7.45	7.45	36.6
9th	8.03	8.03	8.03	8.03	8.03	8.03	8.03	8.03	38.9	8.33	8.33	8.33	8.33	8.33	8.33	8.33	8.33	36.5
10th	9.02	9.02	9.02	9.02	9.02	9.02	9.02	9.02	38.8	9.32	9.32	9.32	9.32	9.32	9.32	9.32	9.32	36.4
11th	10.1	10.1	10.1	10.1	10.1	10.1	10.1	10.1	38.9	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	35.7
12th	11.4	11.4	11.4	11.4	11.4	11.4	11.4	11.4	39.0	11.8	11.8	11.8	11.8	11.8	11.8	11.8	11.8	35.8
13th	12.7	12.7	12.7	12.7	12.7	12.7	12.7	12.7	38.9	13.1	13.1	13.1	13.1	13.1	13.1	13.1	13.1	35.7
14th	13.9	13.9	13.9	13.91	13.9	13.9	13.9	13.9	39.3	14.4	14.4	14.4	14.41	14.4	14.4	14.4	14.4	35.3
15th	15.6	15.6	15.6	15.6	15.6	15.6	15.6	15.6	39.2	16.1	16.1	16.1	16.1	16.1	16.1	16.1	16.1	35.2

Table 4.7aa. Pups and mouse mother weight (gram) before start milking at 08.00 am and after milking at 13.00 pm (15% Torbangun leaves extracts-3)

No / Day	Pups weight (gram) - Weighed at 08 am								Mother (gram)	Pups weigh (gram) - Weighed at 13 pm								Mother (gram)
	1	2	3	4	5	6	7	8		1	2	3	4	5	6	7	8	
1st	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	39.3	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9	37.7
2nd	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	39.2	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	37.6
3rd	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	39.2	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	37.6
4th	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	39.3	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	37.7
5th	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	39.3	4.75	4.75	4.75	4.75	4.75	4.75	4.75	4.75	37.3
6th	5.35	5.35	5.35	5.35	5.35	5.35	5.35	5.35	39.1	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	37.1
7th	6.2	6.2	6.2	6.2	6.2	6.2	6.2	6.2	39.2	6.45	6.45	6.45	6.45	6.45	6.45	6.45	6.45	37.2
8th	7.05	7.05	7.05	7.05	7.05	7.05	7.05	7.05	39.4	7.35	7.35	7.35	7.35	7.35	7.35	7.35	7.35	37.0
9th	8.05	8.05	8.05	8.05	8.05	8.05	8.05	8.05	39.3	8.35	8.35	8.35	8.35	8.35	8.35	8.35	8.35	36.9
10th	9.05	9.05	9.05	9.05	9.05	9.05	9.05	9.05	39.3	9.35	9.35	9.35	9.35	9.35	9.35	9.35	9.35	36.9
11th	10.05	10.05	10.05	10.05	10.05	10.05	10.05	10.05	39.2	10.45	10.45	10.45	10.45	10.45	10.45	10.45	10.45	36.0
12th	11.35	11.35	11.35	11.35	11.35	11.35	11.35	11.35	39.4	11.75	11.75	11.75	11.75	11.75	11.75	11.75	11.75	36.2
13th	12.65	12.65	12.65	12.65	12.65	12.65	12.65	12.65	39.3	13.05	13.05	13.05	13.05	13.05	13.05	13.05	13.05	39.1
14th	13.95	13.95	13.95	13.95	13.95	13.95	13.95	13.95	39.2	14.45	14.45	14.45	14.45	14.45	14.45	14.45	14.45	35.2
15th	15.55	15.55	15.55	15.55	15.55	15.55	15.55	15.55	39.3	16.05	16.05	16.05	16.05	16.05	16.05	16.05	16.05	35.3

Table 4.7ab. Pups and mouse mother weight (gram) before start milking at 08.00 am and after milking at 13.00 pm (15% Torbangun leaves extracts-4)

No / Day	Pups weight (gram) - Weighed at 08 am								Mother (gram)	Pups weigh (gram) - Weighed at 13 pm								Mother (gram)
	1	2	3	4	5	6	7	8		1	2	3	4	5	6	7	8	
1st	1.72	1.71	1.73	1.72	1.73	1.71	1.72	1.73	39.0	1.92	1.91	1.93	1.92	1.93	1.91	1.92	1.93	37.4
2nd	2.44	2.41	2.44	2.45	2.44	2.45	2.43	2.44	39.0	2.64	2.61	2.64	2.65	2.64	2.65	2.63	2.64	37.4
3rd	3.12	3.12	3.12	3.12	3.12	3.12	3.12	3.12	38.9	3.32	3.32	3.32	3.32	3.32	3.32	3.32	3.32	37.3
4th	3.85	3.84	3.85	3.84	3.85	3.84	3.83	3.85	39.0	4.05	4.04	4.05	4.04	4.05	4.04	4.03	4.05	37.4
5th	4.51	4.54	4.53	4.53	4.51	4.51	4.53	4.51	39.1	4.76	4.79	4.78	4.78	4.76	4.76	4.78	4.76	37.1
6th	5.37	5.37	5.37	5.37	5.37	5.37	5.37	5.37	39.2	5.62	5.62	5.62	5.62	5.62	5.62	5.62	5.62	37.2
7th	6.23	6.22	6.24	6.22	6.21	6.23	6.22	6.23	39.1	6.48	6.47	6.49	6.47	6.46	6.48	6.47	6.48	37.1
8th	7.15	7.16	7.16	7.16	7.14	7.15	7.13	7.14	39.0	7.45	7.46	7.46	7.46	7.44	7.45	7.43	7.44	36.6
9th	8.07	8.05	8.05	8.07	8.04	8.07	8.07	8.06	39.0	8.37	8.35	8.35	8.37	8.34	8.37	8.36	8.37	36.6
10th	9.06	9.08	9.08	9.07	9.06	9.07	9.07	9.06	38.9	9.36	9.38	9.38	9.37	9.36	9.37	9.37	9.36	36.5
11th	10.06	10.07	10.07	10.05	10.06	10.05	10.07	10.07	39.2	10.46	10.47	10.47	10.45	10.46	10.45	10.47	10.47	36.0
12th	11.45	11.45	11.44	11.46	11.45	11.46	11.47	11.45	39.1	11.85	11.85	11.84	11.86	11.85	11.86	11.87	11.85	35.9
13th	12.65	12.64	12.63	12.63	12.64	12.65	12.66	12.66	39.2	13.05	13.04	13.03	13.03	13.04	13.05	13.06	13.06	36.0
14th	13.94	13.95	13.95	13.93	13.96	13.95	13.95	13.94	39.2	14.44	14.45	14.45	14.43	14.46	14.45	14.45	14.44	35.2
15th	15.65	15.66	15.64	15.64	15.64	15.65	15.63	15.65	39.1	16.15	16.16	16.14	16.14	16.14	16.15	16.13	16.15	35.1

Table 4.7ac. Pups and mouse mother weight (gram) before start milking at 08.00 am and after milking at 13.00 pm (15% Torbangun leaves extracts-5)

No Day	Pups weight (gram) - Weighed at 08 am								Mother (gram)	Pups weigh (gram) - Weighed at 13 pm								Mother (gram)
	1	2	3	4	5	6	7	8		1	2	3	4	5	6	7	8	
1st	1.75	1.75	1.75	1.75	1.75	1.75	1.75	1.75	38.9	1.95	1.95	1.95	1.95	1.95	1.95	1.95	1.95	37.3
2nd	2.45	2.45	2.45	2.45	2.45	2.45	2.45	2.45	38.9	2.65	2.65	2.65	2.65	2.65	2.65	2.65	2.65	37.3
3rd	3.15	3.15	3.15	3.15	3.15	3.15	3.15	3.15	39.0	3.35	3.35	3.35	3.35	3.35	3.35	3.35	3.35	37.4
4th	3.85	3.85	3.85	3.85	3.85	3.85	3.85	3.85	39.1	4.05	4.05	4.05	4.05	4.05	4.05	4.05	4.05	37.5
5th	4.55	4.55	4.55	4.55	4.55	4.55	4.55	4.55	39.0	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8	37.0
6th	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	39.1	5.65	5.65	5.65	5.65	5.65	5.65	5.65	5.65	37.1
7th	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25	39.2	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	37.2
8th	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	39.2	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4	36.8
9th	8.1	8.1	8.1	8.1	8.1	8.1	8.1	8.1	39.1	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	36.7
10th	9.1	9.1	9.1	9.1	9.1	9.1	9.1	9.1	39.0	9.4	9.4	9.4	9.4	9.4	9.4	9.4	9.4	36.6
11th	10.1	10.1	10.1	10.1	10.1	10.1	10.1	10.1	39.2	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	36.0
12th	11.4	11.4	11.4	11.4	11.4	11.4	11.4	11.4	39.1	11.8	11.8	11.8	11.8	11.8	11.8	11.8	11.8	35.9
13th	12.7	12.7	12.7	12.7	12.7	12.7	12.7	12.7	39.2	13.1	13.1	13.1	13.1	13.1	13.1	13.1	13.1	36.0
14th	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	39.2	14.5	14.5	14.5	14.5	14.5	14.5	14.5	14.5	35.2
15th	15.6	15.6	15.6	15.6	15.6	15.6	15.6	15.6	39.1	16.1	16.1	16.1	16.1	16.1	16.1	16.1	16.1	35.1

Table 4.7ad. Pups and mouse mother weight (gram) before start milking at 08.00 am and after milking at 13.00 pm (15% Torbangun leaves extracts-6)

No / Day	Pups weight (gram) - Weighed at 08 am								Mother (gram)	Pups weigh (gram) - Weighed at 13 pm								Mother (gram)
	1	2	3	4	5	6	7	8		1	2	3	4	5	6	7	8	
1st	1.76	1.76	1.77	1.76	1.75	1.76	1.75	1.76	38.9	1.96	1.96	1.97	1.96	1.95	1.96	1.95	1.96	37.3
2nd	2.44	2.43	2.44	2.43	2.42	2.44	2.42	2.44	39.0	2.64	2.63	2.64	2.63	2.62	2.64	2.62	2.64	37.4
3rd	3.13	3.12	3.13	3.14	3.12	3.13	3.14	3.14	39.0	3.33	3.32	3.33	3.34	3.32	3.33	3.34	3.34	37.4
4th	3.87	3.86	3.85	3.86	3.87	3.86	3.87	3.88	38.9	4.07	4.06	4.05	4.06	4.07	4.06	4.07	4.08	37.3
5th	4.65	4.64	4.64	4.65	4.66	4.65	4.65	4.66	39.1	4.9	4.89	4.89	4.9	4.91	4.9	4.9	4.91	37.1
6th	5.44	5.44	5.45	5.44	5.45	5.44	5.46	5.46	39.2	5.69	5.69	5.70	5.69	5.70	5.69	5.71	5.71	37.2
7th	6.28	6.29	6.29	6.29	6.28	6.27	6.27	6.28	39.1	6.53	6.54	6.54	6.54	6.53	6.52	6.52	6.53	37.1
8th	7.2	7.23	7.23	7.22	7.23	7.24	7.24	7.24	39.0	7.5	7.53	7.53	7.52	7.53	7.54	7.54	7.54	36.6
9th	8.15	8.14	8.14	8.16	8.15	8.16	8.15	8.16	39.0	8.45	8.44	8.44	8.46	8.45	8.46	8.45	8.46	36.6
10th	9.12	9.13	9.12	9.12	9.14	9.14	9.12	9.13	38.9	9.42	9.43	9.42	9.42	9.44	9.44	9.42	9.43	36.5
11th	10.2	10.4	10.2	10.3	10.3	10.1	10.2	10.4	38.9	10.6	10.8	10.6	10.7	10.7	10.5	10.6	10.8	35.7
12th	11.5	11.4	11.4	11.5	11.3	11.3	11.5	11.5	39.1	11.9	11.8	11.8	11.9	11.7	11.7	11.9	11.9	35.9
13th	12.8	12.7	12.7	12.8	12.9	12.9	12.8	12.7	39.2	13.2	13.1	13.1	13.2	13.3	13.3	13.2	13.1	35.9
14th	14.1	14.2	14.1	14.1	14.2	14.1	14.3	14.3	39.2	14.6	14.7	14.6	14.6	14.7	14.6	14.8	14.6	35.3
15th	15.7	15.8	15.8	15.8	15.6	15.7	15.7	156	39.1	16.2	16.3	16.3	16.3	16.1	16.2	16.2	16.1	35.1

Table 4.7ae. Pups and mouse mother weight (gram) before start milking at 08.00 am and after milking at 13.00 pm (20% Torbangun leaves extracts-1)

No / Day	Pups weight (gram) - Weighed at 08 am								Mother (gram)	Pups weigh (gram) - Weighed at 13 pm								Mother (gram)
	1	2	3	4	5	6	7	8		1	2	3	4	5	6	7	8	
1st	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	39.5	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9	37.9
2nd	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	39.5	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	37.9
3rd	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	39.6	3.35	3.35	3.35	3.35	3.35	3.35	3.35	3.35	37.6
4th	3.95	3.95	3.95	3.95	3.95	3.95	3.95	3.95	39.5	4.15	4.15	4.15	4.15	4.15	4.15	4.15	4.15	37.5
5th	4.75	4.75	4.75	4.75	4.75	4.75	4.75	4.75	39.4	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	37.4
6th	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	39.4	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	37.0
7th	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	39.5	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9	37.1
8th	7.6	7.6	7.6	7.6	7.6	7.6	7.6	7.6	39.5	7.9	7.9	7.9	7.9	7.9	7.9	7.9	7.9	37.1
9th	8.6	8.6	8.6	8.6	8.6	8.6	8.6	8.6	39.6	8.9	8.9	8.9	8.9	8.9	8.9	8.9	8.9	37.2
10th	9.6	9.6	9.6	9.6	9.6	9.6	9.6	9.6	39.6	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	36.4
11th	10.9	10.9	10.9	10.9	10.9	10.9	10.9	10.9	39.5	11.3	11.3	11.3	11.3	11.3	11.3	11.3	11.3	36.3
12th	12.2	12.2	12.2	12.2	12.2	12.2	12.2	12.2	39.4	12.6	12.6	12.6	12.6	12.6	12.6	12.6	12.6	36.2
13th	13.5	13.5	13.5	13.5	13.5	13.5	13.5	13.5	39.3	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	35.3
14th	15.1	15.1	15.1	15.1	15.1	15.1	15.1	15.1	39.5	15.6	15.6	15.6	15.6	15.6	15.6	15.6	15.6	35.5
15th	16.7	16.7	16.7	16.7	16.7	16.7	16.7	16.7	39.6	17.3	17.3	17.3	17.3	17.3	17.3	17.3	17.3	33.6

Table 4.7af. Pups and mouse mother weight (gram) before start milking at 08.00 am and after milking at 13.00 pm (20% Torbangun leaves extracts-2)

No / Day	Pups weight (gram) - Weighed at 08 am								Mother (gram)	Pups weigh (gram) - Weighed at 13 pm								Mother (gram)
	1	2	3	4	5	6	7	8		1	2	3	4	5	6	7	8	
1st	1.74	1.74	1.75	1.75	1.76	1.74	1.73	1.74	39.2	1.94	1.94	1.95	1.95	1.96	1.94	1.93	1.94	37.6
2nd	2.43	2.44	2.43	2.42	2.44	2.43	2.42	2.43	39.3	2.63	2.64	2.64	2.62	2.64	2.63	2.62	2.63	37.7
3rd	3.14	3.13	3.13	3.14	3.13	3.13	3.14	3.12	39.3	3.39	3.38	3.38	3.39	3.38	3.38	3.39	3.37	37.3
4th	3.97	3.98	3.98	3.97	3.97	3.98	3.96	3.97	39.4	4.17	4.18	4.18	4.17	4.17	4.18	4.16	4.17	37.4
5th	4.78	4.79	4.79	4.78	4.77	4.78	4.77	4.77	39.3	5.03	5.04	5.04	5.03	5.02	5.03	5.02	5.02	37.3
6th	5.63	5.64	5.63	5.64	5.65	5.65	5.63	5.64	39.5	5.93	5.94	5.93	5.94	5.95	5.95	5.93	5.94	37.1
7th	6.65	6.66	6.67	6.65	6.65	6.66	6.67	6.65	39.6	6.95	6.96	6.97	6.95	6.95	6.96	6.97	6.95	37.2
8th	7.67	7.69	7.68	7.66	7.66	7.69	7.67	7.67	39.4	7.97	7.99	7.98	7.96	7.96	7.99	7.97	7.97	37.0
9th	8.63	8.64	8.65	8.64	8.65	8.63	8.63	8.64	39.4	8.93	8.94	8.95	8.94	8.95	8.93	8.93	8.94	37.0
10th	9.7	9.6	9.7	9.7	9.8	9.6	9.7	9.8	39.5	10.1	10.0	10.1	10.1	10.2	10.0	10.1	10.2	36.2
11th	10.9	10.8	10.9	10.8	10.8	10.9	10.7	10.9	39.3	11.3	11.2	11.3	11.2	11.3	11.3	11.1	11.3	36.1
12th	12.4	12.4	12.3	12.5	12.4	12.3	12.3	12.5	39.5	12.8	12.8	12.7	12.9	12.8	12.7	12.7	12.9	36.1
13th	13.6	13.5	13.5	13.6	13.7	13.6	13.6	13.7	39.4	14.1	14.0	14.0	14.1	14.2	14.1	14.1	14.2	35.4
14th	15.2	15.2	15.3	15.4	15.3	15.4	15.2	15.3	39.5	15.7	15.7	15.8	15.9	15.8	15.9	15.7	15.8	35.5
15th	16.8	16.9	16.7	16.7	16.8	16.7	16.7	16.9	39.6	17.4	17.6	17.3	17.3	17.4	17.3	17.3	17.5	33.6

Table 4.7ag. Pups and mouse mother weight (gram) before start milking at 08.00 am and after milking at 13.00 pm (20% Torbangun leaves extracts-3)

No / Day	Pups weight (gram) - Weighed at 08 am								Mother (gram)	Pups weigh (gram) - Weighed at 13 pm								Mother (gram)
	1	2	3	4	5	6	7	8		1	2	3	4	5	6	7	8	
1st	1.75	1.75	1.75	1.75	1.75	1.75	1.75	1.75	39.5	1.95	1.95	1.95	1.95	1.95	1.95	1.95	1.95	37.9
2nd	2.45	2.45	2.45	2.45	2.45	2.45	2.45	2.45	39.6	2.65	2.65	2.65	2.65	2.65	2.65	2.65	2.65	38.0
3rd	3.15	3.15	3.15	3.15	3.15	3.15	3.15	3.15	39.5	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	37.5
4th	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	39.5	4.25	4.25	4.25	4.25	4.25	4.25	4.25	4.25	37.5
5th	4.85	4.85	4.85	4.85	4.85	4.85	4.85	4.85	39.4	5.1	5.1	5.1	5.1	5.1	5.1	5.1	5.1	37.4
6th	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	39.6	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	37.2
7th	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7	39.6	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	37.2
8th	7.7	7.7	7.7	7.7	7.7	7.7	7.7	7.7	39.7	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	37.3
9th	8.7	8.7	8.7	8.7	8.7	8.7	8.7	8.7	39.5	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	37.1
10th	9.7	9.7	9.7	9.7	9.7	9.7	9.7	9.7	39.6	10.1	10.1	10.1	10.1	10.1	10.1	10.1	10.1	36.4
11th	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	39.6	11.4	11.4	11.4	11.4	11.4	11.4	11.4	11.4	36.4
12th	12.3	12.3	12.3	12.3	12.3	12.3	12.3	12.3	39.5	12.7	12.7	12.7	12.7	12.7	12.7	12.7	12.7	36.3
13th	13.6	13.6	13.6	13.6	13.6	13.6	13.6	13.6	39.5	14.1	14.1	14.1	14.1	14.1	14.1	14.1	14.1	35.5
14th	15.2	15.2	15.2	15.2	15.2	15.2	15.2	15.2	39.7	15.7	15.7	15.7	15.7	15.7	15.7	15.7	15.7	35.7
15th	16.8	16.8	16.8	16.8	16.8	16.8	16.8	16.8	39.7	17.4	17.4	17.4	17.4	17.4	17.4	17.4	17.4	33.7

Table 4.7ah. Pups and mouse mother weight (gram) before start milking at 08.00 am and after milking at 13.00 pm (20% Torbangun leaves extracts-4)

No / Day	Pups weight (gram) - Weighed at 08 am								Mother (gram)	Pups weigh (gram) - Weighed at 13 pm								Mother (gram)
	1	2	3	4	5	6	7	8		1	2	3	4	5	6	7	8	
1st	1.77	1.78	1.78	1.77	1.79	1.79	1.78	1.77	39.3	1.97	1.98	1.98	1.97	1.98	1.98	1.98	1.97	37.7
2nd	2.56	2.56	2.57	2.57	2.56	2.56	2.58	2.58	39.3	2.76	2.76	2.77	2.77	2.76	2.76	2.78	2.78	37.7
3rd	3.25	3.25	3.27	3.27	3.26	3.26	3.25	3.26	39.2	3.5	3.5	3.52	3.52	3.51	3.51	3.5	3.51	37.2
4th	4.04	4.05	4.04	4.05	4.05	4.04	4.06	4.05	39.4	4.29	4.3	4.3	4.29	4.31	4.29	4.31	4.30	37.4
5th	4.87	4.88	4.88	4.87	4.86	4.87	4.88	4.88	39.4	5.12	5.13	5.13	5.12	5.11	5.12	5.13	5.13	37.4
6th	5.74	5.74	5.76	5.75	5.75	5.76	5.75	5.74	39.5	6.04	6.04	6.06	6.05	6.05	6.06	6.05	6.04	37.1
7th	6.72	6.73	6.73	6.74	6.73	6.72	6.74	6.73	39.4	7.02	7.03	7.03	7.04	7.03	7.02	7.04	7.03	37.0
8th	7.73	7.74	7.75	7.75	7.74	7.73	7.74	7.74	39.5	8.03	8.04	8.05	8.05	8.04	8.03	8.04	8.04	37.1
9th	8.78	8.79	8.79	8.77	8.77	8.78	8.79	8.79	39.4	9.08	9.09	9.09	9.07	9.07	9.08	9.09	9.09	27.0
10th	9.9	9.8	9.9	9.7	9.8	9.9	9.7	9.7	39.5	10.3	10.2	10.3	10.1	10.2	10.3	10.1	10.1	36.3
11th	11.1	11.2	11.2	11.3	11.4	11.1	11.3	11.2	39.5	11.5	11.6	11.6	11.7	11.8	11.5	11.7	11.6	36.3
12th	12.4	12.5	12.4	12.3	12.5	12.4	125	12.4	39.4	12.8	12.9	12.8	12.7	12.9	12.8	12.9	12.8	36.2
13th	13.5	13.6	13.7	13.6	13.6	13.5	13.5	13.6	39.6	14.0	14.1	14.2	14.1	14.1	14.0	14.0	14.1	35.6
14th	15.3	15.4	15.4	15.3	15.2	15.3	15.2	15.3	39.6	15.8	15.9	15.9	15.8	15.7	15.8	15.7	15.8	35.6
15th	16.9	16.8	16.9	16.8	16.7	16.9	16.8	16.9	39.5	17.5	17.4	17.5	17.4	17.3	17.5	17.4	17.5	33.5

Table 4.7ai. Pups and mouse mother weight (gram) before start milking at 08.00 am and after milking at 13.00 pm (20% Torbangun leaves extracts-5)

No / Day	Pups weight (gram) - Weighed at 08 am								Mother (gram)	Pups weigh (gram) - Weighed at 13 pm								Mother (gram)
	1	2	3	4	5	6	7	8		1	2	3	4	5	6	7	8	
1st	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	39.4	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	37.8
2nd	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	39.3	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	37.7
3rd	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	39.4	3.45	3.45	3.45	3.45	3.45	3.45	3.45	3.45	37.4
4th	4.05	4.05	4.05	4.05	4.05	4.05	4.05	4.05	39.4	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3	37.4
5th	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9	39.5	5.15	5.15	5.15	5.15	5.15	5.15	5.15	5.15	37.5
6th	5.75	5.75	5.75	5.75	5.75	5.75	5.75	5.75	39.3	6.05	6.05	6.05	6.05	6.05	6.05	6.05	6.05	36.9
7th	6.75	6.75	6.75	6.75	6.75	6.75	6.75	6.75	39.4	7.05	7.05	7.05	7.05	7.05	7.05	7.05	7.05	37.0
8th	7.75	7.75	7.75	7.75	7.75	7.75	7.75	7.75	39.6	8.05	8.05	8.05	8.05	8.05	8.05	8.05	8.05	37.2
9th	8.75	8.75	8.75	8.75	8.75	8.75	8.75	8.75	39.5	9.05	9.05	9.05	9.05	9.05	9.05	9.05	9.05	37.1
10th	9.75	9.75	9.75	9.75	9.75	9.75	9.75	9.75	39.5	10.15	10.15	10.15	10.15	10.15	10.15	10.15	10.15	36.3
11th	11.05	11.05	11.05	11.05	11.05	11.05	11.05	11.05	39.4	11.45	11.45	11.45	11.45	11.45	11.45	11.45	11.45	36.2
12th	12.35	12.35	12.35	12.35	12.35	12.35	12.35	12.35	39.5	12.75	12.75	12.75	12.75	12.75	12.75	12.75	12.75	36.3
13th	13.65	13.65	13.65	13.65	13.65	13.65	13.65	13.65	39.6	14.15	14.15	14.15	14.15	14.15	14.15	14.15	14.15	35.6
14th	15.25	15.25	15.25	15.25	15.25	15.25	15.25	15.25	39.6	15.75	15.75	15.75	15.75	15.75	15.75	15.75	15.75	35.6
15th	16.85	16.85	16.85	16.85	16.85	16.85	16.85	16.85	39.6	17.45	17.45	17.45	17.45	17.45	17.45	17.45	17.45	33.6

Table 4.7aj. Pups and mouse mother weight (gram) before start milking at 08.00 am and after milking at 13.00 pm (20% Torbangun leaves extracts-6)

No / Day	Pups weight (gram) - Weighed at 08 am								Mother (gram)	Pups weigh (gram) - Weighed at 13 pm								Mother (gram)
	1	2	3	4	5	6	7	8		1	2	3	4	5	6	7	8	
1st	1.84	1.84	1.85	1.86	1.84	1.85	1.84	1.85	39.6	2.04	2.04	2.05	2.06	2.04	2.05	2.04	2.05	38.0
2nd	2.53	2.55	2.55	2.54	2.53	2.54	2.53	2.53	39.6	2.73	2.75	2.75	2.74	2.73	2.74	2.73	2.73	38.0
3rd	3.25	3.25	3.24	3.26	3.24	3.25	3.26	3.25	39.5	3.5	3.5	3.49	3.51	3.49	3.5	3.51	3.5	37.5
4th	4.07	4.08	4.06	4.07	4.08	4.06	4.07	4.08	39.4	4.32	4.33	4.31	4.32	4.33	4.31	4.32	4.33	37.4
5th	4.92	4.93	4.92	4.92	4.93	4.92	4.94	4.92	39.5	5.17	5.18	5.17	5.17	5.18	5.17	5.19	5.17	37.5
6th	5.74	5.75	5.74	5.73	5.75	5.74	5.73	5.73	39.6	6.04	6.05	6.04	6.03	6.05	6.04	6.03	6.03	37.2
7th	6.76	6.76	6.75	6.77	6.75	6.76	6.77	6.77	39.5	7.06	7.06	7.05	7.07	7.05	7.06	7.07	7.07	37.1
8th	7.75	7.74	7.73	7.75	7.74	7.73	7.76	7.75	39.5	8.05	8.04	8.03	8.05	8.04	8.03	8.06	8.05	37.1
9th	8.76	8.78	8.76	8.77	8.76	8.76	8.77	8.76	39.7	9.06	9.08	9.06	9.07	9.06	9.06	9.07	9.06	37.3
10th	9.78	9.77	9.78	9.79	9.77	9.78	9.76	9.78	39.6	10.18	10.17	10.18	10.19	10.17	10.18	10.16	10.18	36.4
11th	11.06	11.05	11.06	11.07	11.06	11.05	11.06	11.07	39.6	11.46	11.45	11.46	11.47	11.46	11.45	11.46	11.47	36.4
12th	12.4	12.4	12.5	12.4	12.4	12.6	12.4	12.3	39.4	12.75	12.75	12.85	12.75	12.75	12.95	12.75	12.65	36.2
13th	13.61	13.63	13.61	13.63	13.62	13.63	13.64	13.63	39.6	14.11	14.13	14.11	14.13	14.12	14.13	14.14	14.13	35.6
14th	15.27	15.28	15.28	15.27	15.26	15.26	15.28	15.27	39.7	15.77	15.78	15.78	15.77	15.76	15.76	15.78	15.77	35.7
15th	16.83	16.84	16.85	16.83	16.84	16.83	16.85	16.84	39.7	17.43	17.44	17.45	17.43	17.44	17.43	17.45	17.44	33.7

The difference in weight and 08.00 until at jam13.00 thought to reflect the amount of milk taken or consumed by the puppy milk or milk provided by the mother to the pup, can be seen in (Table 4.8a to Table 4.8f). Summary of mean, standard deviation and standard error of mean data in mice control and treatment on six mice per group were breastfeeding and each mouse had eight pups, see in Table 4.8g and 4.9)

Table 4.8a. Mean, Standard Deviation and Standard Error of Mean Data in Mice Control

Day	Difference = weight of milk consumed or Weight of milk (g)								
	M1	M2	M3	M4	M5	M6	Mean	SD	SEM
1st	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.001	0.001
2nd	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.000	0.000
3rd	0.10	0.10	0.10	0.10	0.30	0.10	0.13	0.082	0.033
4th	0.10	0.10	0.30	0.10	0.04	0.30	0.16	0.113	0.046
5th	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.000	0.000
6th	0.15	0.20	0.15	0.15	0.15	0.15	0.16	0.020	0.008
7th	0.15	0.15	0.15	0.15	0.15	0.14	0.15	0.003	0.001
8th	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.000	0.000
9th	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.000	0.000
10th	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.000	0.000
11th	0.20	0.20	0.20	0.10	0.20	0.20	0.18	0.041	0.017
12th	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.000	0.000
13th	-0.71	0.25	0.25	0.25	0.25	0.35	0.11	0.403	0.165
14th	0.25	0.30	0.30	0.30	0.30	0.30	0.29	0.020	0.008
15th	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.000	0.000

Table 4.8b. Mean, Standard Deviation and Standard Error of Mean Data from Mice Treatment 1% Torbangun Leave Extract

Day	Difference = weight of milk consumed or Weight of milk (g)								
	M1	M2	M3	M4	M5	M6	Mean	SD	SEM
1st	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.001	0.000
2nd	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.000	0.000
3rd	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.001	0.000
4th	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.000	0.000
5th	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.000	0.000
6th	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.000	0.000
7th	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.001	0.000
8th	0.20	0.20	0.20	0.20	0.20	0.19	0.20	0.005	0.002
9th	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.000	0.000
10th	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.000	0.000
11th	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.000	0.000
12th	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.000	0.000
13th	0.30	0.30	0.25	0.30	0.30	0.30	0.29	0.020	0.008
14th	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.000	0.000
15th	0.30	0.30	0.30	0.30	0.24	0.24	0.28	0.029	0.012

Table 4.8c. Mean, Standard Deviation and Standard Error of Mean Data from Mice Treatment 5% Torbangun Leave Extract

Day	Difference = weight of milk consumed or Weight of milk (g)								
	M1	M2	M3	M4	M5	M6	Mean	SD	SEM
1st	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.001	0.000
2nd	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.000	0.000
3rd	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.000	0.000
4th	0.15	0.15	-0.15	-0.15	0.15	0.15	0.05	0.155	0.063
5th	0.20	0.20	0.20	0.20	0.20	0.21	0.20	0.003	0.001
6th	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.000	0.000
7th	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.000	0.000
8th	0.25	0.24	0.25	0.25	0.25	0.25	0.25	0.004	0.002
9th	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.001	0.000
10th	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.000	0.000
11th	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.000	0.000
12th	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.000	0.000
13th	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.000	0.000
14th	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.000	0.000
15th	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.001	0.001

Table 4.8d. Mean, Standard Deviation and Standard Error of Mean Data from Mice Treatment 10% Torbangun Leave Extract

Day	Difference = weight of milk consumed or Weight of milk (g)								
	M1	M2	M3	M4	M5	M6	Mean	SD	SEM
1st	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.000	0.000
2nd	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.000	0.000
3rd	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.001	0.000
4th	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.000	0.000
5th	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.000	0.000
6th	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.000	0.000
7th	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.000	0.000
8th	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.000	0.000
9th	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.000	0.000
10th	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.000	0.000
11th	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.000	0.000
12th	0.30	0.30	0.30	0.31	0.30	0.30	0.30	0.004	0.001
13th	0.30	0.29	0.30	0.30	0.30	0.30	0.30	0.004	0.002
14th	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.000	0.000
15th	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.000	0.000

Table 4.8e. Mean, Standard Deviation and Standard Error of Mean Data from Mice Treatment 15% Torbangun Leave Extract

Day	Difference = weight of milk consumed or Weight of milk (g)								
	M1	M2	M3	M4	M5	M6	Mean	SD	SEM
1st	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.000	0.000
2nd	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.000	0.000
3rd	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.000	0.000
4th	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.000	0.000
5th	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.000	0.000
6th	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.000	0.000
7th	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.000	0.000
8th	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.000	0.000
9th	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.000	0.000
10th	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.000	0.000
11th	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.000	0.000
12th	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.000	0.000
13th	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.000	0.000
14th	0.50	0.50	0.50	0.50	0.50	0.48	0.50	0.010	0.004
15th	0.06	0.50	0.50	0.50	0.50	0.50	0.43	0.179	0.073

Table 4.8f. Mean, Standard Deviation and Standard Error of Mean Data from Mice Treatment 20% Torbangun Leave Extract

Day	Difference = weight of milk consumed or Weight of milk (g)								
	M1	M2	M3	M4	M5	M6	Mean	SD	SEM
1st	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.001	0.000
2nd	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.001	0.000
3rd	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.000	0.000
4th	0.20	0.20	0.25	0.25	0.25	0.25	0.23	0.026	0.011
5th	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.000	0.000
6th	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.000	0.000
7th	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.000	0.000
8th	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.000	0.000
9th	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.000	0.000
10th	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.000	0.000
11th	0.40	0.41	0.40	0.40	0.40	0.40	0.40	0.005	0.002
12th	0.40	0.40	0.40	0.40	0.40	0.35	0.39	0.020	0.008
13th	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.000	0.000
14th	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.000	0.000
15th	0.60	0.61	0.60	0.60	0.60	0.60	0.60	0.005	0.002

Table 4.8g. Mean data with standard deviation of pups milk consumed.

Day	Control	1% Torbangun Leave Extract	5% Torbangun Leave Extract	10% Torbangun Leave Extract	15% Torbangun Leave Extract	20% Torbangun Leave Extract
1st	0.10 ± 0.001	0.10±0.000	0.15±0.000	0.15±0.000	0.20±0.000	0.20±0.000
2nd	0.10 ± 0.000	0.10±0.000	0.15±0.000	0.15±0.000	0.20±0.000	0.20±0.000
3rd	0.13 ±0.033	0.10±0.000	0.15±0.000	0.15±0.000	0.20±0.000	0.25±0.000
4th	0.16±0.046	0.15±0.000	0.05±0.063	0.20±0.000	0.20±0.000	0.23±0.011
5th	0.15±0.000	0.15±0.000	0.20±0.001	0.20±0.000	0.25±0.000	0.25±0.000
6th	0.16±0.008	0.15±0.000	0.20±0.000	0.20±0.000	0.25±0.000	0.30±0.000
7th	0.15±0.001	0.20±0.000	0.20±0.000	0.25±0.000	0.25±0.000	0.30±0.000
8th	0.20±0.000	0.20±0.002	0.25±0.002	0.25±0.000	0.30±0.000	0.30±0.000
9th	0.20±0.000	0.20±0.000	0.25±0.000	0.25±0.000	0.30±0.000	0.30±0.000
10th	0.20±0.000	0.20±0.000	0.25±0.000	0.30±0.000	0.30±0.000	0.40±0.000
11th	0.18±0.017	0.25±0.000	0.30±0.000	0.30±0.000	0.40±0.000	0.40±0.002
12th	0.25±0.000	0.25±0.000	0.30±0.000	0.30±0.001	0.40±0.000	0.39±0.008
13th	0.11±0.165	0.29±0.008	0.30±0.000	0.30±0.002	0.40±0.000	0.50±0.000
14th	0.29±0.008	0.30±0.000	0.35±0.000	0.40±0.000	0.50±0.004	0.50±0.000
15th	0.30±0.000	0.28±0.012	0.35±0.001	0.40±0.000	0.43±0.073	0.60±0.002

Table 4.9. Summary of mean, standard deviation and standard error of mean data in mice control and mice treatment, in clinical trial.

Day	0%			1%				5%		10%			15%			20%		
	Mean	SD	SEM	Mean	SD	SEM	Mean	SD	SEM	Mean	SD	SEM	Mean	SD	SEM	Mean	SD	SEM
1st	0.10	0.001	0.001	0.10	0.001	0.000	0.15	0.001	0.000	0.15	0.000	0.000	0.20	0.000	0.000	0.20	0.001	0.000
2nd	0.10	0.000	0.000	0.10	0.000	0.000	0.15	0.000	0.000	0.15	0.000	0.000	0.20	0.000	0.000	0.20	0.001	0.000
3rd	0.13	0.082	0.033	0.10	0.001	0.000	0.15	0.000	0.000	0.15	0.001	0.000	0.20	0.000	0.000	0.25	0.000	0.000
4th	0.16	0.113	0.046	0.15	0.000	0.000	0.05	0.155	0.063	0.20	0.000	0.000	0.20	0.000	0.000	0.23	0.026	0.011
5th	0.15	0.000	0.000	0.15	0.000	0.000	0.20	0.003	0.001	0.20	0.000	0.000	0.25	0.000	0.000	0.25	0.000	0.000
6th	0.16	0.020	0.008	0.15	0.000	0.000	0.20	0.000	0.000	0.20	0.000	0.000	0.25	0.000	0.000	0.30	0.000	0.000
7th	0.15	0.003	0.001	0.20	0.001	0.000	0.20	0.000	0.000	0.25	0.000	0.000	0.25	0.000	0.000	0.30	0.000	0.000
8th	0.20	0.000	0.000	0.20	0.005	0.002	0.25	0.004	0.002	0.25	0.000	0.000	0.30	0.000	0.000	0.30	0.000	0.000
9th	0.20	0.000	0.000	0.20	0.000	0.000	0.25	0.001	0.000	0.25	0.000	0.000	0.30	0.000	0.000	0.30	0.000	0.000
10th	0.20	0.000	0.000	0.20	0.000	0.000	0.25	0.000	0.000	0.30	0.000	0.000	0.30	0.000	0.000	0.40	0.000	0.000
11th	0.18	0.041	0.017	0.25	0.000	0.000	0.30	0.000	0.000	0.30	0.000	0.000	0.40	0.000	0.000	0.40	0.005	0.002
12th	0.25	0.000	0.000	0.25	0.000	0.000	0.30	0.000	0.000	0.30	0.004	0.001	0.40	0.000	0.000	0.39	0.020	0.008
13th	0.11	0.403	0.165	0.29	0.020	0.008	0.30	0.000	0.000	0.30	0.004	0.002	0.40	0.000	0.000	0.50	0.000	0.000
14th	0.29	0.020	0.008	0.30	0.000	0.000	0.35	0.000	0.000	0.40	0.000	0.000	0.50	0.010	0.004	0.50	0.000	0.000
15th	0.30	0.000	0.000	0.28	0.029	0.012	0.35	0.001	0.001	0.40	0.000	0.000	0.43	0.179	0.073	0.60	0.005	0.002

The statistic calculated to take the average milk consumed, standard deviation and standard error of mean from each treatment group. Calculation of data between the control group with the combined treatment group 1%, 5%. 10%, 15% and 20% using SPSS programme. The result of statistic calculation with Table t, df = 5 ; P <0.05 and F = 7514 as follow:

1. The comparisons value between control and 1% Torbangun leave extract = 0.993 that mean > 0.05 and not significant.
2. The comparisons value between control and 5% Torbangun leave extract = 0.338 that mean > 0.05 and not significant.
3. The comparisons value s between control and 10% Torbangun leave extract = 0.127 that mean > 0.05 and not significant.
4. The comparisons value between control and 15% Torbangun leave extract = 0.001 that mean < 0.05 and significant.
5. The comparisons value between control and 20% Torbangun leave extract = 0.001 that mean < 0.05 and significant.
6. The ANOVA value of between groups is 0.000 that mean < 0.05and significant.

Statistic calculation by SPSS program

Descriptive

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1.00	15	1833	.06986	.01804	.1446	.2220	10	30
2.00	15	.1967	07188	01856	1569	.2365	10	30
3.00	15	2367	07188	01856	.1969	.2765	15	35
4.00	15	2533	.08121	.02097	.2084	.2983	15	40
5.00	15	3100	10556	.02726	2515	3685	20	50
6.00	15	.3433	11932	.03081	.2773	.4094	20	60
Total	90	.2539	10365	.01093	.2322	.2756	10	60

Test of Homogeneity of Variances

VAR00001

Levene Statistic	df1	df2	Sig.
2.069	5	84	077

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between	.295	5	.059	7.514	.000
Groups	.661	84	.008		
Within Groups	.956	89			
Total					

Post Hoc Tests

Multiple Comparisons

VAR00001

Dunnett t (2-sided)[a]

(I) VA00002	(J) VA00002	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
2.00	1.00	.01333	.03238	.993	-.0696	.0963
3.00	1.00	.05333	.03238	.338	-.0296	.1363
4.00	1.00	.07000	.03238	.127	-.0130	.1530
5.00	1.00	.12667*	.03238	.001	.0437	.2096
6.00	1.00	.16000*	.03238	.000	.0770	.2430

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

*. The mean difference is significant at the 0.05 level.

Clinical trial statistic calculation by ANOVA.

1. Mice control

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
VAR00001	6	.1000	.00000	.00000
VAR00002	6	.1000	.00000	.00000
VAR00003	6	.1333	.08165	.03333
VAR00004	6	.1567	.11343	.04631
VAR00005	6	.1500	.00000	.00000
VAR00006	6	.1583	.02041	.00833
VAR00007	6	.1483	.00408	.00167
VAR00008	6	.2000	.00000	.00000
VAR00009	6	.2000	.00000	.00000
VAR00010	6	.2000	.00000	.00000
VAR00011	6	.1833	.04082	.01667
VAR00012	6	.2500	.00000[a]	.00000
VAR00013	6	.1067	.40208	.16415
VAR00014	6	.2917	.02041	.00833
VAR00015	6	.3000	.00000	.00000

t cannot be computed because the standard deviation is 0.

One-Sample Test

	Test Value = 0					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
VAR00001	5.779E16	5	.000	.10000	.1000	.1000
VAR00002	5.779E16	5	.000	.10000	.1000	.1000
VAR00003	4.000	5	.010	.13333	.0476	.2190
VAR00004	3.383	5	.020	.15667	.0376	.2757
VAR00005	8.661E16	5	.000	.15000	.1500	.1500
VAR00006	19.000	5	.000	.15833	.1369	.1798
VAR00007	89.000	5	.000	.14833	.1440	.1526
VAR00008	5.779E16	5	.000	.20000	.2000	.2000
VAR00009	5.779E16	5	.000	.20000	.2000	.2000
VAR00010	5.779E16	5	.000	.20000	.2000	.2000
VAR00011	11.000	5	.000	.18333	.1405	.2262
VAR00013	.650	5	.544	.10667	-.3153	.5286
VAR00014	35.000	5	.000	.29167	.2702	.3131
VAR00015	8.661E16	5	.000	.30000	.3000	.3000

2. Mice Treatment 1%

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
VAR00001	6	.1000	.00000	.00000
VAR00002	6	.1000	.00000	.00000
VAR00003	6	.1000	.00000	.00000
VAR00004	6	.1500	.00000	.00000
VAR00005	6	.1500	.00000	.00000
VAR00006	6	.1500	.00000	.00000
VAR00007	6	.2000	.00000	.00000
VAR00008	6	.1983	.00408	.00167
VAR00009	6	.2000	.00000	.00000
VAR00010	6	.2000	.00000	.00000
VAR00011	6	.2500	.00000[a]	.00000
VAR00012	6	.2500	.00000[a]	.00000
VAR00013	6	.2917	.02041	.00833
VAR00014	6	.3000	.00000	.00000
VAR00015	6	.2800	.03098	.01265

a. t cannot be computed because the standard deviation is 0.

One-Sample Test

	Test Value = 0					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
VAR00001	5.779E16	5	.000	.10000	.1000	.1000
VAR00002	5.779E16	5	.000	.10000	.1000	.1000
VAR00003	5.779E16	5	.000	.10000	.1000	.1000
VAR00004	8.661E16	5	.000	.15000	.1500	.1500
VAR00005	8.661E16	5	.000	.15000	.1500	.1500
VAR00006	8.661E16	5	.000	.15000	.1500	.1500
VAR00007	5.779E16	5	.000	.20000	.2000	.2000
VAR00008	119.000	5	.000	.19833	.1940	.2026
VAR00009	5.779E16	5	.000	.20000	.2000	.2000
VAR00010	5.779E16	5	.000	.20000	.2000	.2000
VAR00013	35.000	5	.000	.29167	.2702	.3131
VAR00014	8.661E16	5	.000	.30000	.3000	.3000
VAR00015	22.136	5	.000	.28000	.2475	.3125

3. Mice Treatment 5%

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
VAR00001	6	.1500	.00000	.00000
VAR00002	6	.1500	.00000	.00000
VAR00003	6	.1500	.00000	.00000
VAR00004	6	.0500	.15492	.06325
VAR00005	6	.2017	.00408	.00167
VAR00006	6	.2000	.00000	.00000
VAR00007	6	.2000	.00000	.00000
VAR00008	6	.2483	.00408	.00167
VAR00009	6	.2500	.00000[a]	.00000
VAR00010	6	.2500	.00000[a]	.00000
VAR00011	6	.3000	.00000	.00000
VAR00012	6	.3000	.00000	.00000
VAR00013	6	.3000	.00000	.00000
VAR00014	6	.3500	.00000	.00000
VAR00015	6	.3500	.00000	.00000

a. t cannot be computed because the standard deviation is 0.

One-Sample Test

	Test Value = 0					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
VAR00001	5.779E16	5	.000	.10000	.1000	.1000
VAR00002	5.779E16	5	.000	.10000	.1000	.1000
VAR00003	5.779E16	5	.000	.10000	.1000	.1000
VAR00004	8.661E16	5	.000	.15000	.1500	.1500
VAR00005	8.661E16	5	.000	.15000	.1500	.1500
VAR00006	8.661E16	5	.000	.15000	.1500	.1500
VAR00007	5.779E16	5	.000	.20000	.2000	.2000
VAR00008	119.000	5	.000	.19833	.1940	.2026
VAR00009	5.779E16	5	.000	.20000	.2000	.2000
VAR00010	5.779E16	5	.000	.20000	.2000	.2000
VAR00013	35.000	5	.000	.29167	.2702	.3131
VAR00014	8.661E16	5	.000	.30000	.3000	.3000
VAR00015	22.136	5	.000	.28000	.2475	.3125

4. Mice Treatment 10%

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
VAR00001	6	.1500	.00000	.00000
VAR00002	6	.1500	.00000	.00000
VAR00003	6	.1500	.00000	.00000
VAR00004	6	.2000	.00000	.00000
VAR00005	6	.2000	.00000	.00000
VAR00006	6	.2000	.00000	.00000
VAR00007	6	.2500	.00000[a]	.00000
VAR00008	6	.2500	.00000[a]	.00000
VAR00009	6	.2500	.00000[a]	.00000
VAR00010	6	.3000	.00000	.00000
VAR00011	6	.3000	.00000	.00000
VAR00012	6	.3017	.00408	.00167
VAR00013	6	.2983	.00408	.00167
VAR00014	6	.4000	.00000	.00000
VAR00015	6	.4000	.00000	.00000

a. t cannot be computed because the standard deviation is 0.

One-Sample Test

	Test Value = 0					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
VAR00001	8.661E16	5	.000	.15000	.1500	.1500
VAR00002	8.661E16	5	.000	.15000	.1500	.1500
VAR00003	8.661E16	5	.000	.15000	.1500	.1500
VAR00004	5.779E16	5	.000	.20000	.2000	.2000
VAR00005	5.779E16	5	.000	.20000	.2000	.2000
VAR00006	5.779E16	5	.000	.20000	.2000	.2000
VAR00010	8.661E16	5	.000	.30000	.3000	.3000
VAR00011	8.661E16	5	.000	.30000	.3000	.3000
VAR00012	181.000	5	.000	.30167	.2974	.3060
VAR00013	179.000	5	.000	.29833	.2940	.3026
VAR00014	5.779E16	5	.000	.40000	.4000	.4000
VAR00015	5.779E16	5	.000	.40000	.4000	.4000

5. Mice Treatment 15%

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
VAR00001	6	.2000	.00000	.00000
VAR00002	6	.2000	.00000	.00000
VAR00003	6	.2000	.00000	.00000
VAR00004	6	.2000	.00000	.00000
VAR00005	6	.2500	.00000[a]	.00000
VAR00006	6	.2500	.00000[a]	.00000
VAR00007	6	.2500	.00000[a]	.00000
VAR00008	6	.3000	.00000	.00000
VAR00009	6	.3000	.00000	.00000
VAR00010	6	.3000	.00000	.00000
VAR00011	6	.4000	.00000	.00000
VAR00012	6	.4000	.00000	.00000
VAR00013	6	.4000	.00000	.00000
VAR00014	6	.4967	.00816	.00333
VAR00015	6	.4267	.17963	.07333

a. t cannot be computed because the standard deviation is 0.

One-Sample Test

	Test Value = 0					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
VAR00001	5.779E16	5	.000	.20000	.2000	.2000
VAR00002	5.779E16	5	.000	.20000	.2000	.2000
VAR00003	5.779E16	5	.000	.20000	.2000	.2000
VAR00004	5.779E16	5	.000	.20000	.2000	.2000
VAR00008	8.661E16	5	.000	.30000	.3000	.3000
VAR00009	8.661E16	5	.000	.30000	.3000	.3000
VAR00010	8.661E16	5	.000	.30000	.3000	.3000
VAR00011	5.779E16	5	.000	.40000	.4000	.4000
VAR00012	5.779E16	5	.000	.40000	.4000	.4000
VAR00013	5.779E16	5	.000	.40000	.4000	.4000
VAR00014	149.000	5	.000	.49667	.4881	.5052
VAR00015	5.818	5	.002	.42667	.2382	.6152

6. Mice Treatment 20%

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
VAR00001	6	.2000	.00000	.00000
VAR00002	6	.2000	.00000	.00000
VAR00003	6	.2500	.00000a	.00000
VAR00004	6	.2333	.02582	.01054
VAR00005	6	.2500	.00000a	.00000
VAR00006	6	.3000	.00000	.00000
VAR00007	6	.3000	.00000	.00000
VAR00008	6	.3000	.00000	.00000
VAR00009	6	.3000	.00000	.00000
VAR00010	6	.4000	.00000	.00000
VAR00011	6	.4017	.00408	.00167
VAR00012	6	.3917	.02041	.00833
VAR00013	6	.5000	.00000[a]	.00000
VAR00014	6	.5000	.00000[a]	.00000
VAR00015	6	.6017	.00408	.00167

a. t cannot be computed because the standard deviation is 0.

One-Sample Test

	Test Value = 0					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
VAR00001	5.779E16	5	.000	.20000	.2000	.2000
VAR00002	5.779E16	5	.000	.20000	.2000	.2000
VAR00004	22.136	5	.000	.23333	.2062	.2604
VAR00006	8.661E16	5	.000	.30000	.3000	.3000
VAR00007	8.661E16	5	.000	.30000	.3000	.3000
VAR00008	8.661E16	5	.000	.30000	.3000	.3000
VAR00009	8.661E16	5	.000	.30000	.3000	.3000
VAR00010	5.779E16	5	.000	.40000	.4000	.4000
VAR00011	241.000	5	.000	.40167	.3974	.4060
VAR00012	47.000	5	.000	.39167	.3702	.4131
VAR00015	361.000	5	.000	.60167	.5974	.6060

.2.10 Result of Histology Description

Histological description results of mammary glands in control mice showed that the lobe's (a), lobules (b), lobules wall were thick (c) and there were fat between lobe's and there were blood vessel showed in between lobe's, it's under normal circumstances, see Figure 4.8a.

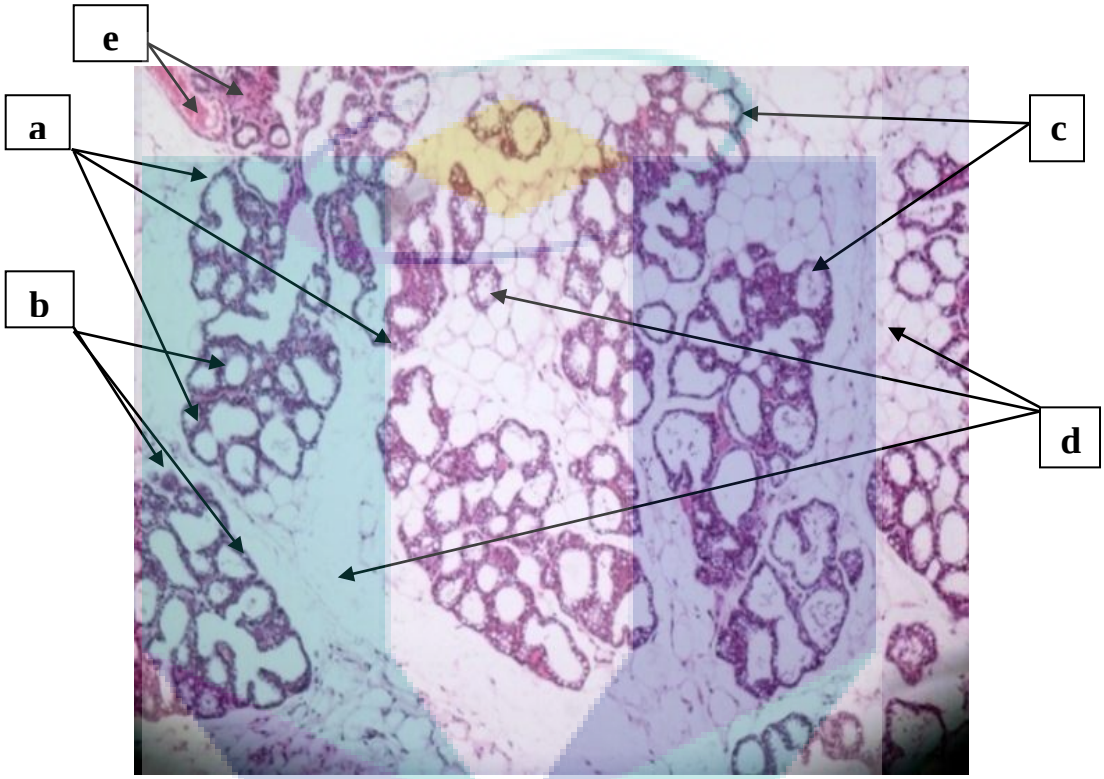


Figure 4.8a. Histology description of mammary gland tissues in mouse control

Histological description results of mammary glands in treatment mice with 1% Torbangun leave extarct showed that the lobe's (a), lobules (b), lobules wall were thick (c) and there were fat between lobe's (d) and there was blood vessel showed in between lobe's (e), it's under normal circumstances, see Figure 4.8b.

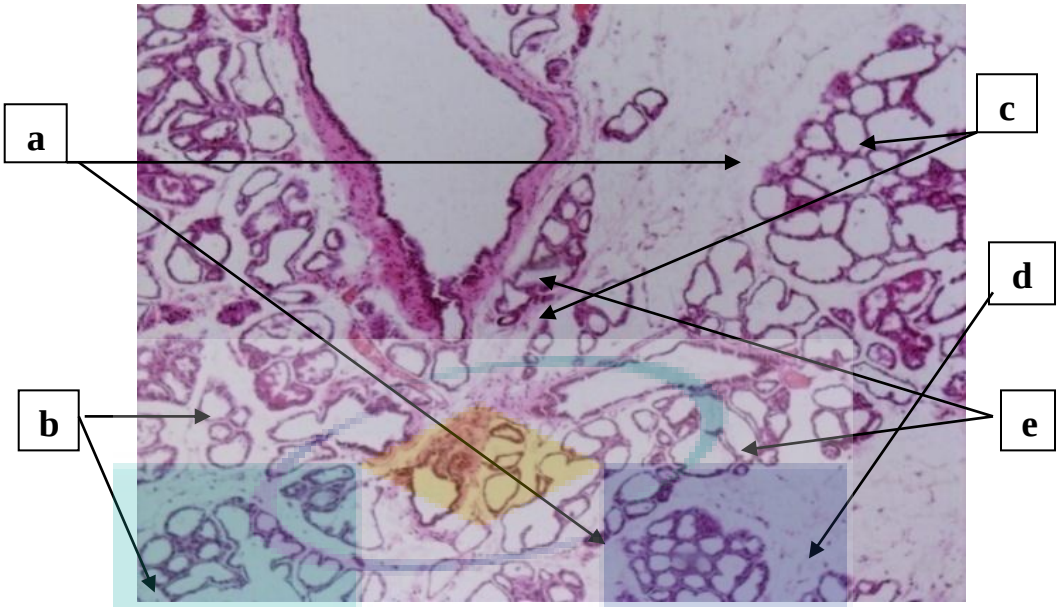


Figure 4.8b. Histology description of mammary gland tissue in mouse treatment with 1% Torbangun leave extract.

Histological description results of mammary glands in treatment mice with 5% Torbangun leave extract showed that the lobe's (a), lobules (b), lobules wall were rather thin (c), there were blood vessel showed in between lobules and there were little milk in the lobules, it's under normal circumstances, see Figure 4.8c.

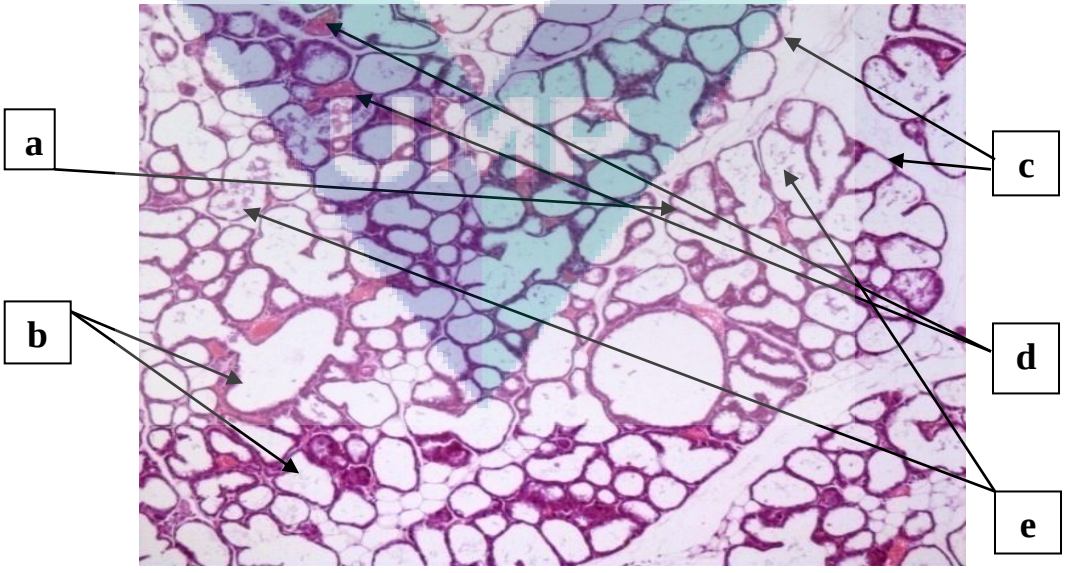


Figure 4.8c. Histology description of mammary gland tissues in treatment mouse with 5% Torbangun leave extract.

Histological description results of mammary glands in treatment mice with 10% Torbangun powder showed that the lobe's (a), lobule (b), lobules wall were thick (c), visible the milk in lobules, fibricyt (e) and myocyt (f), it's under normal circumstances, see Figure 4.8d.

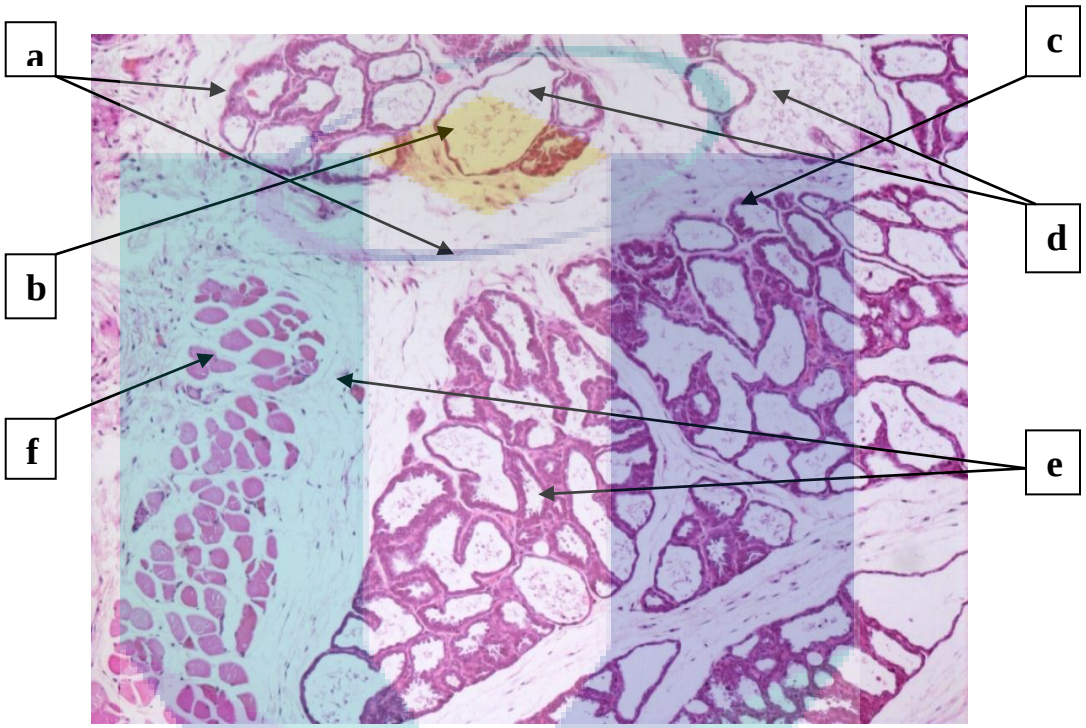


Figure 4.8d. Histology description of mammary gland tissue in treatment mouse with 10% Torbangun leave extract.

Histological description results of mammary glands in treatment mice with 15% Torbangun leave extract showed that the lobe's (a), lobules (b), lobules wall rather thin (c) and visible the much milk in lobules (d), fibrosis (e) and there were blood vessel between lobules, it's under normal circumstances, see Figure 4.8e.

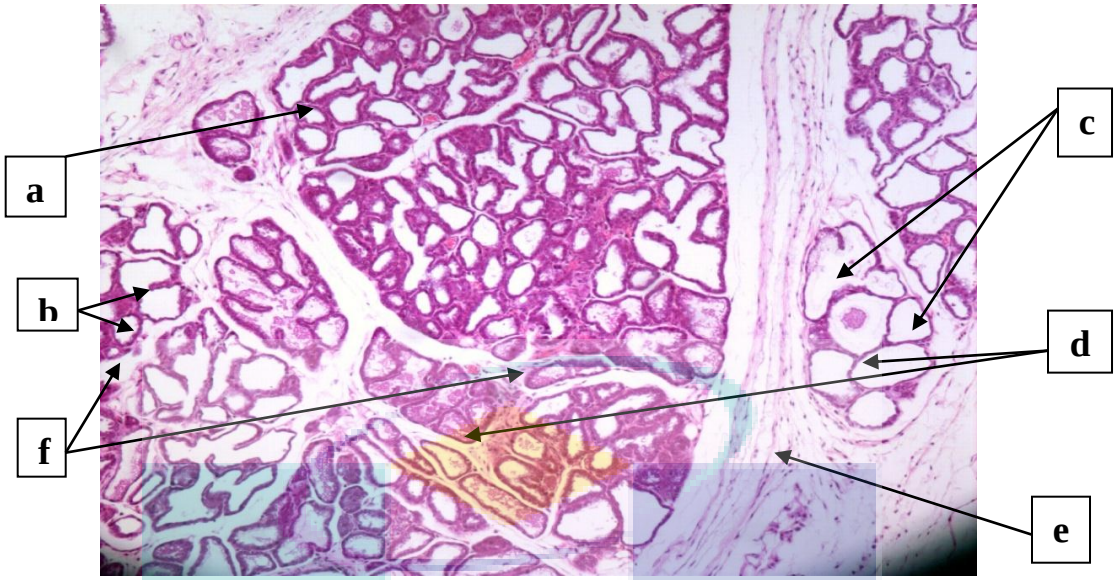


Figure 4.8e. Histology description of mammary gland tissue in treatment mouse with 15% Torbangun leave extract.

Histological description results of mammary glands in treatment mice with 20% Torbangun leave extract showed that the lobe's (a), lobules rather wide and lobules wall were thick (b), visible the much milk in lobules (c), there were blood vessel showed in between lobe's and there was fibrosis (e), it's under normal circumstances, see Figure 4.8f.

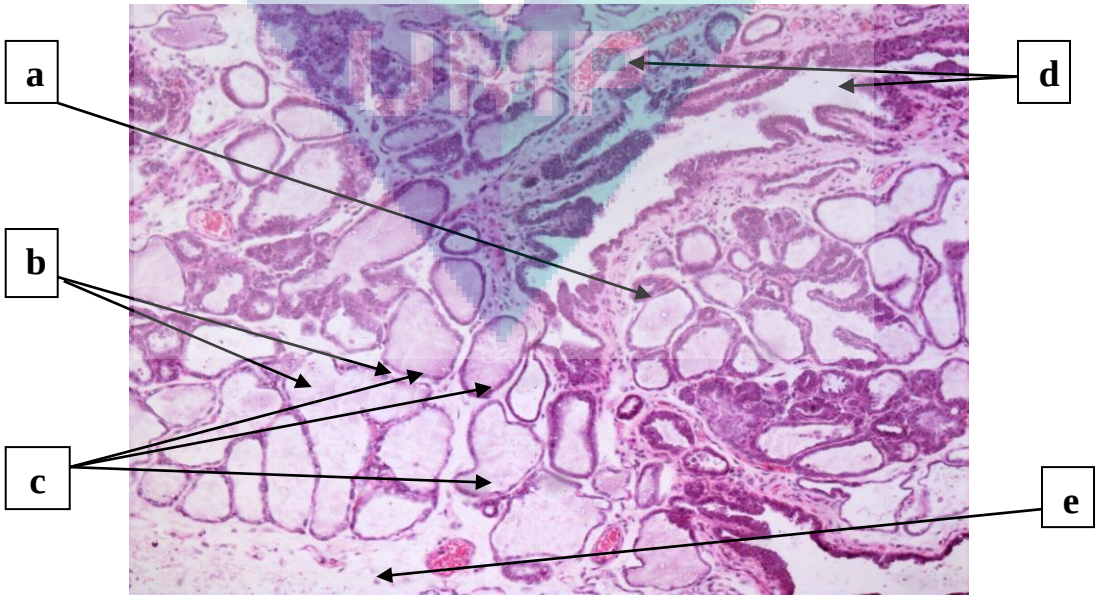


Figure 4.8f. Histology description of mammary gland tissue in treatment mouse with 20% Torbangun powders.

The wide areas of the mammary glands and enlarged lobes would mean the amount of milk had increased, because the contents of the mammary gland lobes contain milk (Julie *et.al*., 2005).

4.3 Discussion

4.3.1 Recovery of Active Substances on Mammary Gland

The active ingredients in the plant that plays a lot Torbangun is phytosterol and fatty acid (oktadekanoid acid). Advantages of phytosterol the mammary gland that may promote the development of the mammary gland and uterus. For example, a very high intake of phytosterols, sitosterol and campesterol in particular can inhibit the growth of breast, prostate, cardiovascular (Karl, 1997) and breast cancer (Atif *et al*., 2003). Sterols and stanols are beneficial to health in reducing serum cholesterol levels.

The fatty acid is one of the active substance in the plant Torbangun indirect function in the mammary gland, particularly the provision of fatty acids in women who are pregnant can increase memory performance and brain are normal in infants. In addition, fatty acids can also reduce Inflammation, prevent heart disease, cancer and arthritis (Steven, 2009).

Meanwhile, other active ingredients directly no role in the mammary gland, such as benzoic acid, and alpha amyrin benzenediol, chances are too small.

4.3.2 Deleterious Effect of Active Substances on Mammary Gland

Effect of damage due to the active substance contained in Torbangun crops in the mammary gland there is no common, but few reported side effects of sterol compounds that heartburn or indigestion, diarrhea, and nausea (Rubis *et al.*, 2008).

Loss of fatty acids in the body of the person who is easily injured, and bleeding due to the high doses of fatty acids can increase bleeding, cause gas, bloating, belching, and diarrhea (Steven, 2009). Meanwhile, the man who had a heart attack and stroke, saturated fatty acids are major risk factors. A diet high in fatty acids can lead to

increased production of cholesterol and together form a precipitate proteins in the body and can occur obesity.

4.3.3 Beneficial and Deleterious of Other Supporting Results: Antibioti, Phenolic, Flavonoid, and Antioxidant

(A) Beneficial of Other Supporting Substances on Mammary Gland

Antibiotic activity contained in Torbangun plants can prevent mammary gland inflammatory disease caused by bacteria. Giving Torbangun during pregnancy or prenatal effective to eliminate infection of the mammary gland during late pregnancy and to reduce the prevalence of mastitis during early lactation and throughout lactation (Oliver *et al.*, 2003).

Benefits of phenolic compounds in the body, especially in the mammary gland can prevent intramammary infections during lactation. Compounds called polyphenols (catechins) found in plant protection Torbangun beneficial for degenerative diseases and prevent breast cancer. In addition, the polyphenolic compounds are also useful for preventing cardiovascular diseases, anti-inflammatory, anti-arthritis, antibacterial, anti-angiogenic, antioxidant, antiviral, nerves, and cholesterol lowering effects (Sabu et al., 2010).

Certain flavonoid compounds have been reported that could potentially protect against some types of cancer, including breast cancer. Intake of flavonoid compounds demonstrated the ability to inhibit aromatise activity, so the level of circulating estrogen and estrogen biosynthesis lower, inhibit tumor cell proliferation and inhibits the formation of oxygen species reactive cancer affect breast development (Brian et al., 2006).

Antioxidant enzyme activity and concentrations of vitamins A and C increases during lactation. Antioxidants serve to the best defense against oxidative stress in the newborn and the mother at birth. In addition, the antioxidant found in the mammary gland, and breast milk kalostrum can prevent the occurrence of mastitis, or inflammation of the mammary gland (Justyna et al., 2012) and apoptosis (Su et al.,

2002). In addition, the antioxidant may prevent damage to cells caused by cancer, aging, and various diseases (Richard, 2003).

(B) Deleterious of Other Supporting Substances on Mammary Gland

In fact, loss of use of antibiotics on the mammary gland is not directly, but the side effects of antibiotics on the body, especially allergic reactions, contribute to cancer, destruction of microflora in the gut, the development of resistant species of micro-organism, immune suppression, an overgrowth of *Candida albicans* and more dangerous intestinal infections, chronic fatigue syndrome, diarrhea, leading to the loss of essential minerals (Lawrence, 2012).

Based on data from previous studies and to date, it is likely that the phenolic compounds, flavonoids and antioxidants are not harmful to the body and mammary glands (Richard, 2003)

4.3.4 Beneficial and Deleterious of Heavy Metals on Mammary Gland

(A) Beneficial of Heavy Metals on Mammary Gland

In a very small amount or below the threshold of tolerance, heavy metal harmless to humans. Some of them referred to as elements eg, iron, copper, manganese, zinc and selenium can be as essential nutrients for a healthy life (International Occupational Safety and Health Information Centre 1999). Especially, magnesium is necessary for biochemical reactions in the body and regulate muscle and nerve function normally, maintaining the rhythm of the heart, the immune system and bone strength manjaga. In addition, the function of magnesium also regulate sugar in the blood, increases blood pressure in normal energy metabolism and protein synthesis. The advantage of magnesium in plants Torbangun to prevent and control hypertension, cardiovascular disease and biabetes (Darius, 2009).

Although, the copper contained in the plant enzyme cofactor Torbangun useful as antioxidants to protect against oxygen free radicals generated during oxidative stress

(Leung 1998), decrease nausea, vomiting, vertigo and seizures caused by chemicals and drugs used in chemotherapy (Pakdaman 1998).

(B) Deleterious of Heavy Metals on Mammary Gland

Heavy metals are metallic chemical elements that have a high density and is toxic at low concentrations, eg heavy metals harmful to humans include timbale, arsenic and mercury. In amounts above the threshold of tolerance, heavy metals are very dangerous in the world. Heavy metals become toxic when they are not metabolized by the body and accumulate in the soft tissues (Roberts 1999). Damage caused by heavy metals that reduce vitality and health, such as degenerative neurological diseases, cancer, heart disease, autoimmune diseases and disorders of the skin.

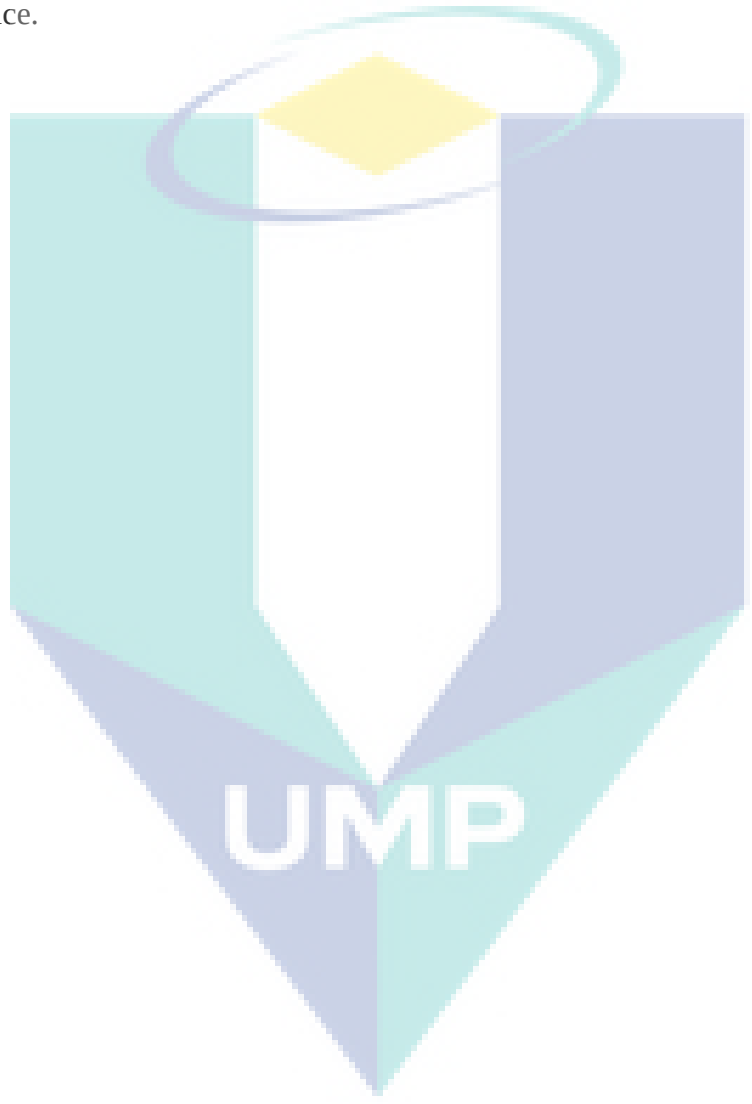
In addition, it can also damage the blood composition, internal organs such as the lungs, liver, kidneys and other vital organs. For a long time, heavy metals in the body can result in physical Progressing slowly, muscular, and Alzheimer's, Parkinson's disease, muscular dystrophy, and multiple sclerosis ((International Occupational Safety and Health Information Centre 1999) .

4.3.5 Discussion of Clinical Test Result

Data from clinical trial results were processed using SPSS program with a P <0.05, df = 5 and F = 7514 found that, in mice treated 1%, 5% and 10% Torbangun leave extract did not significant, then the provision of 1%, 5% and 10 % Torbangun leave extract were not effects to increase milk production in lactating mice, while giving 15% and 20% Torbangun were significant, that meaning to the provision of 15% and 20% Torbangun leave extract affected to increase milk production in lactating mice. the higher the percentage of Torbangun leave extract higher increase in milk production in the mice mother, whereas group (batch) of mice were not significant or groups of mice not effect to increased milk production in mother mice.

4.3.6 Discussion of Histology Description

The results of histological description on mammary glands in control mice, and mice treated 1%, 5% and 10% Torbangun no changes are prominent or equal. While the mice by administering 15% and 20% Torbangun leave extract seen a lot of milk in the lobules. This suggests that the provision of 15% from an increase in milk production in lactating mice.



CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Introduction

This chapter presents the conclusion and recommendations of the analysis result and discussion of compounds in Torbangun plants by using GC-MS and FTIR, phytochemical test (antibiotic activity, phenolics, total flavonoids and antioxidant activity), heavy metal analysis, clinical trial in lactating mice, description of the histology in mice mammary gland.

5.2 Conclusion

Torbangun plants (*Coleus amboinicus* Lour) have 10 compound as active substances that affect in medicinal plant. In addition, Torbangun plants contain compounds alkanes, aromatic ring compounds, alcohols, ethers, carboxylic acids and esters were caught on infrared.

Torbangun plants have phytochemical such as antibiotic, total phenolic, total flavonoid compound and antioxidant activity that could as an anti-inflammatory function in the mammary gland caused by bacteria, prevent intramammary infections, oxidative stress, and protects breast cancer degenerative diseases. In addition, these plants can prevent cardiovascular disease, antiartritis, antiangiogenic, antiviral, brain memory, lowering cholesterol effect, inhibiting tumor cell proliferation and inhibits the formation of oxygen species reactive. Treatment of Torbangun leave during pregnancy or prenatal effective to eliminate infection of the mammary gland during late pregnancy and to reduce the prevalence of mastitis during early lactation and throughout lactation.

Torbangun plant can consumed as food and food additives are safe, despite the heavy metals found in amounts below the threshold of tolerance. In addition, the plant also serves as a nutrition and decrease nausea, vomiting, vertigo and seizures caused by chemicals and drugs used in chemotherapy.

Clinical trial basically, Torbangun plants have effect on milk production increasing in mice. Although, treatment with 15% Torbangun leave extract after one day birth for 15 days could imilk production ncreasing in mice.

The higher dose of Torbangun leave extract giving more affect the increased milk production in mice that can be proven by clinical tests and changes in histology of the mammary glands of mice.

5.3 Recommendation and Further Works

Torbangun plants as medicinal plants contain many substances and compounds such as phytochemicals, phytosterols, proteins, fats, vitamins and minerals that are used for humans. Substances and compounds in plants has been studied and analyzed. However, the specific components and mechanisms involved in the plant lactagogue Torbangun be studied and researched in detail.

Previous research by Damanik *et al,* (2006). Reported that plants can be contained lactagogue Torbangun stimulant nursing mothers. In areas Bataknesse, North Sumatra, Indonesia has a tradition of consuming leaves Torbangun after birth and believed that consumption Torbangun leaves for a month after birth increase their milk production. Traditionally, the leaves of plants Torbangun and consumed in the form of soup to eat. Recommendations by other research studies continue to make capsules Torbangun plants and commercialization of women to increase lactation milk in Malaysia in particular and the world in general.

Future work, after completion of the study followed by a study made of the leaf extract capsules Torbangun and research will continue in cattle or goats. The purpose of this study to increase milk production in cows and goats.

REFERENCES

Acosta L., R. Menéndez, V. Fuentes, C. Rodríguez I. Hechevarria & C. Carballo. 1998. Instructivo técnico del cultivo de *Plecthranthus amboinicus* (Lour.) Spreng (orégano francés). Revista Cubana de Plantas Medicinales 3:51- 53.

Adams, R., 1995. Identification of Essential oil Component by Gas Chromatography/ Mass Spectroscopy. Allured Publishing Co., Carol Steam, IL.

Adaramoye O.A., Farombi E.O., Adeyemi E.O. & Emerole G.O., 2005. Comparative Study on Antioxidant Properties of Flavonoids of *Garcinia Kola.* Kola Seeds. J Med Sci July-September 2005 Vol. 21 No. 3 331-339.

Adegboye,M.F;Akinpelu, D.A and Okoh, A.I., 2008. The Bioactive and Phytochemical Properties of Gracinia kola (Heckel) seed Extract on some Phatogens. African Journal of Biotechnology vol 7 (21) pp 3934-3938.

Adlard M.W., B.M. Gordon , T. Keshavarz , C. Bucke 1, M.D. Lilly, A.T. Bull and G. Holt, 1991. An HPLC Procedure for Monitoring Penicillin G Fermentations. *Biotechnology Techniques Vol 5 No 2 121-126 (1991).*

Afolabi C. Akinmoladun1, E.O. Ibukun1 and I.A. Dan-Ologe, 2007. Phytochemical constituents and antioxidant properties of extracts from the leaves of *Chromolaena odorata.* Academic Journals *Full Length Research Paper.*

Afzal, M. and Armstrong D., 2002. "Fractionation of herbal medicine for identifying antioxidant activity". In: Armstrong, D. (Ed.) Methods in Molecular Biology, vol. 186: Oxidative Stress Biomarkers and Antioxidant Protocols, Humana Press Inc.

Agency for Toxic Substances and Disease Registry (ATSDR),1998. *Toxicological Profile for Phenol (Update).* Public Health Service, U.S. Department of Health and Human Services, Atlanta, GA.

Aiello, A. Mays (Editors), Veterinary Merck Index, Eighth Edition, Merck and Co. Inc., Washington. 1998. p. 1779.

Akoh, C.C and Min, D.B. 1998. *Food lipids; chemistry, nutrition and biotechnology, Part IV.* Marcel Dekker. New York. pp 432-437.

Alade, P.I. and O.N. Irobi, 1993. Antimicrobial activities of crude leaf extract of *Acalypha wilkensiana.* Journal of Ethnopharmacology, 39: 171-174.

Almeida, W. L. C.; Vitor, D. N.; Pereira, M. R. G; de Sá, D. S.; Alvarez, L. D. G.; Pinheiro, A. M.; Costa, S. L.; Costa, M. F. D.; Rocha, Z. N.; El-Bachá, R. S. (2007).Redox properties of ruthenium complex with catechol are involved in toxicity to glial cells. J. Chil. Chem. Soc. 52 (3): 1240-1243.

Alloway, B.J., 1995. Heavy Metal in Soils. Jhon Willey and Sons Inc., New York.

Alonso B, Angeles M, Zono L, Dominico A, Guillen, Garcio BC., 2003. Study of the polyphenol content of red and white grape varieties by liquid Chromatography_Mass spectrometry and its relationship to antioxidant power. J. Chromatogr. 1012: 31-38.

Anttonen, Mikko J., 2007. Evaluation of mean to increase the content of bioactivephenolic compounds in soft fruits. Kuopio University Publications C. Natural andEnvironmental Sciences 208. 93 p.

Aragao, G. F., Carneiro, L. M., Junior, A. P., et al. (2006). A possible mechanism for anxiolytic and antidepressant effects of alpha- and beta-amyrin from Protiuheptaphyllum (Aubl.) March, *Pharmacol. Biochem. Behav* **85**, 827–34.

Arrington, L. R., 1972. Introductory Laboratory Animal Science, the Breeding, Care and Management of Experimental Animal. The Interstate Printers and Publishers, Inc. Danvile, Illionis.

Association Office Analysis Chemical "AOAC" International, 1995. Published and Distributed by 481 Nort Frederick Avenue, Suite 500 Gaitherburg MD 20877 USA.

Atif B. Awad and Carol S. Fink, 2000. Phytosterols as Anticancer Dietary Components: Evidence and Mechanism of Action. The American Society for Nutritional Science. *Journal of Nutrition.* 130:2127-2130.

Atif B Awad, Heinric Williams, Carol S Fink, 2003. Effect of phytosterols on cholesterol metabolism and MAP kinase in MDA-MB-231 human breast cancer cells. The American Society for Nutritional Science. *Journal of Nutrition.*

Audrey Meikle, L. Anne Clover, Kenneth Killham and James I. Prosser, 1994. Potential Luminescence an Indicator of Activation of Genetically Modified *PSEUDOMONAS FLUORESCENS* IN Liquid Culture and Soil. *Soil Bid. Biochem.* Vol. 26, No. 6, pp. 147-755, 1994. Elsevier Science Ltd.

Avorn J., 2004. Powerful Medicines: The Benefits, Risks, and Costs of Prescription Drugs. New York, NY: Alfred A Knopf.

Bach D and Wachtel E., 2003.. "Phospholipid/cholesterol model membranes: formation of cholesterol crystallites". *Biochim Biophys Acta* **1610** (2): 187–97.

Baillie, J K; A A R Thompson, J B Irving, M G D Bates, A I Sutherland, W Macnee, S R J Maxwell, D J Webb, 2009. "Oral antioxidant supplementation does not prevent acute mountain sickness: double blind, randomized placebo-controlled trial". *QJM: Monthly Journal of the Association of Physicians* **102** (5): 341–8.

Backer, A. J. M.; Brooks, R. R., (1989). Terrestrial higher plants which hyperaccumulate metallic elements. A review of their distribution, ecology and phytochemistry. Biorecovery, 1, 81-126 (46 pages).

Barner B. A., 2004. "Catechol" in Encyclopedia of Reagents for Organic Synthesis (Ed: L. Paquette), J. Wiley & Sons, New York. DOI: 10.1002/047084289.

Barrie, L.A., Gregor. D., Hargrave., B., Lake. R., Muir. D., Sheater. R., Tracey. B and Bidleman. T., 1992. Arctic Contaminants—Sources, occuranceand pathways. *Sci. Total Environ.*, 122, 1 – 74.

Bartholomew, Edwin F.; Martini, Frederic; Ober, William B., 2007. *Essentials of anatomy & physiology*. San Francisco: Pearson/Benjamin Cummings. pp. 340. ISBN 0-8053-7303-9.

Baslas, R.K., Kumar, P., 1981. Chemical examination of essential oil of *Coleus aromaticus* Benth. Journal of Indian Chemistry Society 58, 103–104.

Beal, M. F., 2003. Mitochondria, oxidative damage, and inflammation in Parkinson's disease Ann. N.Y. Acad. Sci. 991 120 131.

Beare-Rogers, J.; Dieffenbacher, A.; Holm, J.V. 2001. "Lexicon of lipid nutrition (IUPAC Technical Report). *Pure and Applied Chemistry* 73 (4): 685–744.

Beecher GR., 2003. Overview of dietary flavonoids: Nomenclature,occurrence and intake. J Nutr;133:3248S-54S.

Bender and David A., 1992. *Nutritional Biochemistry of the Vitamins.* Cambridge University Press.

Benito-Peña E., J.L. Urraca and M.C. Moreno-Bondi, 2008. Quantitative determination of penicillin V and amoxicillin in feed samples by pressurised liquid extraction and liquid chromatography with ultraviolet detection. Elsevier B.V. All rights reserved.

Bhatnagar, D. and P. N. Durrington 2003. "Omega-3 fatty acids: their role in the prevention and treatment of atherosclerosis related risk factors and complications." Int J Clin Pract **57**(4): 305-14.

Bjelakovic G; Nikolova, D; Gluud, LL; Simonetti, RG; Gluud, C., 2007. "Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis". *JAMA* 297 (8): 842–57.

Bodwell, C.E. 1979. "Evaluation of plant proteins to solve nutritional problems of the third world". *Plant Foods for Human Nutrition* **29**: 135–162.

Boening, D.W., 2000. Ecological effects, transport, and fate of mercury: A general review. Chemosphere, 40, 1335 – 1351.

Boguslawa A. Stelmach, Axel Müller, Peter Hennig, Dietmar Laudert, Leo Andert and Elmar W. Weiler, 1998. Quantitation of the octadecanoid 12-oxo-phytodienoic acid, a signalling compound in plant mechanotransduction Phytochemistry Volume 47, Issue 4, Pages 539-546.

Brereton R. G. and Ellis Horwood Ltd., 1990. Chemometrics - Application of Mathematics and Statistics to Laboratory Systems.

Brian N. Fink, Susan E. Steck, Mary S. Wolff, Julie A. Britton, Geoffrey C. Kabat, Jane C. Schroeder, Susan L. Teitelbaum, Alfred I. Neugut, and Marilie D. Gammon, (2006). Dietary Flavonoid Intake and Breast Cancer Risk among Women on Long Island. Epidemiology, School of Public Health, University of North Carolina, Chapel Hill, NC

Brisson-Noel A., P. Trieu-Cuot and P. Courvalis, 1988. *J. Antimicrob. Chemother.* **22** Suppl. B , p. 13.

Brocchieri L, Karlin S., 2005. "Protein length in eukaryotic and prokaryotic proteomes". *Nucleic Acids Research* **33** (10): 3390–3400.

Brody, T. 1998. *Nutritional Biochemistry*. San Diego: Academic Press.

Brown D., 1997. Grenada: isle of spices, Herbs 22 (1997), pp. 6–7.

Bullard RW, Dill DB, Yousef MK., 1970. "Response of the burro to desert heat stress". *J Appl Physiol* 29: 159–167.

Butt, E.M., Nusbaum, R.E., Gilmour. T.C., Mariano. S.X., and Didio. S.L., 1964. Trace metal level in human serum and blood. *Arch. Environ. Health, 8, 52-57.*

Buszewski B, A. Jastrzebska,T. Kowalkowski, A and Gorna-Binkul (2000) Monitoring of selected Heavy Metal Up take by Plants and soils in The area of Torm, Poland. Polish J of Environmental studies Vol,9 No.6, 511-515.

Buznego MT, Perez-Saad H., 1999. Antiepileptic effect of *Plectranthus amboinicus* (lour.) Spreng. (French marjoram). Rev Neurol 1999;29:388-9.

Calderon CB, Sabundayo BP., 2007. Antimicrobial Classifications: Drugs for Bugs. In Schwalbe R, Steele-Moore L, Goodwin AC. Antimicrobial Susceptibility Testing Protocols. CRC Press. Taylor & Frances group.

Caldwell HK, Young WS 3rd, 2006. "Oxytocin and Vasopressin: Genetics and Behavioral Implications". In Lajtha, Abel; Ramon Lim. *Handbook of Neurochemistry and Molecular Neurobiology* (3rd ed.). Berlin: Springer. pp. 573–607.

Campbell and Neil A., 2000. *Biology*, 4th edition. San Francisco: Benjamin/ Cummings.

Carol L Wagner, Eric M Graham, William W Hope, 2006. Human Milk and Lactation. Department of Pediatrics, Medical University of South Carolina Department of Surgery, Greenville Memorial Hospital.

Caroline Mourão Melo, Karine Maria Martins Bezerra Carvalho, Julliana Catharina de Sousa Neves, Talita Cavalcante Morais, Vietla Satyanarayana Rao, Flávia Almeida Santos, Gerly (2010). Alpha-Beta Amyrin.

Carolyn Glatz (2009).The article :Why Magnesium is Important to your Health, Filed under Healthy Livingno comments Written on April 8, 2009 at 8:31 pm.

Cathrin Brisken1 and Bert O'Malley, 2010. Hormone Action in the Mammary Gland. *Cold Spring Harbor Perspectives in Biology.* Cold Spring Harbor Laboratory Press.

Chang C, Yang M, Wen H, Chern J., 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J. Food Drug Analaysis, 10: 178-182.

Chantry, Caroline J.; Howard, Cynthia R.; Montgomery, Anne; Wight, Nancy, 2004. Useof galactogogues in initiating or augmenting maternal milk supply. The Academy Of Breastfeeding Medicine. Archived from the original on 2007-06-28.

Charlena 2004. Pencemaran Logam Berat Timbal (Pb) dan cadmium (Cd) pada sayuran, Falsafah Sain (PSL-702) Program pasca Sarjana (S.3) IPB (posted 30 Desember 2004).

Charles Owens Wilson; Ole Gisvold; John H. Block, 2004. *Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical*. Lippincott Williams & Wilkins. pp. 234.

Chaturvedi, R. K.; Beal, M. F., 2008. PPAR: a therapeutic target in Parkinson's disease J. Neurochem. 106 506 518.

Chopra I, Roberts M., 2001. Tetracycline antibiotics: mode of action, application, molecular biology, and epidemiology of bacterial resistance. Microbiol Mol Biol Rev 65:232–260. DOI 10.1128/MMBR.65.2.232-260.

Chopra RN, Nayar SL and Chopra IC (1999). Glossary of Indian Medicinal Plants. C.S.I.R. Publications, New Delhi, India. p. 74.

Christie W.W., 2011. Sterol 1. Cholesterol and Choleterol esters Structure, Occurrence, Biochemistry and Analysis. *Scottish Crop Research Institute (and Mylnefield Lipid Analysis), Invergowrie,Dundee (DD2 5DA).*

Christine Moers**,** 2007. How to Induce Lactation - the Well May Not Be Dry!. Sources used for this article: http://www.fourfriends.com/abrw/

Cobbett CS, Hussain D, Haydon MJ. (2003) Structural and functional relationships between type 1B heavy metal-transporting P-type ATPases in Arabidopsis. *New Phytologist* **159**: 315-321.

Codex Alimentarius, 2001a. Codex maximum levels for Cadmium in Cereals, Pulses and Legumes, Joint FAO/WHO Standards, CAC/GL 39-2001. http//www. codexalimentarius. net/standards_search.asp. (accessed March 2004).

Codex Alimentarius, 2001b. Maximum levels for Lead, Joint FAO/WHO Standards, Codex STAN 230-2001, http//www.codexalimentarius.net/standards_search.asp (accessed March 2004).

Codex Alimentarius Committee, 1996. Heavy Metals. CAOBISCO, IOCCC. *E-mail : ioccc@caobisco.be*.

Colak, E., 2008. New markers of oxidative damage to macromolecules J. Med. Biochem. 27 1 16.

Couse JF, Korach KS. 1999. Estrogen receptor null mice:What havewe learned and where will they lead us? Endocard Review 20: 358–417.

Cox DB, Owens RA, Hartmann PE., 1996: Blood and milk prolactin and the rate of milk synthesis in women. *Exp Physiol*, 81**:**1007-1020.

Cregan M, Mitoulas L, Hartmann P., 2002. "Milk prolactin, feed volume and duration between feeds in women breastfeeding their full-term infants over a 24 h period". *Exp Physiol* **87** (2): 207–14.

Crisp TM, Clegg ED, Cooper RL, Wood WP, Anderson DG, Baetcke KP, Hoffmann JL, Morrow MS, Rodier DJ, Schaeffer JE, Touart LW, Zeeman MG, Patel YM 1998. "Environmental endocrine description ; An effects assessment and analysis". *Environ. Health Perspect.* 106 (Suppl 1): 11–56.

Cristobal Miranda and Donald R. Buhler, 200.Antioxidant Activities of Flavonoids. Department of Environmental and Molecular Toxicology Oregon State University

Croteau R, Kutchan TM, Lewis NG., 2000. Natural products (secondarymetabolites), In: Buchanan BB, Gruissem W, Jones RL, eds. Biochemistry & Molecular

Biology of Plants.Rockville, MD, USA: American Society of Plants Physiologists;1250-1318.

Curtis, L. R., Smith, B. W., 2002. Heavy metals in fertilizers: Considerations for setting regulations in Oregon, Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis, Oregon, August 2, 2002 http://www.oregon. gov/ODA/PEST/docs/pdf/fertheavymet.pdf.

Dahan A., Altman H. (2004): Food–drug interaction: grapefruit juice augments drug bioavailabilityFmechanism, extent and relevance. European Journal of Clinical Nutrition 58: 1-9.

Daly SE, Owens RA, Hartmann PE., 1993: The short-term synthesis and infantregulated removal of milk in lactating women. *Exp Physiol*, **78:**209-220.

Damanik R, Damanik N, Daulay Z, Saragih S, R Premier, N Wattanapenpaiboon, Wahlqvist ML., 2004. Consumption of Bangun-Bangun Leaves (*Coleus amboinicus* Lour) to Increase Breast Milk Production Among Bataknesse Women in North Sumatra Island, Indonesia.

Damanik R; Wahlqvist ML; Wattanapenpaiboon N., 2006. Lactagogue effects of Torbangun, a Bataknese traditional cuisine. Dept. of Community Nutrition, Faculty of Human Ecology IPB University, Kampus IPB Darmaga, Bogor-16610, Indonesia. damanik@ipb.ac.id.

Dana and James D., 1985. Hurlbut, Cornelius S.; Klein, Cornelis. eds. *Manual of Mineralogy* (20 ed.). John Wiley & Sons Inc.. free older version: 1912 edition.

Darius.Vd'Rhys (2009**)**, http://ods.od.nih.gov/factsheets /magnesium.asp, *March 18, 2009.*

Darmono. 1995. Logam dalam Sistem Biologi. Universitas Indonesia Press.

Davey PG., 2000. "Antimicrobial chemotherapy". in Ledingham JGG, Warrell DA. Concise Oxford Textbook of Medicine. Oxford: Oxford University Press. pp. 1475. ISBN 0192628704.

da Silva OP and Knoppert DC., 2004.”Domperidone for lactating women”. *CMAJ* 171 (7): 725–6.

deCarvalho M, Anderson D, Giangreco A, Pittard W., 1985. "Frequency of milk expression and milk production by mothers of non-nursing premature neonates". *Am J Dis Child* **139** (5): 483–5.

Dellman, H.D. and Brown, D.H., 1987. Text book of Veteriner Histology. 3rd Edn. Lea and Febige, Philadelphia, pp. 193 – 194.

Devi Rianti and Sri Yogyarti, 2006. Antimicrobial effects of Coleus amboinicus lour Folium infusum towards Candida albicans and Streptococcus mutans. Majalah Kedokteran Gigi (Dent. Journ) vol. 39 no.1. 12-15.

Devi Rianti, 2006. The transverse strength of acrylic resin after Coleus amboinicus lour extract solution immersion. Majalah Kedokteran Gigi (Dent. Journ) vol. 39 no.4. 156-160.

Di Matteo V, Esposito E., 2003. "Biochemical and therapeutic effects of antioxidants in the treatment of Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis". *Curr Drug Targets CNS Neurol Disord* **2** (2): 95–107.

Djingova. R; Wagner. G. and Kuleff. I., 1999. Screening of heavy metal pollution in Bulgaria using *Populus nigra* 'Italica'. *Sci. Total Environ*. 234: 175-184.

Dobson CM., 2000. "The nature and significance of protein folding". In Pain RH. (ed.). *Mechanisms of Protein Folding*. Oxford, Oxfordshire: Oxford University Press.ISBN 0-19-963789-X.

Donald P. Briskin, 2000. Medicinal Plants and Phytomedicines. Linking Plant Biochemistry and Physiology to Human Health. Update on Phytomedicines. Plant Physiol, October 2000, Vol. 124, pp. 507-514.

Duke, 2000. Dr. Duke's Contituens and Ethnobotanical Databases, Phytochemical database, United State Drug and Agriculture-ARS-NGRI.

Edeoga H,O.D ,E Okwu and B,O Mbaebie, 2005. Phytochemical Constituents of Nigerian medicinal plants, African journal of Biotechnology vol 4 (7), pp 685-688.

Edgar Folk G.Jr. and A. Semken Jr. 1991. "The evolution of sweat glands". *Int. J. of Biometeorology* **35**: 180–186.

Edge DS, Segatore M., 1993. Assessment and management of galactorrhea. *Nurse Pract*. 18356, 38,43–4, passim.

Elks J., C.R. Ganelin (Eds.), 1991. Dictionary of Drugs, Chapman. and Hall, London, 1991.

Emma Lloyd, 2009. Functions of Protein in the Human Body. Article, Edited & published by Leigh A. Zaykoski (54,036 pts) on Oct 4, 2009.

Ernawita, (2008)). Bioassay guided fraction and identification of antioxidant and antimicrobial compound from *Callestemon viminalis* (Gaeten)G.don. Thesis of Master Science.School of Biology Science USM.

Eschrich W. 1980. Free space invertase, its possible role in phloem unloading. *Berichte der Deutschen Botanischen Gesellschaft* 93, 363-78.

European Commission, 2005. Opinion on Benzoic Acid and Sodium Benzoate. Sciencitific Committee on Consumer Products (SCCP). Health & Consumer Protection Directorate-General.

Europe Food Safety Authority, 2009. For citation purposes: Scientific Opinion of the Panel on Contaminants in the Food Chain on a request from the European Commission on cadmium in food. *The EFSA Journal* (2009) 980, 1-139.

Evans. I.J., 1989. Chemistry of metal retention by soil—Several processes are explained. *Environ. Sci. Technol.*, 23, 1046 – 1056.

Fahy E, Subramaniam S, Brown HA, *et al.* (2005). " A comprehensive classification system for lipids". *J. Lipid Res.* **46** (5): 839–61.

FAO/WHO., 1984. Contaminants. In Codex Alimentarius, vol. XVII,Edition 1. FAO/WHO, Codex Alimentarius Commision, Rome.

Farrington WWH, Tarbin J, Sheraer G.1991. Analysis of trace residues of tetracyclines in animal tissues and fluids using metal chelate affinity chromatography/HPLC. Food Addit Content 8:595–614.

Frédérique L. van Holthoon, Martien L. Essers, Patrick J. Mulder, Sara L. Stead, Marianne Caldow, Helen M. Ashwin and Matthew Sharman, 2008. A generic method for the quantitative analysis of aminoglycosides (and spectinomycin) in animal tissue using methylated internal standards and liquid chromatography tandem mass spectrometry. Elsevier B.V. All rights reserved.

Finberg RW, Moellering RC, Tally FP., 2004. "The importance of bactericidal drugs: future directions in infectious disease". *Clin. Infect. Dis.* 39 (9): 1314–20.

Food and Nutrition Board, 2005. *A Report of the Panel on Macronutrients, Subcommittees on Upper Reference Levels of Nutrients and Interpretation and Uses of Dietary Reference Intakes, and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes*. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients). The National Academies Press Washington, D.C.

Frances Sizer; Ellie Whitney, 2007. *Nutrition: Concepts and Controversies.* Cengage Learning. pp. 26.

Friedland A.J. 1990. The movement of metals through soils and ecosystems. In : Shaw AJ (ed) Heavy metals tolerance implants : evolutionary aspects. CRC Press, Boca Raton, pp. 7-19.

Fritioff, A., Greger, M., 2007. Fate of cadmium in Elodea Canadensis. Chemosphere, 6: 365-375.

Fukuwatari T and Shibata K., 2008. "Urinary water-soluble vitamins and their metabolite contents as nutritional markers for evaluating vitamin intakes in young Japanese women". *J. Nutr. Sci. Vitaminol.* 54 (3): 223–9.

Ganesh K. Agrawal, Shigeru Tamogami, Oksoo Han, Hitoshi Iwahashi[e] and Randeep Rakwal, 2004. Rice octadecanoid pathway. Biochemical and Biophysical Research Communications. Volume 317, Issue 1, Pages 1-15.

Gargh P, R. D. Triphati, U. N. Rai, S. Sinha and P. Chandra, 1997. *Environmental Monitoring and Assessment* **47:** 167–173.

Gembong T. Taksonomi Tumbuhan (Spermatophyta), 2004. Gadjah Mada University Press, Yogyakarta.

Genton, Laurence; Melzer, Katarina; Pichard, Claude, 2010. "Energy and macronutrient requirements for physical fitness in exercising subjects". *Clinical Nutrition* 29 (4): 413–423.

Ghinwa M.Naja and Bohumil Volesky, 2009. Toxicity and Sources of Pb, Cd, Hg, Cr ,As and Radionuclide in the Environment. Text book Heavy Metals in The Environment.CRC Press. Taylor and Francis Group. London. New York.

Gislene GF Nascimento, Locatelly J, Paulo C.F and Giuliana L.S., 2000. Antibacterial Activity of Plant Extract and Phytochemicals on Antibiotic- Resistant Bacteria.Braz J.Microbiol vol.31 no.4 Sao Paulo.

Goda Y, Shimuzi T, Kato Y, Nakamura M, Maitani T, Yamada T, Terehara N, Yamaguchi M., 1996. Two acylated anthocynanins from purple sweet potato. Phytochem. 44(1): 183-186.

Goffin V, Binart N, Touraine P and Kelly PA., 2002. Prolactin: the new biology of an old hormone. Annual Review of Physiology 64 47–67.

Gregg A. Howe, 2001. Cyclopentenone signals for plant defense: Remodeling the jasmonic acid response. PNAS vol. 98 u no. 22 u 12317–12319.

Gregg C, Shikar V, Larsen P., 2007. "White matter plasticity and enhanced remyelination in the maternal CNS". *J. Neurosci.* **27** (8): 1812–23. Gretchen Humphries, 2000. Herbal Galactagogues

Gretchen Humphries, 2000. Herbal Galactagogues. MOBI Motherhood Intl

Goldhaber SB., 2003. Trace element risk assessment: essentiality vs. toxicity. Regulatory Toxicology and Pharmacology.;38:232-42.

Guggenbuhl, P., Seón, A., Piñón Quintana, A., Simões Nunes, C., 2007. Effects of dietary supplementation with benzoic acid (VevoVitall®) on the zootechnical performance, the gastrointestinal microflora and the ileal digestibility of the young pig. Livest. Sci. 108, 218–221.

Gupta.D.K, Srivastava.A, and A.P. Singh, 2004. Phytoremiditiation of Induced Lead Toxycity in *Vigna Mungo* L Hepper by Vetiver Grass in UGC project No 7-30-147/2004 (SR) New Delhi (cited from Goegle.com).

Hall. J.L., 2001. Cellular mechanisms for heavy metal detoxification and tolerance School of Biological Sciences, University of Southampton, Biomedical Sciences Building, Southampton SO16 7PX, UK.

Halliwell, B. & Gutteridge, J. M. C., 1989. *Free radicals in biology and medicine* (2nd ed.). Oxford: Clarendon press.

Han, C., J. Li and Q. Hui, 2008. Determination of trace elements in Jinqi a traditional Chinese medicine. Biol. Trace Elem. Res., 122: 122-126.

Harborne J. (2001), Twenty-five years of chemical ecology. Nat. Prod. Reports 18, 361D379.

Harborne J., Williams C. (2000): Advances in flavonoid research since 1992. Phytochemistry **55**: 481-504.

Heinecke, J. W., 1997. Mechanisms of ocidative damage of low density lipoprotein in human atherosclerosis Curr. Opin. Lipidol. 8 268 274.

Helal Uddin A.B.M., 2009. Infra Red (IR) Spectroscopy. Pharmaceutical Analysis. Laboratory Manual Practical-4. Departement of Pharmaceutical Chemistry. Kulliyah of Pharmacy, International Islamic Universiti Malaysia, Kuantan.

Helmut Fiegel, Heinz-Werner Voges, Toshikazu Hamamoto, Sumio Umemura, Tadao Iwata, Hisaya Miki, Yasuhiro Fujita, Hans-Josef Buysch, Dorothea Garbe, Wilfried Paulus, 2002. "Phenol Derivatives" in Ullmann's Encyclopedia of Industrial Chemistry, Wiley-VCH,: Weinheim.

Hermann, Janice R.. "Protein and the Body". *Oklahoma Cooperative Extension Service, Division of Agricultural Sciences and Natural Resources • Oklahoma State University*: T–3163–1 – T–3163–4.

Heyne K., 1999. Tumbuhan Berguna Indonesia. Jilid 1. Diterjemahkan oleh Badan Litbang Kehutanan Jakarta. Jakarta: Yayasan Sarana Jaya.

Hoebend D., C. Burvenich, and R. Heyneman. 1998. Antibiotics Commonly Used to Treat Mastitis and Respiratory Burst of Bovine Polymorphonuclear Leukocytes. Journal of Dairy Science Vol. 81, No. 2, 1998.

Hole. R.C , A.R. Juvekar, G. Roja, , Susan Eapen and S.F. D'Souza, 2008. Positive inotropic effect of the leaf extracts of parent and tissue culture plants of Coleus amboinicus on an isolated perfused frog heart preparation. Department of Pharmaceutical Sciences and Technology, Institute of Chemical Technology, University of Mumbai. India.

Holland, M.S and Holland, R.E, 2005. The cellular perspective on mammary gland development; stem/ progenitor cells and beyond. Journal Dairy Science 88 (E suppl.): E_1 – E8.

Holmgren. G.G.S., Meyer. M.W., Chaney. R.I., and Daniel. R.B., 1993. Cadmium, lead, zinc, copper and nikel in agricultural soils of the United Stated of America. J. Environ , Qual, 22, 335 – 348.

Holtz von R.L., C.S. Fink, A.B. , 1998. A wad β-Sitosterol activates the sphingomyelin cycle and induces apoptosis in LNCaP human prostate cancer cells Nutr Cancer, 32 (1998), pp. 8–12.

Hopkinson J, Schanler R, Garza C., 1988. "Milk production by mothers of premature infants". *Pediatrics* 81 (6): 815–20.

Hotta K, Okami Y., 1996. Diversity in aminoglycoside antibiotic resistance of actinomycetes
and its exploitation in the search for novel antibiotics. *J. Ind. Microbiol. Biotechnol. 17:352-358.*

Hsieh, F. S. (2004). Model and control holonic manufacturing systems based on fusion of contract nets and petri nets. *Automatica, 40*, 51–57.

Huang D, Ou, B. and Prior, R.L. 2005. The chemistry behind antioxidant capacity assays. J Agric Food Chem 53:1841. doi:10.1021/jf030723

Ibrahim Jantan 2004. Medicinal plants research in malaysia Scientific and advance. Jurnal Sains Kesehatan Malaysia 2 (2) 27-46.

Institute of Medicine. Food and Nutrition Board, 2001. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. National Academy Press, Washington, DC.

Irwandi, J. and Farida, O. 2009. Mineral and heavy metal contents of marine fin fish in Langkawi island, Malaysia. *International Food Research Journal,* 16,pp 105-112.

Irena Baranowska, and Dominika Raróg, 2001. Application of derivative spectrophotometry to determination of flavonoid mixtures.

Jacqueline C. Kent, 2007. How Breastfeeding Works. American College of Nurse-Midwives Published by Elsevier Inc. *Journal of Midwifery & Women's Health*.Volume 52, Issue 6, November-December 2007, Pages 564-570.

Jain, S. K., 2006. Superoxide dismutase overexpression and cellular oxidative damage in diabetes. A commentary overexpression of mitochondrial superoxide dismutase in mice protects the retina from diabetes Free Radical Biol. Med. 41 1187 1190.

Jain S.K., S Lata. 1996. Unique indigoneus amazonian uses of same plants growing in India. IK Manitor. 4 (3) Article 1996. *http://www.nuffic.nl./ciran/ikdm*.

Jarup L., 2003. Hazards of heavy metal contamination. Department of Epidemiology and Public Health, Imperial College, London, UK. l.jarup@imperial.ac.uk. PubMed - indexed for MEDLINE

Jeffrey T. Maehr, 2010. What Are Phytochemicals?. Pure Health Systems.

Jeffery Schwartz; Bryant, Carol A.; DeWalt, Kathleen Musante; Anita Courtney, 2003. *The cultural feast: an introduction to food and society*. Belmont, California: Thomson/Wadsworth. pp. 282, 283.

Jelena Janićijević1, Svetlana Tošić, Tatjana Mitrović, 2007. Flavonoids in plants. 9th Symposium on Flora of Southeastern Serbia and Neighbouring Regions. *Niš (Serbia), September 01-03, 2007. Proceeding, 153-156.*

Joe Singleton, 2011. Phytosterols, Cholesterol, Healthy Hearts – Best Kept Secrets. Ubscure.

John B. Ohlrogge and Jan G. Jaworski, 1997. Regulation of Fatty Acid Synthesis. *Annu. Rev. Plant Physiol. Plant Mol. Biol. 1997. 48:109–136.*

John Coates, 2000. Interpretation of Infrared Spectra, A Practical Approach. In *Encyclopedia of Analytical Chemistry.* R.A. Meyers (Ed.). Ó John Wiley & Sons Ltd, Chichester, pp. 10815–10837.

John H. Duffus 2002. "Heavy metals" a meaningless term? (IUPAC Technical Report)" Pure and Applied Chemistry, Vol. 74, pp. 793-807.

Johnson I., 2011. Phytochemicals Immune System Support. Woodhead Publishing Limited, 80 High Street, Sawston, Cambridge, CB22 3HJ, UK. Registered in England No. 2395953. Registered office: as above. VAT Reg No GB 538 2109 53.

Joon-Kwan Moon and Takayuki Shibamoto, 2009. REVIEWS: Antioxidant Assays for Plant and Food Components. *J. Agric. Food Chem.*, *57*, 1655–1666 1655.

Jose MA, Ibrahim, Janardhanan S., 2005. Modulatory effect of *Plectranthus amboinicus* Lour. on ethylene glycol induced nephrolithiasis in rats. Indian J Pharmacol 2005;37:43-4.

Joy PP, Thomas J, Mathew S, Skaria BP (1998). Zingiberaceous Medicinal and Aromatic Plants. Aromatic and Medicinal Plants Research Station, Odakkali, Asamannoor PO, Kerala, India, 236-238.

Julie Mennella, 2008. Alcohol's Effect on Lactation. *Monell Chemical Senses Center, Philadelphia, Pennsylvania.*

Juo, P. 1996. *Concise Dictionary of Biomedicine and Molecular Biology*. CRC Press: Boca Raton, FL, 1996.

Kabata-Pendias and S. Dudka, 1991. A Baseline data for cadmium and lead in soils and some cereals in Poland, *Water Air Soil Pollut.* **57–58** (1991), pp. 723–731.

Kaliappan ND, Viswanathan PK., 2008. Pharmacognostical studies on the leaves of Plectranthus amboinicus (Lour) spring. Int J Green Pharm.Vol 2, issue 3:182-184.

Karadag, A., Ozcelik, B. and Saner, S. 2009. Review of methods to determine antioxidant capacities. *Food Analysis Methods* (**2**): pp.41–60.

Karl-Georg Fahlbusch, Franz-Josef Hammerschmidt, Johannes Panten, Wilhelm Pickenhagen, Dietmar Schatkowski, Kurt Bauer, Dorothea Garbe, Horst Surburg 2005. Flavors and Fragrances" in *Ullmann's Encyclopedia of Industrial Chemistry*, Wiley-VCH,: Weinheim.

Karl H. Pegel, 1997. The importance of sitosterol and sitosterolin in human and animal nutrition. *outh African Journal of Science Vol. 93 June 1997*

Katerere, D. R., Gray, A. I., Nash, R. J., et al. (2003). Antimicrobialactivity of pentacyclic triterpenes isolated from African Combretaceae, *Phytochemistry* **63**, 81–8.

Katz, S. E., 1986. Microbiological Assay Procedures for Antibiotic Residues. In *Agricultural Uses of Antibiotics*; Moats, W. A., Ed.; ACS Symposium Series 320; American Chemical Society: Washington, DC,; pp 142-153.

Katznelson L, Klibanski A., 1999. Hyperprolactinemia: physiology and clinical approach. In: Krisht AF, Tindall GT, eds. Pituitary disorders: comprehensive management. Baltimore: Lippincott Williams & Wilkins. 189–98.

Kaur. C and Kapoor, H., 2001. "Review: antioxidants in fruits and vegetables – the millennium's health". *International Journal of Food Science and Technology* **36**: 703–725.

Kelvin Chan.K,.Sze-Yin.L and Sandy SZ., 2009. Harmonization of monographic Standards is needed to ensure the quality of Chinese medicine material, Bio med Central LTD.

Ken Burnside, 1990. Uses of Benzoic Acid in a Pharmacy Contributing to publications as diverse as "Pyramid" and "Training & Simulations Journal.

Khairi M.S. Fahelelbom, 2008. Analysis of Certain Tetracyclines and Oxytetracyclines through Charge Transfer Complexation. Science Publications. American Journal of Pharmacology and Toxicology 3 (3): 212-218.

Khalil MY, Moustafa AA and Naguib NY., 2007. Growth Phenolic compound and antioxidant activity of Some Medicinal Plants grown Under Organic Farming Condition. World Journal of agricultural Science 3(4): 451-457.

Kinsella JE, Lokesh B, Stone RA (1990). Dietary n_3 polyunsaturated fatty acids and amelioration of cardiovascular disease: possible mechanisms. Am. J. Clin. Nutr., 52(1): 1-28.

Kiss A, Mikkelsen JD., 2005. "Oxytocin anatomy and functional assisment: a minireview". *Endocrine Regulations* **39** (3): 97–105.

Kitagawa Shinji, Michio Takenaka and Yoshikazu Fukuyama, 2011. Recent Optimization Techniques and Applications to Customer Solution. Fuji Electric Review. Vol 50. No.3.

Kluge, H., Broz, J., Eder, K., 2006. Effect of benzoic acid on growth performance, nutrient digestibility, nitrogen balance, gastrointestinal microflora and parameters of microbial metabolism in piglets. J. Anim. Physiol. Anim. Nutr. 90, 316–324.

Koch, K.E., 1996. Carbohydrate-modulated gene expression in plants, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 47, pp. 509–540.

Kopke RD, Jackson RL, Coleman JK, Liu J, Bielefeld EC, Balough BJ (2007). "NAC for noise: from the bench top to the clinic". *Hear. Res.* **226** (1-2): 114–25.

Kris-Etherton, P.M., Hecker, K.D., Bonanome, A., Coval, S.M., Binkoski, A.E., Hilpert, K.F., Griel, A.E. and Etherton, T.D. 2002. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *American Journal of Medicine* **113:** pp. 71S–88S.

Krishnaiah Abburi, 2003. Adsorption of phenol and *p*-chlorophenol from their single and bisolute aqueous solutions on Amberlite XAD-16 resin. Elsevier B.V. All rights reserved.

Lacasse P., K. Lauzon, M. S. Diarra, and D. Petitclerc.2008. Utilization of lactoferrin to fight antibiotic-resistant mammary gland pathogens. *American Society of Animal Science. All rights reserved.* J. Anim. Sci. 2008. 86(Suppl. 1):66–71.

Laron Z, Klinger B, Erster B, Silbergeld A, 1989. Serum GH binding protein activity identifies for Laron type dwarfism. Acta Endocrinol 121: 603-608

Lau, W.S., (1999). Infrared characterization for microelectronics. World Scientific.

Lawrence Wilson, MD, 2012. Beyond antibiotics. The Center For Development

Lee HJ, Macbeth AH, Pagani JH, Young WS., 2009. "Oxytocin: the great facilitator of life". *Progress in Neurobiology* **88** (2): 127–51.

Lester A. Mitscher, Thomas L. Lemke, and Elmer J. Gentry, 2008. Antibiotic and Antimicrobial Agents in FOYE'S Principles of Medicinal Chemistry, Sixth Edition. Lippincott Williams & Wilkins, a Wolters Kluwer Business. Printed ini the USA, pp . 1028.

Leung FY. Trace elements that act as antioxidants in parenteral micronutrition. J Nutr Biochem. 1998;9:304–7.

Lia, P. K. and Roy, J. 2004. Antimicrobial and chemopreventive properties of herbs and spices. Current Medical Chemistry 47(2): 234-238.

Ljiljana Bjelakovic, Gordana Kocic, Ivana Stojanovic, Tatjana Jevtovic-Stoimenov, Stevo Najman, Dusan Sokolovic, Svetlana Stojanovic, Gordana Bjelakovic, 2012. Folic Acid Effect on Arginase Activity in Human Colostrum and Mature Milk

Li, Thomas S. C.; Beveridge, Thomas H.J., Drover, John C.G., 2006. Phytochemical content of sea buckthorn (Hyppophae rhamnoides L) seed oil: Extraction and identification". Food Chemistry (Elsevier). 101 (4): 1633–1639.

Liang. Y.L, M.B. Denton and R.B. Bates, 1998. *Journal. Chromatograph. Aplication* **827**, pp. 45–55..

Lin C.C., Huang P.C., 2000. Antioxidant and hepatoprotective effects of Acathopanax senticosus. Phytother. Res. 14:489-494.

Lide, D., 1992. CRC Handbook of Chemistry and Physics, 73rd Edition, Boca Raton, FL: CRC Press.

Lieberman, S, Bruning, N., 1990. The Real Vitamin & Mineral Book. NY: Avery Group, 3.

Lippard, Stephen J.; Jeremy M. Berg, 1994. *Principles of Bioinorganic Chemistry*. Mill Valley, CA: University Science Books. pp. 411.

Liu, J.; Mori, A., 2006. Oxidative damage hypothesis of stress-associated aging acceleration: neuroprotective effects of natural and nutritional antioxidants Res. Commun. Biol. Psychol. Psychiat. Neurosci. 30−31 103 119.

Liu, RH., 2004.. "Potential synergy of phytochemicals in cancer prevention: mechanism of action". *The Journal of nutrition* 134 (12 Suppl): 3479S–3485S.

Lukas Krahenbuhl, Jurg Reichen, Christine Talos and Stephan Krahenbuhl, 1997. Benzoic Acid Metabolism Reflects Hepatic Mitochondrial Function in Rats With Long-Term Extrahepatic Cholestasis. the American Association for the Study of Liver Diseases. Hepatology Vol. 25, No. 2, 1997.

Lukhobaa Catherine W., Monique S.J. Simmondsb, , and Alan J. Patonb, 2006. Plectranthus: A review of ethnobotanical uses. Journal of Ethnopharmacology Volume 103, Issue 1, 3 January 2006, Elsevier Ireland Ltd All rights reserved. Pages 1-24.

Lusk, J.E.; Williams, R.J.P., and Kennedy, E.P. (1968). "Magnesium and the growth of Escherichia coli". *Journal of Biological Chemistry* 243 (10): 2618–2624.

Macintyre, A., 2011. The Herbal For Mother And Child, ISBN 1852302445.

Mahmud M, K Slamet, DS Apriyantono, RR Hermana, 1990. Komposisi Zat Gizi Pangan Indonesia. Depkes RI., Direktorat Bina Gizi Masyarakat dan Pusat Penelitian dan Pengembangan Gizi, Jakarta.

Majaw.S and J.Moirangthem, 2009. Qualitative and Quantitative Analysis of *Clerodendron colebrookkianum* Walp. Leaves and *Zingiber cassumurnar* Roxb. Rhizomes, Department of Biotechnology and Bioinformatics, North Eastern Hill University, Meghalaya, India.

Malole MB, CS Pramono. 1989. *Penggunaan Hewan Percobaan di Laboratorium*. Departemen Pendidikan dan Kebudayaan. Direktorat Jenderal Pendidikan Tinggi. Pusat Antar Universitas Bioteknologi. Institut Pertanian Bogor, Bogor.

Manach C., Scalbert A., Morand C., Rémésy C., Jiménez L. (2004): Polyphenols: food sources and bioavailability. American Journal of Clinical Nutrition 79 (5): 727-747.

Maqbool A, Schall JI, Garcia-Espana JF., 2008. Serum linoleic acid status as a clinical indicator of essential fatty acid status in children with cystic fibrosis. J Pediatr Gastroenterol Nutr; 47:635.

Mardisiswojo S, H Rajakmangundarso. 1985. Cabe Puyang Warisan Nenek Moyang. Jakarta: PN Balai Pustaka.

Markaryan A. A., 2006. A New Method for Express Analysis of Flavonoid Fraction in Nephrophite Plant Extract and Prediction of Its Antioxidant Effects. Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 141, No. 6, pp. 715-717.

Marschner, H. (1995). *Mineral Nutrition in Higher Plants*. San Diego: Academic Press. ISBN 0-12-473542-8.

Mary K. Campbell, Shawn O. Farrell (2006). *Biochemistry* (5th ed.). Cengage Learning. p. 579.

Masakazu Horie, Harumi Takegami, Kazuo Toya and Hiroyuki Nakazawa, 2003. Determination of macrolide antibiotics in meat and fish by liquid chromatography–electrospray mass spectrometry. Elsevier B.V. All rights reserved.

Mathews, CK and van Holde, K. E., 1990. "Integration and control of metabolic processes". in Bowen, D.. *Biochemistry*. The Benjamin/Cummings publishing group. pp. 790–792.

Maton A, Hopkins J, McLaughlin CW, Johnson S, Warner MQ, LaHart D, Wright JD (1993). *Human Biology and Health*. Englewood Cliffs, Michigan, USA: Prentice Hall. ISBN 0-13-981176-1. OCLC 32308337.

Matsumuran, Minami S, Watanabe Y, Iyobe S, Mitsuhashi S., 1999. Roles of permeability in the activites of beta-lactams against gram-negative bacteria which produce a group 3 beta-lactamase. Antimicrob Agents Chemother 43: 2084-2086.

Mazza G, Miniati E., 1993. Anthocyanins in fruits, vegetables and grains. Boca Raton, Fla, USA: CRC Press; 1993.

McCrady, J. K. and Maggard, S. P.(1993). Uptake and Photodegradation of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin Sorbed to Grass Foliage. *Environ.Sci.Technol.* vol.27, pp. 343-350.

McDonnell DP, Norris JD., 1997. Analysis of the molecular pharmacology of estrogen receptor agonists and antagonists provides insights into the mechanism of action of estrogen in bone. Osteoporos Int 7: S29–S34.

McNeilly, A. S., 1997. "Lactation and fertility". *Journal of Mammary Gland Biology and Neoplasia* **2** (3): 291–298.

Meharg A.A and J. Hartley-Whitaker, 2002. Arsenic uptake and metabolism in arsenic resistant and nonresistant plant species, *New Phytol.* 154 (2002), pp. 29–43.

Mensor LL, Menezes FS, Leitao GG, Reis AS, dos Santos TC, Coube CS and Leitao SG., 2001. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytother. Res.*, **15**: 127-130.

.Mepham, T. B. 1987. Physiology of lactation. Open university press, Philadelphia, USA .

Middleton E Jr, Kandaswami C, Theoharides TC., 2000. The effects of plant flavonoids on mammalian cells: Implications for inflammation, heart disease, and cancer. Pharmacol Rev;52:67-751.

Mikko J Antonen, 2007. Evaluation of Means to Increase the Content of Bioactive Phenolic Compounds in Soft Fruits. Kuopio University Publications C. Natural and Environmental Sciences 208.. 93 p.

Milo Wiltbank, Hermando Lopez, Roberto Sartori, Siwat Sangsritavong, Ahmet Gumen. 2005. Changes in reproductive physiology of lactating dairy cows due to elevated steroid metabolism. Department of Dairy Science, University of Wisconsin-Madison, 1675 Observatory Drive, Madison, WI 53706, USA.

Miloje Savic, Josip Lovrić, Tatjana Ilic Tomic, Branka Vasiljevic and Graeme L. Conn, 2009. Determination of the target nucleosides for members of two families of 16S rRNA methyltransferases that confer resistance to partially overlapping groups of aminoglycoside antibiotics cited in Schatz A, Bugie E, Waksman SA. *Streptomycin, a substance exhibiting antibiotic activity against gram-positive and gram-negative bacteria. Proc. Soc. Exp. Biol. Med. 1944;55:65-69.*

Ministry of Agriculture, Fisheries and Food, 1982. Survey of arsenic in food, Her Majesty's Stationary Office, London.

Mohrbacher, Nancy; Stock, Julie, 2003. *The Breastfeeding Answer Book* (3rd ed. (revised) ed.). La Leche League International.

Monmarche Philippe and Laurent Sousselier, 2001. New insights into the cosmetic benefits of shea butter. *The World Directory for the Cosmetics Industry (Teknoscienze ed).*

Moreira, P.; Smith, M. A.; Zhu, X.; Honda, K.; Lee, H.-G.; Aliev, G.; Perry, G., 2005. Since oxidative damage is a key phenomenon in Alzheimer's disease, treatment with antioxidants seems to be a promising approach for slowing disease progression. Oxidative damage and Alzheimer's disease: are antioxidant therapies useful? Drug News Perspect. 18,13–19.

Moriwaki K, Sagai T, Shiroishi T, Bonhomme F, Wang C-H, He X-Q, Jin M-L, Wu Z-G. 1990. Mouse subspecies differentiation and H-2 polymorphism. Biol J Linn Soc 41:125-139.

Morton J.F., 1992. Country borage (*Coleus amboinicus* Lour.): a potent flavoring and medicinal plant, Journal of Herbs, Spices Medicinal Plants 1 (1992), pp. 77–90.

Moss G. P., 1989. " Nomenclature of Steroids (Recommendations 1989). *Pure & Appl. Chem.* 61 (10): 1783–1822. "IUPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN). The nomenclature of steroids. Recommendations 1989". *Eur. J. Biochem.* 186 (3): 429–58. December 1989.

Mukherjee, A. B.; Zhang, Z.; Chilton, B. S., 2007. Uteroglobin: a steroid-inducible immunomodulator protein that founded the Secretoglobin superfamily Endocrine Rev. 28 707 725.

Must, A., et al. 1999. "The Disease Burden Associated with Overweight and Obesity." *Journal of the American Medical Association* 282: 1523.

Nadkarni AK (2002). Indian Materia Medica. Vol.1. 3rd ed. Popular Prakashan, Bombay, India;. p. 371-72.

Naito, Y.; Uchiyama, K.; Yoshikawa, T., 2006. Oxidative stress involvement in diabetic nephropathy and its prevention by astaxanthin Oxid. Stress Disease 21 235 242.

Nakamura. Y, S. Watanabe, N. Miyake, H. Kohno, T.Osawa, 2003. J.Agric Food Chem., 51, 2003,3309-3312. In D. Marinova, F.Ribarova and M.Atanassova, (2005). Total Phenolics and Total Flavonoids in Bulgarian Fruits and Vegetables. Journal of the University of Chemical Technology and Metallurgy, 40, 3, 2005, 255-260.

Namiesnik J, Wardenski W (2000) ."Application of plants for monitoring of environmental pollution", Herba Polonica, 46:198-212.

Nancy M. Hurst, 2007. Feature Recognizing and Treating Delayed or Failed Lactogenesis II. Journal of Midwifery & Women's Health. Volume 52, Issue 6, November-December 2007, Pages 588-594.

Nature's Sunshine Products Follows Numerous Manufacturing Guidelines, 2001. MAGNESIUM, Nervous System Support. Nature's Sunshine Products, Inc.

Neetu Vijay 1, Pramod Sairkar, Nipun Silawat, R. K. Garg1 and N. N. Mehrotra, 2009. Genetic variability in *Asparagus racemosus* (Willd.) from Madhya Pradesh, India by random amplified polymorphic DNA. African Journal of Biotechnology Vol. 8 (14), pp. 3135-3140.

Neil K. Kaneshiro, MD, MHA, 2009. Fata. Clinical Assistant Professor of Pediatrics, University of Washington School of Medicine.

Nelson D. R., Kamataki T., Waxman D. J., Guengerich F. P., Estabrook R. W., Feyereisen R., Gonzalez F. J., Coon M. J., Gunsalus I. C., Gotoh O., Okuda K., Nebert D. W.1993. *DNA Cell Biol. 12:1–51.*

Neve J. In: P. Collery, P. Bratter, V. Negretti de Bratter, I. Khassanova and J.C. Etienne, 1998. Editors, *Bioavailability and Safety of Selenium Supplements*, John Libbey Eurotext, Paris , p. 79.

Nijveldt RJ, van Nood E, van Hoorn DE, Boelens PG, van Norren K, van Leeuwen PA 2001. Flavonoids: a review of probable mechanisms of action and potential applications. Am J Clin Nutr.;74:418-25.

Neville. M.C, Mrton. J, Umemura. S., 2001. Lactogenesis. The transition from pregnancy to lactation. Pediatr Clin North Am 2001 Feb;48(1):35-52.

Nriagu. J.O., 1989. A global assessment of natural sources of atmospheric trace metals. *Nature*, 338, 47 – 49.

Nuria Rodriguez Æ., So` nia Roig Æ., Nu´ ria Olmedo, Jose´ G. Lore´n Æ Nu´ ria Rius, 2004. A new bioassay method for quantitative analysis of tetracyclines. Society for Industrial Microbiology.

Okwu.D.E., 2001. Evaluation of the chemical composition of indigenous spices and flavoring agents. Global J.Pure Appall Si. 7 (3) : 455-459.

Oliver SP, Lewis MJ, Gillespie BE, Dowlen HH, Jaenicke EC, Roberts RK., 2003. Prepartum pengobatan antibiotik sapi-sapi: produksi susu, kualitas susu dan manfaat ekonomi. J Dairy Sci. 2003 Apr, 86 (4) :1187-93.

Oliveira, F. A., Vieira-Junior, G. M., Chaves, M. H., et al. (2004). Gastroprotective effect of the mixture of alpha- and beta-amyrin from. Protium heptaphyllum: role of capsaicin-sensitive primary afferent neurons, *Planta. Med* **70**, 780–2.

Oliveira, F. A, Chaves, M. H, Almeida, F. R., et al. (2005). Protective effect of alpha- and beta-amyrin, a triterpene mixture from *Protium heptaphyllum* (Aubl.) March. Trunk wood resin, against acetaminophen-induced liver injury in mice, *J. Ethnopharmacol* **98**,103–8.

Omolo M.O., D. Okinyo, I.O. Ndiege, W. Lwande and A. Hassanali, 2004. Repellency of essential oils of some Kenyan plants against Anopheles gambiae, Phytochemisty 65 (2004), pp. 2797–2802.

Onyilagha JC, Grotewold E., 2004. The biology and structural distribution of surface flavonoids. Recent Res Devel Plant Sci;2:53-71.

Osawa T (1999). Protective role of dietary polyphenols in oxidative stress. Mech. Ageing Dev. 111(2-3): 133-139.

Ostlund RE, Racette SB, Stenson WF., 2003. "Inhibition of cholesterol absorption by phytosterol-replete wheat germ compared with phytosterol-depleted wheat germ". *Am. J. Clin. Nutr.* **77** (6): 1385–9.

Padma PR, Bhuvaneswari V, Chelvi SK (1988). The activities of enzymic antioxidant in selected green leaves. Indian J Nutr Diet 1988;35:1-3.

Pankey GA and Sabath LD.,. 2004. "Clinical relevance of bacteriostatic versus bactericidal mechanisms of action in the treatment of Gram-positive bacterial infections.". Clin Infect Dis. 38 (6): 864–870. doi:10.1086/381972. PMID 14999632.

Parekh, J., Chanda, S., 2006. Screening of some Indian medicinal plants for antibacterial activity., Indian J Pharm Sci,:68, 835-838.

Pascual M.E, Carretero ME, Slowing K.V, Villar A., 2002. Simplified screening by TLC of plant drugs. Pharmaceutical Biology, 40(2): 139-142.

Pastrorova I, de Koster CG, Boom JJ (1997). "Analytic Study of Free and Ester Bound Benzoic and Cinnamic Acids of Gum Benzoin Resins by GC-MS HPLC-frit FAB-MS". *Phytochem Anal* **8**: 63–73.

Patel A.K., P.G. Koringa, K.N. Nandasana, U.V. Rahmani, H.V. Goriya and K.M. Panchal, 2007. Comparative Histology of Mammary Gland in Heifer, Pregnant, Lactating and Dry Buffaloes. Indian Journal of Veterinary Anatomu 19 (1): 71-78 June 2007.

Patricia A. Masso-Welch,1,2 Kathleen M. Darcy,1 Nannette C. Stangle-Castor,1 and Margot M. Ip, 2000. A Developmental Atlas of Rat Mammary Gland Histology*Journal of Mammary Gland Biology and Neoplasia, Vol. 5, No. 2, 2000.*

Paz-Elizur, T.; Sevilya, Z.; Leitner-Dagan, Y.; Elinger, D.; Roisman, L. C.; Livneh, Z., 2008. DNA repair of oxidative DNA damage in human carcinogenesis: Potential application for cancer risk assessment and prevention Cancer Lett. 266 60 72.

Pennington and Doglas, 2005. Food Values of Portions Commonly Used, 18th Ed

Percival, M., 1998. "Antioxidants". *Clinical Nutrition Insights* 1/96 Rev. 10/98. http://acudoc.com/

Perry E.K., Pickering A.T., Wang W.W., Houghton P.J., Perru N.S., 1999. *Medicinal plants and Alzheimer's disease: from ethnobotany to phytotherapy. J. Pharm. Pharmacol. 1999;51:527-534.*

Peterson J, Dwyer J. 1998. Taxonomic classification helps identify flavonoid-containing foods on a semiquantitative food frequency questionnaire. *J Am Diet Assoc* 1998;98:682-5.

Pilon Smits, E.A.H.; Desouza, M.P.; Hong, G.; Amini, A.; Bravo, R.C.; Payabyab, S.T. and Terry, N., 1999. Selenium volatilization and accumulation by twenty aquatic plant species. *Journal of Environmental Quality*, vol. 28, no. 3, p. 1011-1017.

Pino J.A., J. Garcia and M.A. Martinez, 1996. Comparative chemical composition of the volatiles of Coleus aromaticus produced by steam distillation, solvent extraction and supercritical carbon dioxide extraction, Journal of Essential Oil Research 8 (1996), pp. 373–375.

Pirotta MV, Garland SM., 2006. "Genital Candida species detected in samples from women in Melbourne, Astralia, before and after treatment with antibiotic". *J Clin Microbiol*. 44 (9): 3213–3217.

Prasad S, Naik P, Vijayalaxmi KK., 2002. Efficiency of *Coleus aromaticus* extract in modifying cyclophosphamide and mitomycin-C induced clastogenicity in mouse bone marrow cells. Indian J Exp Biol 2002;40:1020-5.

Price, N. M. and Morel, F. M. M., 1990. 'Cadmium and cobalt substitution for zinc in a marine diatom, *Nature* 344, 658–660.

Preedy, V. R.; Reilly, M. E.; Mantle, D.; Peters, T. J., 1998. Oxidative damage in liver disease J. Int. Fed. Clin. Chem. 10 16 20.

Prior RL, Wu X, Schaich K., 2005. Standardized methods for the determination of Antioxidant capacity and phenolics in foods and dietary supplements. J. Agric. Food Chem., 53(10): 4290-4302.

Prudent, D., Perineau, F., Bessiere, J.M., Michel, G.M., Baccou, J.C., 1995. Analysis of the essential oil of wild oregano from Martinique (*Coleus aromaticus* Benth.): evaluation of its bacteriostatic and fungistatic properties. Journal of Essential Oil Research 7, 165–173.

Purseglove J.W., 1987. Tropical Plants. Dicotyledons, Longman Scientific & Technical. In

Qin G.W., Xu R.S. 1998: Recent advances on bioactive natural products from Chinese medicinal plants. Med. Res. Rev. Nov., 18: 375–382.

Quinn., M.J., and Sherlock. J.C., 1990. The correspondence between U.K."action levels" for lead in blood and in water. *Food Addit Contain*. 7(3), 387 – 424.

Ramar Perumal Samy, Peter Natesan Pushparaj and Ponnampalam Gopalakrishnakone, 2008. A compilation of bioactive compounds from Ayurveda. Bioinformation by Biomedical Informatics Publishing Group.

Rangasamy ,O .Raoelison,G. Fransisco,E , Rakoloiriana, K,Chenk, Suzane U.R, Jullie,O.L, Ammenah G,F and Anwar H,S., 2007. Screening for anti infective properties of several medicinal plants of the Mauritians flora. Journal of Etnopharmacology Vol 109 issue 2, pp 331-337.

Rao, AV; Rao, LG., 2007. "Carotenoids and human health". *Pharmacological research* **55** (3): 207–216.

Rao Y.K., S.H. Fang, Y.M. Tzeng, 2006. Anti-inflammatory activities of constituents isolated from *Phyllanthus polyphyllus*J. Ethnopharmacol., 103 (2) (2006), pp. 181–186.

Rao. A and Balachandran B., 2002. "Role of oxidative stress and antioxidants in neurodegenerative diseases". *Nutr Neurosci* **5** (5): 291–309.

Rao A.V, S.A. Janezic, 1992. The role of dietary phytosterols in colon carcinogenesis Nutr Cancer, 18 (1992), pp. 43–52.

Ray-Yu Yang, Shou Lin and George Kuo., 2008. Content and distribution of flavonoids among 91 edible plant species. *Asia Pac J Clin Nutr 2008;17(S1):275-279.*

Rayman M.P., 1997. *Br. Med. J.* **314**, p. 387. View Record in Scopus Cited By in Scopus (144).

Ressang A.A., 1984. *Patologi Khusus Veteriner*. Second edition. Denpasar – Bali, Indonesia. N. V. Percetakan.

Rhee KY, Gardiner DF., 2004. "Clinical relevance of bacteriostatic versus bactericidal activity in the treatment of gram-positive bacterial infections". Clin. Infect. Dis. 39 (5): 755–6. doi:10.1086/422881. PMID 15356797.

Ridley, M., 2006. *Genome*. New York, NY: Harper Perennial. ISBN 0-06-019497-9.

Roberts, J.R., 1999. Metal toxicity in children. In Training Manual on Pediatric Environmental Health: Putting It into Practice 1999 Jun. Emeryville, CA: Children's Environmental Health Network (http://www.cehn.org/ cehn/ trainingmanual/pdf/manual-full.pdf).

Robinson, Corinne H.; Weigley, Emma S.; and Mueller, Donna H., 1993. *Basic Nutrition and Diet Therapy*, 7th edition. New York: Macmillan.

Rodriguez-Amaya D., 2003. "Food carotenoids: analysis, composition and alterations during storage and processing of foods". *Forum Nutr* **56**: 35–7.

Rojas, A., Duque, E., Schmid, A., Hurtado, A., Ramos, J.-L., Segura. A., 2004. Biotransformation in Double-Phase Systems: Physiological Responses of Pseudomonas putida DOT-T1E to a Double Phase Made of Aliphatic Alcohols and Biosynthesis of Substituted Catechols. *Appl. Environ. Microbiol.* 70: 3637-3643.

Rolland F. *et al.*, 2002. Sugar sensing and signaling in plants, *Plant Cell* 14 (Suppl.), pp. S185–S205.

Rongjie Fu, 2009. Analysis of Aminoglycoside Antibiotics by Reversed-Phase HPLC Agilent Technologies, Inc., Printed in the USA.

Roshan P., Naveen M., Manjul PS, Gulzar A., Anita S., Sudarshan S., 2009. *Plectranthus amboinicus* (Lor) Spreng: An Overview. The Pharma Research (T. Ph. Res.), (2010), 4; 01-15.

Rossier MF., 2006. "T channels and steroid biosynthesis: in search of a link with mitochondria". *Cell Calcium.* 40 (2): 155–64.

Rubis B, Paszel A, Kaczmarek M, Rudzinska M, Jelen H, M. Rybczynska, 2008. Menguntungkan atau merugikan pengaruh pitosterol pada sel manusia?. Br J Nutr. 2008 Dec, 100 (6) :1183-91. Epub 2008 Apr 29.

Russo J, Russo IH, 2004. *Molecular Basis of Breast Cancer: Prevention and Treatment*. Heidelberg: Springer-Verlag; 2004.

Sabu M Chacko1, Priya T Thambi1, Ramadasan Kuttan and Ikuo Nishigaki, 2010. Beneficial effects of green tea: A literature review. Chinese Medicine 2010, 5:13 doi:10.1186/1749-8546-5-13.

Said HM, Mohammed ZM (2006). Intestinal absorbtion of water-soluble vitamin: an update.. *Curr. Opin. Gastroenterol.* 22 (2): 140–6.

Salisbury, in H. Oka, H. Nakazawa, K. Harada, J.D. McNeil (Editors), *Chemical analysis for antibiotics used in agriculture*, AOAC Int., Arlington. 1995. Ch. 6 p.165.

Salt DE, M Blay lock NPB, D,.Kumar, V Duncheukov, BD Ensley, I Chet and I Raskin, 1995. Phytoremediation. A novel strategy for the Removal of Toxic metal from the Environment using Plant. Biotechnology 13:468-474.

Sandalio, L.M., Dalurzo, H.C., Gomez, M., Romero-Puertas, M.C., del Rio, L.A., 2001. Cadmium-inducedchanges in the growth and oxidative metabolism of pea plants. J. Exp. Bot. 52, 2115-2126.

Scientific Committee on Consumer Products (SCCP), 2005. Opinion on Benzoic Acid and Sodium Benzoate. European Commission. Health &Consumer Protection Directorate General.

Scully KM, Gleiberman AS, Lindzey J, Lubahn DB, Korach KS, Rosenfeld MG. 1997. Role of estrogen receptor-a in the anterior pituitary gland.Mol Endocrinol 11: 674–681.

Sepulveda, R. T.; Watson, R. R., 2002. Treatment of antioxidant deficiencies in AIDS patients Nutr. Res. (N.Y.) 22 27 37.

Shahidi F, Naczk M., 2003. Phenolics in food and nutraceuticals. Boca Raton, Fla, USA: CRC Press; 2003.

Shalini Joshi, 2002. HPLC separation of antibiotics present in formulated and unformulated samples. Department of Chemistry, K.L.D.A.V. College, Roorkee 247 667, India. Cited from Korolkovas. A and J.H. Burckhalter In: *Essentials of Medicinal Chemistry*, John Wiley & Sons, New York (1976), pp. 502–503.

Sherman Hsu, C.-P., 2011. Infrared Spectroscopy. Handbook of Instrumental Techniques for Analytical Chemistry. *Separation Sciences Research and Product Development Mallinckrodt, Inc.Mallinckrodt Baker Division*

Sies H., 1997. "Oxidative stress: oxidants and antioxidants (PDF). *Exp Physiol* **82** (2): 291–5.

Singh A.P., 2006. Short review : Distribution of steroid like Compound in Plant Flora. Pgarmacognosy magazine, 2(6) :87-89.

Singgih, Maria, 2007. Uji pootensi antibiotic http://digilib.si.itb.ac.id/go .php? id=jbptitbpp-gdl-s2-1990-sudding-1734.

Slama TG, Amin A, Brunton SA., 2005. "A clinician's guide to the appropriate and accurate use of antibiotics: the Council for Appropriate and Rational Antibiotic Therapy (CARAT) criteria". *Am. J. Med.* 118 Suppl 7A: 1S–6S.

Stampfer MJ,Colditz GA,WillettWC,Manson JE, Rosner B,Speizer FE, Hennekens CH., 1991. Postmenopausal estrogen therapy and cardiovascular disease. Ten-year follow up from the nurses' health study. N Engl J Med 325:756–762.

Stapleton PD, Shah S, Anderson JC, Hara Y, Hamilton-Miller JMT, Taylor PW., 2004. Modulation of β-lactam resistance in *Staphylococcus aureus* by catechins and gallates. Int J Antimic Agents 23:462-467.

Stephen Boyd and Lieven Vandenberghe, 2004. Convex Optimization. Printed in the United Kingdom at the University Press, Cambridge.

Steven M Anderson, Michael C Rudolph, James L McManaman and Margaret C Neville, 2007. Key stages in mammary gland development Secretory activation in the mammary gland: it's not just about milk protein synthesis!. This article is online at http://breast-cancer-research.com/content/9/1/204.BioMed Central Ltd.

Steven D. Ehrlich, NMD, 2009. Solutions Acupuncture, a private practice specializing in complementary and alternative medicine, Phoenix, AZ. Review provided by VeriMed Healthcare Network.

Subhas C. Joshi, Arti R. Verma and Chandra S. Mathela, 2010. Antioxidant and antibacterialactivities of the leaf essential oils of Himalayan Lauraceae species. Journa Food and Chemical Toxicology. Volume 48, Issue 1, 1 January 2010, Page 37 – 40.

Suwari and Darmakusuma, 2005. Uji Toksisitas Ekstrak Ethanol *Sida acuminate*, Journal of Phytochemistry and Nutraceutical Research- ISSN 1829-9792, No.1 Vol.2.

Suzuki K, Nagai K, Shimizu Y, Suzuki Y., 1994. Search for actinomycetes in screening for new bioactive compounds. Actinomycetologica ;8:122-127.

Sweetman SC., 2002. Antibacterials. In: Martindale (ed) The complete drug reference, 33rd edn. Pharmaceutical Press, London, pp 258–261.

Tanaka N. In: H. Umezawa and I.R. Hooper, 1982. Editors, *Aminoglycoside Antibiotics*, Springer–Verlag, Berlin, p. 221.

Tapeiro. H, K.D Tew, G.N Ba, G. Mathe, 2002. Biomed Pharmacother., 56, 2002, 200-207. In D. Marinova, F.Ribarova and M.Atanassova, 2005. Total Phenolics and Total Flavonoids in Bulgarian Fruits and Vegetables. Journal of the University of Chemical Technology and Metallurgy, 40, 3, 2005, 255-260.

Tapsell LC, Hemphill I, Cobiac L., 2006. "Health benefits of herbs and spices: the past, the present, the future". *Med. J. Aust.* 185 (4 Suppl): S4–24.

Terri Armstrong, RN, MS, NP, CS., 1997. Clinical Trials for Brain Tumor. The National Brain Tumor Foundation. Neuro-Oncology Nurse Practitioner Emory University, Atlanta, Georgia.

Tewari. D.N., 2000. Report of the Task Force on Conservation and Sustainable use of medicinal plants. Government of India planning Commission (In March, 2000).

Thomson Gale, 2006. Mammary Glands and Lactation. *World of Anatomy and Physiology*.

Thomson CD., 2004. Assessment of requirements for selenium and adequacy of selenium status: a review. Eur J Clin Nutr;58:391-402.

Tikkanen MJ, Wahale K, Ojala S, Vihma V, Aldercreutz, 1998. Effect of soya bean phytoestrogen intake on low density lipoprotein oxidation resistance. Proc. Natl. Acad. Sci. U.S.A. 95: 3106-3110.

Torrallardona, D., Badiola, I., Broz, J., 2007. Effects of benzoic acid on performance and ecology of gastrointestinal microbiota in weanling piglets. Livest. Sci. 108, 210–213.

Umezawa H and I.R. Hooper, 1982. *Aminoglycoside Antibiotics*, Springer–Verlag, Berlin.

University of Michigan Health System, 2006. Antibiotic-Associsted Diarrhea.

Valvo L., L. Manna, R. Alimenti, S. Alimonti, P. Bertocchi and E. Cirrani *J. Pharm. Biomed. Anal.* **21** (1999), pp. 9–14. In Shalimi, 2002.

Van de Wiel, H. J. (2003). Determination of elements by ICPAES and ICP-MS. Bilthoven: National Institute of Public Health and the Environment (RIVM).

Van Spall HG, Toren A, Kiss A, Fowler RA., 2007. "Eligibility criteria of randomized controlled trials published in high-impact general medical journals: a systematic sampling review". *JAMA* 297 (11): 1233–40.

Varahalarao Vadlapudi and K. Chandrasekhr Naidu, 2009. Evaluation of Antioxidant potential of selected Mangrove Plants. Journal of Pharmacy Reseach 2009, 2(11), 1742-1745.

Varsha M., Madaan .N and Mudgal, A., 2010. Heavy Metal in Plants : Phytoremediation plants used to remedied heavy metal pollution. Agricultured and Biology Journal of North America, ISSn print , 2151-7517 ISSN online, 2151-7525.

Vasquez EA, W Kraus, AD Solsoloy, BM Rejesus, 2000. The use of Species and Medicinal : antifungal, antibacterial, anthelmentic, and molluscicidal constituents of phillippine plant, http://www.faoorg/dorcep/x2230e/x2230e8.

Veenema AH, Neumann ID., 2008. "Central vasopressin and oxytocin release: regulation of complex social behaviours". *Progress in Brain Research* 170: 261–76.

Vincent du Vigneaud, Ressler C, Swan JM, Roberts CW, Katsoyannis PG, Gordon S 1953. "The synthesis of an octapeptide amide with the hormoneal activity of oxytocin". *J. Am. Chem. Soc.* 75 (19): 4879–80.

Walters, F. H., Parker, L. R., Morgan, S. L., Deming, S. N., 1991. *Sequential Simplex Optimization.* CRC Press, Boca Raton, Florida.

Warner D, Sheng H, Batinić-Haberle I., 2004. "Oxidants, antioxidants and theischemic brain". *J Exp Biol* 207 (Pt 18): 3221–31.

Warrier PK, Nambiar VP, Ramankutty, 1996, editors. Indian medicinal plants, 1 st ed, Orient Logman Limited: Madras; 1995. p. 315.

Warth. A.D., 1991. "Mechanism of action of benzoic acid on Zygosaccharomyces bailii; effects on glycolytic metabolite levels, energy production, and intracellular pH". *Appl Environ Microbiol. 1991 December* **1** (12): 1.

Watanabe, T.; Shimbo, S.; Moon, C.S.; Zhang, Z.W.; Ikeda, M., 1996. Cadmium contents in rice samples available from various areas in the world. *Sci. Total Environ., 184*, 191-196.

Waterman, P.G and Mole, S., 1994. Analysis of Phenolic Plant Metabolites, pp 83 – 85. The method is modified to suite the number of students involved in the practical work and laboratory facilities.

Weiler, E.W., Laudert, D., Stelmach, B.A., Hennig, P., Biesgen, C. and Kubigsteltig, I. (1999) Octadecanoid and hexadecanoid signalling in plant defence. In Insect-plant interactions and induced plant defence. Wiley, Chichester (Novartis Foundation Symposium 223), 191-204.

Whitney, Elanor and Sharon Rolfes, 2005. *Understanding Nutrition, 10th edition*, p 6. Thomson-Wadsworth.

Wijayakusuma H, Dalimartha S, Wirian AG., 1996. Tanaman obat berkhasiat Indonesia IV. Edisi 1. Jakarta: Pustaka Kartini.

Wildman REC., 2001. Nutraceuticals: A brief review of historical and teleological aspects, In: Wildman REC ed. Handbook of Nutraceuticals and Functional Foods. CRC press: Boca Raton, FL;1-12.

Williams, D. and L. Thomas, 2002. Foye's Principles of Medicinal Chemistry. 5th Edn., Lippincott Williams and Wilkins, Philadelphia, USA., ISBN: 0683307371, pp:1114.

Winkel-Shirley B., 2001. Flavonoid biosynthesis. A colourful model for genetics, biochemistry, cell biology, and biotechnology. Plant Physiol.;126:485-93.

Wiseman H, Okeilly JD, Aldlercreutz H, Mallet AJ, Bowery EA, Sanders AB., 2000. Isoflavones phytoestrogen consumed in soya decrease F2-isoprostane concentrations and increase resistance of low-density lipoprotein to oxidation in humans. Am. J. Clin. Nutr. 72: 397- 400.

Wobus U. and H. Weber, 1999. Sugars as signal molecules in plant seed development, *Biol. Chem.* **380**, pp. 937–944.

World Health Organization, Food an Agriculture Organization, of the United Nation, United Nation University, 2007. "Protein and amino acid requirements in human nutrition" (PDF). WHO Press.

World Health Organization (WHO), 2007. *Monographs on selected medicinal plants, Volume 3* (ISBN 978 92 4 154702 4).

Worley, J. and Kvech, S. 2000. ICP-MS. High resolution inductively coupled plasma mass spectrometry. http://zonvark.wustl.edu/icpms/hires.html. Visited January 2011.

Xamplified, (2010). Functions of Carbohydrate. All Rights are Reserved. Designed by Tact9 Media.

Xianquan S, Shi J, Kakuda Y, Yueming J., 2005. "Stability of lycopene during food processing and storage". *J Med Food* **8** (4): 413–22.

Yao JDC, Moellering RC., 2003. Antibacterial agents. In: Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken RH (eds) Manual of clinical microbiology, 8th edn. ASM Press, Washington, D.C., pp 1039–1073.

Yasue, H., T. Itoh, T. Mizuno Y and E. Harada, 2007. Severe hypokalemia, rhabdomyolysis, muscle paralysis and respiratory impairment in a hypertensive patient taking herbal medicines containing licorice. Int. Med., 46; 575-578.

Yazigi RA, Quintero CH, Salameh WA., 1997. Prolactin disorders. *Fertil Steril*. ;67:215–25.

Yoshizawa K., W.C. Willett, S.J. Morris, M.J. Stampfer, D. Spiegelman, E.B. Rimm and E. Giovannucci, 1998. .*J. Natl. Cancer Inst.* 90, p. 1219.

Yuh-Shan Ho and Mohammad I. El-Khairy, 2009. Metal Research trend in Environmental Field Text book Heavy Metals in The Environment.CRC Press. Taylor and Francis Group. London. New York.

Zhang G. , B. Keita, A. Dolbecq, P. Mialane, F. Se'cheresse and F. Miserques, 2007. Green chemistry-type one step synthesis of silver nanostructures based on MoV-MoVI mixed-valence polyoxometales, *Chem Mater* **19**, pp. 5821–5823.

Zhu BT,. 2002. Catechol-*O*-Methyltransferase (COMT)–mediated methylation metabolism of endogenous bioactive catechols and modulation by endobiotics and xenobiotics: importance in pathophysiology and pathogenesis. Curr Drug Metab;3:321–49.

APPENDIX A1

RESULT OF TORBANGUN PLANT IDENTIFICATION

LEMBAGA ILMU PENGETAHUAN INDONESIA
(Indonesian Institute of Sciences)
PUSAT PENELITIAN BIOLOGI
(Research Center for Biology)
Jl. Raya Jakarta - Bogor Km. 46 Cibinong 16911, Indonesia P.O Box 25 Cibinong
Telp. (021) 87907636 - 87907604 Fax. 87907612

Cibinong, 23 Agustus 2010

Nomor : 1033/IPH.1.02/If.8/VIII/2010
Lampiran : -
Perihal : *Hasil identifikasi/determinasi Tumbuhan*

Kepada Yth.
Bpk./Ibu/Sdr(i). Awalludin Risch
BOGOR

Dengan hormat,

Bersama ini kami sampaikan hasil identifikasi/determinasi tumbuhan yang Saudara kirimkan ke "Herbarium Bogoriense", Bidang Botani Pusat Penelitian Biologi-LIPI Bogor, adalah sebagai berikut :

No.	No. Kol.	Jenis	Suku
1	-	*Coleus amboinicus* Lour.	Lamiaceae

Demikian, semoga berguna bagi Saudara.

Kepala Bidang Botani
Pusat Penelitian Biologi-LIPI,

Prof. Dr. Eko Baroto Walujo
NIP. 195111041975011001

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APPENDIX. A2

PRINCIPAL COMPONENT ANALYSIS

Observations/variables table: Workbook = XLSTAT.TORBANGUN.xlsx / Sheet = Sheet1 / Range = Sheet1!$B$1:$BE$6 / 5 rows and 56 columns. Observation labels: Workbook = XLSTAT.TORBANGUN.xlsx / Sheet = Sheet1 / Range = Sheet1!$A$1:$A$6 / 5 rows and 1 column. PCA type: Pearson (n).

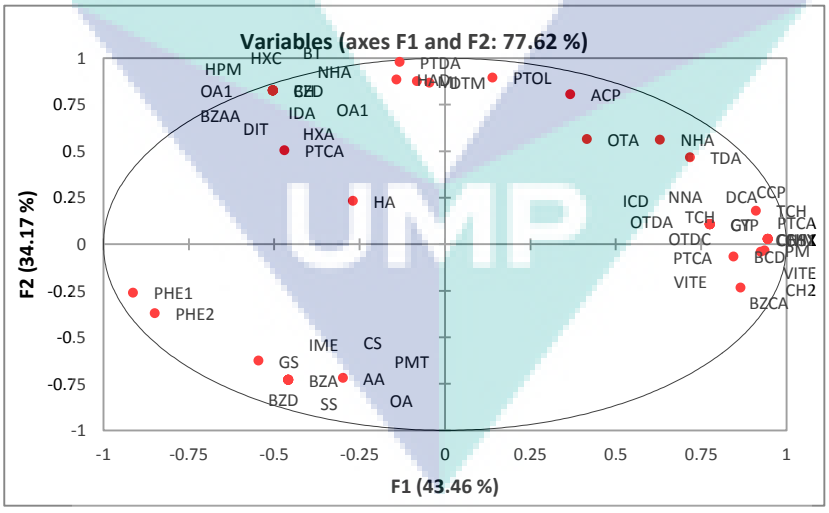
Rotation: Varimax (Kaiser normalization) / Number of factors = 13

Type of biplot: Correlation biplot / Coefficient = n/p

Correlations between variables and factors:

	F1	F2	F3	F4
PM	0.936	-0.034	-0.098	0.336
MV	0.946	0.028	-0.052	0.320
CCHX	0.946	0.028	-0.052	0.320
PHE1	-0.912	-0.260	-0.281	-0.145
ACP	0.367	0.807	-0.265	0.380
CHSX	0.946	0.028	-0.052	0.320
CH1	0.946	0.028	-0.052	0.320
PHE2	-0.849	-0.371	0.095	-0.364
CH2	0.946	0.028	-0.052	0.320
TDA	0.718	0.467	-0.123	0.501
BCD	0.846	-0.066	0.526	-0.066
PTDA	-0.132	0.980	0.148	0.009
NHA	0.630	0.562	0.533	-0.048
HA	-0.269	0.233	0.782	-0.512
PTOL	0.140	0.895	0.415	-0.079
OTA	0.416	0.565	-0.504	0.503
PTCA	-0.470	0.505	0.589	-0.421
PTCA	0.946	0.028	-0.052	0.320
BZCA	0.867	-0.233	-0.240	0.371
TCH	0.911	0.180	-0.097	0.358
VITE	0.925	-0.041	0.374	0.052
VITE	0.946	0.028	-0.052	0.320
GT	0.776	0.108	-0.420	-0.458
MI	-0.081	0.876	-0.453	-0.147
CYP	0.776	0.108	-0.420	-0.458
DTM	-0.045	0.868	-0.466	-0.165
PTCA	0.776	0.108	-0.420	-0.458
CCP	0.776	0.108	-0.420	-0.458
ICD	0.776	0.108	-0.420	-0.458
HAD	-0.142	0.884	-0.430	-0.115
OTDC	0.776	0.108	-0.420	-0.458

OTDA	0.776	0.108	-0.420	-0.458
NNA	0.776	0.108	-0.420	-0.458
DCA	0.776	0.108	-0.420	-0.458
TCH	0.776	0.108	-0.420	-0.458
BZD	-0.503	0.827	-0.231	0.100
CH	-0.503	0.827	-0.231	0.100
BZAA	-0.503	0.827	-0.231	0.100
IDA	-0.503	0.827	-0.231	0.100
NHA	-0.503	0.827	-0.231	0.100
OA1	-0.503	0.827	-0.231	0.100
OA1	-0.503	0.827	-0.231	0.100
HPM	-0.503	0.827	-0.231	0.100
HXC	-0.503	0.827	-0.231	0.100
HXA	-0.503	0.827	-0.231	0.100
GS	-0.545	-0.626	-0.553	0.076
AA	-0.298	-0.718	-0.338	0.530
BT	-0.503	0.827	-0.231	0.100
DIT	-0.503	0.827	-0.231	0.100
BZA	-0.458	-0.729	-0.505	0.060
BZD	-0.458	-0.729	-0.505	0.060
PMT	-0.458	-0.729	-0.505	0.060
SS	-0.458	-0.729	-0.505	0.060
OA	-0.458	-0.729	-0.505	0.060
IME	-0.458	-0.729	-0.505	0.060
CS	-0.458	-0.729	-0.505	0.060



Contribution of the variables (%):

	F1	F2	F3	F4
PM	3.603	0.006	0.125	2.277
MV	3.674	0.004	0.036	2.072
CCHX	3.674	0.004	0.036	2.072
PHE1	3.421	0.353	1.038	0.425
ACP	0.554	3.401	0.923	2.920
CHSX	3.674	0.004	0.036	2.072

CH1	3.674	0.004	0.036	2.072
PHE2	2.963	0.719	0.118	2.677
CH2	3.674	0.004	0.036	2.072
TDA	2.119	1.141	0.198	5.080
BCD	2.938	0.023	3.642	0.088
PTDA	0.072	5.021	0.288	0.002
NHA	1.630	1.653	3.751	0.047
HA	0.296	0.283	8.058	5.309
PTOL	0.080	4.191	2.271	0.128
OTA	0.712	1.668	3.353	5.122
PTCA	0.906	1.333	4.579	3.583
PTCA	3.674	0.004	0.036	2.072
BZCA	3.085	0.283	0.756	2.784
TCH	3.411	0.170	0.125	2.587
VITE	3.517	0.009	1.844	0.054
VITE	3.674	0.004	0.036	2.072
GT	2.476	0.061	2.322	4.241
MI	0.027	4.007	2.706	0.434
CYP	2.476	0.061	2.322	4.241
DTM	0.008	0.061	2.322	4.241
PTCA	2.476	3.940	2.859	0.552
CCP	2.476	0.061	2.322	4.241
ICD	2.476	0.061	2.322	4.241
HAD	0.082	4.086	2.437	0.266
OTDC	2.476	0.061	2.322	4.241
OTDA	2.476	0.061	2.322	4.241
NNA	2.476	0.061	2.322	4.241
DCA	2.476	0.061	2.322	4.241
TCH	2.476	0.061	2.322	4.241
BZD	1.040	3.573	0.701	0.203
CH	1.040	3.573	0.701	0.203
BZAA	1.040	3.573	0.701	0.203
IDA	1.040	3.573	0.701	0.203
NHA	1.040	3.573	0.701	0.203
OA1	1.040	3.573	0.701	0.203
OA1	1.040	3.573	0.701	0.203
HPM	1.040	3.573	0.701	0.203
HXC	1.040	3.573	0.701	0.203
HXA	1.040	3.573	0.701	0.203
GS	1.221	2.047	4.024	0.118
AA	0.364	2.694	1.509	5.693
BT	1.040	3.573	0.701	0.203
DIT	1.040	3.573	0.701	0.203
BZA	0.862	2.779	3.359	0.072
BZD	0.862	2.779	3.359	0.072
PMT	0.862	2.779	3.359	0.072
SS	0.862	2.779	3.359	0.072
OA	0.862	2.779	3.359	0.072
IME	0.862	2.779	3.359	0.072
CS	0.862	2.779	3.359	0.072

Squared cosines of the variables:

	F1	F2	F3	F4
PM	0.877	0.001	0.010	0.113
MV	0.894	0.001	0.003	0.102
CCHX	0.894	0.001	0.003	0.102
PHE1	0.833	0.068	0.079	0.021
ACP	0.135	0.651	0.070	0.144
CHSX	0.894	0.001	0.003	0.102
CH1	0.894	0.001	0.003	0.102
PHE2	0.721	0.138	0.009	0.132
CH2	0.894	0.001	0.003	0.102
TDA	0.516	0.218	0.015	0.251
BCD	0.715	0.004	0.276	0.004
PTDA	0.017	0.961	0.022	0.000
NHA	0.397	0.316	0.285	0.002
HA	0.072	0.054	0.611	0.262
PTOL	0.020	0.802	0.172	0.006
OTA	0.173	0.319	0.254	0.253
PTCA	0.220	0.255	0.347	0.177
PTCA	0.894	0.001	0.003	0.102
BZCA	0.751	0.054	0.057	0.138
TCH	0.830	0.033	0.009	0.128
VITE	0.856	0.002	0.140	0.003
VITE	0.894	0.001	0.003	0.102
GT	0.602	0.012	0.176	0.210
MI	0.007	0.767	0.205	0.021
CYP	0.602	0.012	0.176	0.210
DTM	0.002	0.754	0.217	0.027
PTCA	0.602	0.012	0.176	0.210
CCP	0.602	0.012	0.176	0.210
ICD	0.602	0.012	0.176	0.210
HAD	0.020	0.782	0.185	0.013
OTDC	0.602	0.012	0.176	0.210
OTDA	0.602	0.012	0.176	0.210
NNA	0.602	0.012	0.176	0.210
DCA	0.602	0.012	0.176	0.210
TCH	0.602	0.012	0.176	0.210
BZD	0.253	0.684	0.053	0.010
CH	0.253	0.684	0.053	0.010
BZAA	0.253	0.684	0.053	0.010
IDA	0.253	0.684	0.053	0.010
NHA	0.253	0.684	0.053	0.010
OA1	0.253	0.684	0.053	0.010
OA1	0.253	0.684	0.053	0.010
HPM	0.253	0.684	0.053	0.010
HXC	0.253	0.684	0.053	0.010
HXA	0.253	0.684	0.053	0.010
GS	0.297	0.392	0.305	0.006
AA	0.089	0.515	0.114	0.281
BT	0.253	0.684	0.053	0.010
DIT	0.253	0.684	0.053	0.010
BZA	0.210	0.532	0.255	0.004

BZD	0.210	0.532	0.255	0.004
PMT	0.210	0.532	0.255	0.004
SS	0.210	0.532	0.255	0.004
OA	0.210	0.532	0.255	0.004
IME	0.210	0.532	0.255	0.004
CS	0.210	0.532	0.255	0.004

Values in bold correspond for each variable to the factor for which the squared cosine is the largest

Contribution of the observations (%):

	F1	F2	F3	F4
ETH1	48.199	0.937	14.092	16.772
ETH2	3.100	1.398	51.139	24.363
AC1	11.665	0.434	10.123	57.778
AC2	20.246	54.693	4.258	0.802
WTR	16.790	42.537	20.388	0.286

Squared cosines of the observations:

	F1	F2	F3	F4
ETH1	0.850	0.013	0.077	0.060
ETH2	0.124	0.044	0.635	0.197
AC1	0.434	0.013	0.117	0.436
AC2	0.313	0.664	0.021	0.003
WTR	0.296	0.590	0.112	0.001

Values in bold correspond for observation to the factor for which the squared cosine is the largest

Results after the Varimax rotation: Rotation matrix:

	D1	D2	D3	D4
D1	0.675	0.370	0.339	0.541
D2	0.091	0.815	0.565	0.090
D3	-0.197	-0.427	0.727	-0.501
D4	0.706	0.130	-0.194	-0.669

Percentage of variance after Varimax rotation:

	D1	D2	D3	D4
Variability (%)	24.979	31.258	23.399	20.364
Cumulative %	24.979	56.237	79.636	100.000

Factor loadings after Varimax rotation:

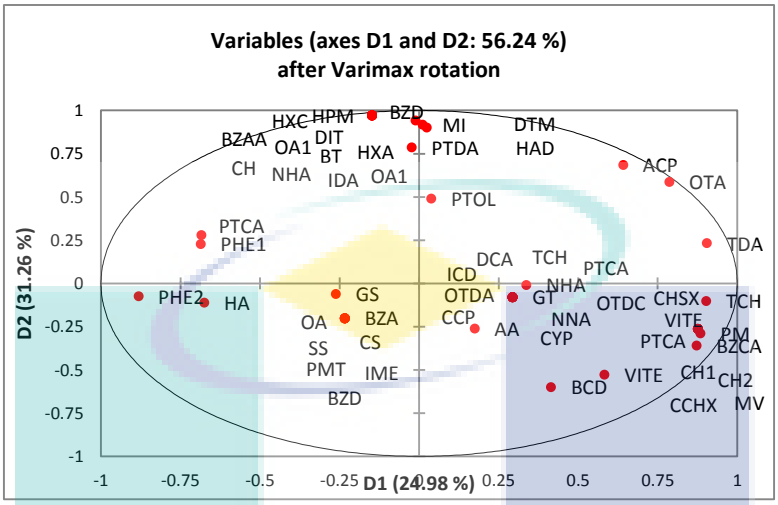
	D1	D2	D3	D4
PM	0.885	-0.289	0.162	0.328
MV	0.877	-0.263	0.236	0.326
CCHX	0.877	-0.263	0.236	0.326
PHE1	-0.686	0.227	-0.632	-0.280

ACP	0.641	0.683	0.315	0.150
CHSX	0.877	-0.263	0.236	0.326
CH1	0.877	-0.263	0.236	0.326
PHE2	-0.882	-0.075	-0.358	-0.297
CH2	0.877	-0.263	0.236	0.326
TDA	0.905	0.232	0.321	0.157
BCD	0.414	-0.600	0.644	0.232
PTDA	-0.023	0.786	0.615	-0.063
NHA	0.337	-0.009	0.928	0.157
HA	-0.676	-0.111	0.708	-0.173
PTOL	0.038	0.490	0.871	0.001
OTA	0.787	0.587	-0.003	0.192
PTCA	-0.684	0.279	0.636	-0.222
PTCA	0.877	-0.263	0.236	0.326
BZCA	0.872	-0.360	-0.084	0.320
TCH	0.903	-0.102	0.271	0.319
VITE	0.583	-0.529	0.552	0.275
VITE	0.877	-0.263	0.236	0.326
GT	0.293	-0.079	0.108	0.947
MI	0.011	0.918	0.167	0.360
CYP	0.293	-0.079	0.108	0.947
DTM	0.024	0.901	0.169	0.398
PTCA	0.293	-0.079	0.108	0.947
CCP	0.293	-0.079	0.108	0.947
ICD	0.293	-0.079	0.108	0.947
HAD	-0.011	0.942	0.162	0.295
OTDC	0.293	-0.079	0.108	0.947
OTDA	0.293	-0.079	0.108	0.947
NNA	0.293	-0.079	0.108	0.947
DCA	0.293	-0.079	0.108	0.947
TCH	0.293	-0.079	0.108	0.947
BZD	-0.148	0.971	0.110	-0.149
CH	-0.148	0.971	0.110	-0.149
BZAA	-0.148	0.971	0.110	-0.149
IDA	-0.148	0.971	0.110	-0.149
NHA	-0.148	0.971	0.110	-0.149
OA1	-0.148	0.971	0.110	-0.149
OA1	-0.148	0.971	0.110	-0.149
HPM	-0.148	0.971	0.110	-0.149
HXC	-0.148	0.971	0.110	-0.149
HXA	-0.148	0.971	0.110	-0.149
GS	-0.262	-0.062	-0.955	-0.126
AA	0.175	-0.262	-0.855	-0.411
BT	-0.148	0.971	0.110	-0.149
DIT	-0.148	0.971	0.110	-0.149
BZA	-0.234	-0.201	-0.946	-0.101
BZD	-0.234	-0.201	-0.946	-0.101
PMT	-0.234	-0.201	-0.946	-0.101
SS	-0.234	-0.201	-0.946	-0.101
OA	-0.234	-0.201	-0.946	-0.101
IME	-0.234	-0.201	-0.946	-0.101
CS	-0.234	-0.201	-0.946	-0.101

Correlations between variables and factors after Varimax rotation:

	D 1	D 2	D 3	D 4
PM	0.885	-0.289	0.162	0.328
MV	0.877	-0.263	0.236	0.326
CCHX	0.877	-0.263	0.236	0.326
PHE1	-0.686	0.227	-0.632	-0.280
ACP	0.641	0.683	0.315	0.150
CHSX	0.877	-0.263	0.236	0.326
CH1	0.877	-0.263	0.236	0.326
PHE2	-0.882	-0.075	-0.358	-0.297
CH2	0.877	-0.263	0.236	0.326
TDA	0.905	0.232	0.321	0.157
BCD	0.414	-0.600	0.644	0.232
PTDA	-0.023	0.786	0.615	-0.063
NHA	0.337	-0.009	0.928	0.157
HA	-0.676	-0.111	0.708	-0.173
PTOL	0.038	0.490	0.871	0.001
OTA	0.787	0.587	-0.003	0.192
PTCA	-0.684	0.279	0.636	-0.222
PTCA	0.877	-0.263	0.236	0.326
BZCA	0.872	-0.360	-0.084	0.320
TCH	0.903	-0.102	0.271	0.319
VITE	0.583	-0.529	0.552	0.236
VITE	0.877	-0.263	0.275	0.326
GT	0.293	-0.079	0.108	0.947
MI	0.011	0.918	0.167	0.360
CYP	0.293	-0.079	0.108	0.947
DTM	0.024	0.901	0.169	0.398
PTCA	0.293	-0.079	0.108	0.947
CCP	0.293	-0.079	0.108	0.947
ICD	0.293	-0.079	0.108	0.947
HAD	-0.011	0.942	0.162	0.295
OTDC	0.293	-0.079	0.108	0.947
OTDA	0.293	-0.079	0.108	0.947
NNA	0.293	-0.079	0.108	0.947
DCA	0.293	0.293	0.108	0.947
TCH	0.293	0.293	0.108	0.947
BZD	-0.148	0.293	0.110	-0.149
CH	-0.148	0.293	0.110	-0.149
BZAA	-0.148	0.971	0.110	-0.149
IDA	-0.148	0.971	0.110	-0.149
NHA	-0.148	0.971	0.110	-0.149
OA1	-0.148	0.971	0.110	-0.149
OA1	-0.148	0.971	0.110	-0.149
HPM	-0.148	0.971	0.110	-0.149
HXC	-0.148	0.971	0.110	-0.149
HXA	-0.148	0.971	0.110	-0.149
GS	-0.262	-0.262	-0.955	-0.126
AA	0.175	-0.262	-0.855	-0.411
BT	-0.148	0.971	0.110	-0.149
DIT	-0.148	0.971	0.110	-0.149
BZA	-0.234	-0.201	-0.946	-0.101
BZD	-0.234	-0.201	-0.946	-0.101
PMT	-0.234	-0.201	-0.946	-0.101
SS	-0.234	-0.201	-0.946	-0.101

OA	-0.234	-0.201	-0.946	-0.101
IME	-0.234	-0.201	-0.946	-0.101
CS	-0.234	-0.201	-0.946	-0.101



Contribution of the variables (%) after Varimax rotation:

	D 1	D 2	D 3	D 4
PM	5.593	0.478	0.201	0.944
MV	5.492	0.396	0.427	0.934
CCHX	5.492	0.396	0.427	0.934
PHE1	3.365	0.294	3.049	0.686
ACP	2.941	2.669	0.756	0.197
CHSX	5.492	0.396	0.427	0.934
CH1	5.492	0.396	0.427	0.934
PHE2	5.560	0.032	0.980	0.773
CH2	5.492	0.396	0.427	0.934
TDA	5.850	0.308	0.788	0.216
BCD	1.226	2.058	3.162	0.474
PTDA	0.004	3.526	2.887	0.035
NHA	0.811	0.000	6.578	0.215
HA	3.264	0.071	3.824	0.263
PTOL	0.010	1.374	5.786	0.000
OTA	4.424	1.967	0.000	0.324
PTCA	3.345	0.445	3.089	0.434
PTCA	5.492	0.396	0.427	0.934
BZCA	5.440	0.741	0.054	0.897
TCH	5.823	0.060	0.559	0.892
VITE	2.429	1.596	2.329	0.664
VITE	5.492	0.396	0.427	0.934
GT	0.615	0.036	0.089	7.857
MI	0.001	4.813	0.212	1.136
CYP	0.615	0.036	0.089	7.857
DTM	0.004	4.643	0.219	1.387
PTCA	0.615	0.036	0.089	7.857
CCP	0.615	0.036	0.089	7.857
ICD	0.615	0.036	0.089	7.857
HAD	0.001	5.065	0.200	0.763
OTDC	0.615	0.036	0.089	7.857

OTDA	0.615	0.036	0.089	7.857
NNA	0.615	0.036	0.089	7.857
DCA	0.615	0.036	0.089	7.857
TCH	0.615	0.036	0.089	7.857
BZD	0.157	5.391	0.092	0.196
CH	0.157	5.391	0.092	0.196
BZAA	0.157	5.391	0.092	0.196
IDA	0.157	5.391	0.092	0.196
NHA	0.157	5.391	0.092	0.196
OA1	0.157	5.391	0.092	0.196
OA1	0.157	5.391	0.092	0.196
HPM	0.157	5.391	0.092	0.196
HXC	0.157	5.391	0.092	0.196
HXA	0.157	5.391	0.092	0.196
GS	0.490	0.022	6.958	0.138
AA	0.219	0.391	5.585	1.483
BT	0.157	5.391	0.092	0.196
DIT	0.157	5.391	0.092	0.196
BZA	0.390	0.231	6.828	0.089
BZD	0.390	0.231	6.828	0.089
MT	0.390	0.231	6.828	0.089
SS	0.390	0.231	6.828	0.089
OA	0.390	0.231	6.828	0.089
IME	0.390	0.231	6.828	0.089
CS	0.390	0.231	6.828	0.089

Squared cosines of the variables after Varimax rotation:

	D 1	D 2	D 3	D 4
PM	0.782	0.084	0.026	0.108
MV	0.768	0.069	0.056	0.106
CCHX	0.768	0.069	0.056	0.106
PHE1	0.471	0.052	0.400	0.078
ACP	0.411	0.467	0.099	0.022
CHSX	0.768	0.069	0.056	0.106
CH1	0.768	0.069	0.056	0.106
PHE2	0.778	0.006	0.128	0.088
CH2	0.768	0.069	0.056	0.106
TDA	0.818	0.054	0.103	0.025
BCD	0.171	0.360	0.414	0.054
PTDA	0.001	0.617	0.378	0.004
NHA	0.113	0.000	0.862	0.024
HA	0.457	0.012	0.501	0.030
PTOL	0.001	0.240	0.758	0.000
OTA	0.619	0.344	0.000	0.037
PTCA	0.468	0.078	0.405	0.049
PTCA	0.768	0.069	0.056	0.106
BZCA	0.761	0.130	0.007	0.010

TCH	0.815	0.010	0.073	0.102
VITE	0.340	0.279	0.305	0.076
VITE	0.768	0.069	0.056	0.106
GT	0.086	0.006	0.012	0.896
MI	0.000	0.843	0.028	0.130
CYP	0.086	0.006	0.012	0.896
DTM	0.001	0.813	0.029	0.158
PTCA	0.086	0.006	0.012	**0**.896
CCP	0.086	0.006	0.012	0.896
ICD	0.086	0.006	0.012	0.896
HAD	0.000	0.887	0.026	0.087
OTDC	0.086	0.006	0.012	0.896
OTDA	0.086	0.006	0.012	0.896
NNA	0.086	0.006	0.012	0.896
DCA	0.086	0.006	0.012	0.896
TCH	0.086	0.006	0.012	0.896
BZD	0.022	0.944	0.012	0.022
CH	0.022	0.944	0.012	0.022
BZAA	0.022	0.944	0.012	0.022
IDA	0.022	0.944	0.012	0.022
NHA	0.022	0.944	0.012	0.022
OA1	0.022	0.944	0.012	0.022
OA1	0.022	0.944	0.012	0.022
HPM	0.022	0.944	0.012	0.022
HXC	0.022	0.944	0.012	0.022
HXA	0.022	0.944	0.012	0.022
GS	0.022	0.944	0.012	0.022
AA	0.031	0.068	0.732	0.169
BT	0.022	0.944	0.012	0.022
DIT	0.022	0.944	0.012	0.022
BZA	0.055	0.041	0.895	0.010
BZD	0.055	0.041	0.895	0.010
PMT	0.055	0.041	0.895	0.010
SS	0.055	0.041	0.895	0.010
OA	0.055	0.041	0.895	0.010
IME	0.055	0.041	0.895	0.010
CS	0.055	0.041	0.895	0.010

Values in bold correspond for each variable to the factor for which the squared cosine is the largest

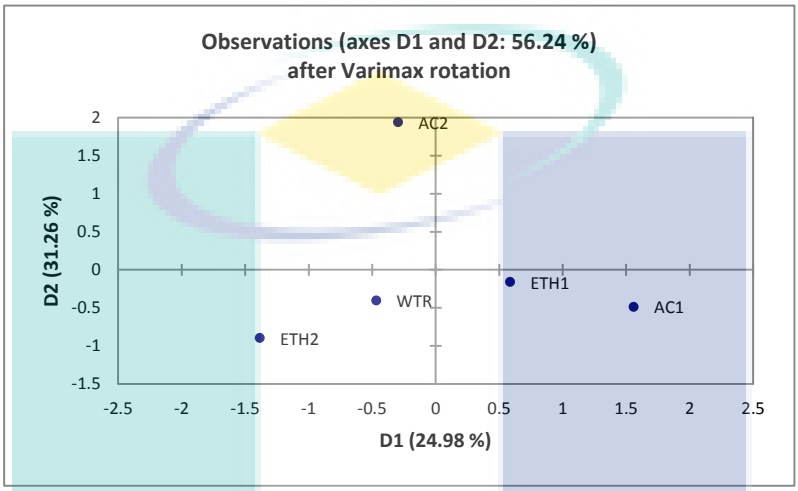
Component score coefficients after Varimax rotation:

	D 1	D 2	D 3	D 4
PM	0.076	-0.001	-0.010	-0.018
MV	0.073	-0.002	-0.004	-0.019
CCHX	0.073	-0.002	-0.004	-0.019
PHE1	-0.040	0.015	-0.042	0.017
ACP	0.075	0.054	0.011	-0.022
CHSX	0.073	-0.002	-0.004	-0.019
CH1	0.073	-0.002	-0.004	-0.019
PHE2	-0.080	-0.018	0.001	0.022
CH2	0.073	-0.002	-0.004	-0.019
TDA	0.097	0.029	-0.008	-0.042

BCD	0.000	-0.047	0.063	-0.007
PTDA	-0.002	0.036	0.041	-0.009
NHA	-0.001	-0.017	0.078	-0.012
HA	-0.100	-0.043	0.098	0.013
PTOL	-0.014	0.011	0.071	-0.009
OTA	0.099	0.059	-0.046	-0.023
PTCA	-0.086	-0.016	0.081	0.010
PTCA	0.073	-0.002	-0.004	-0.019
BZCA	0.082	0.000	-0.032	-0.016
TCH	0.080	0.009	-0.005	-0.021
VITE	0.023	-0.035	0.045	-0.011
VITE	0.073	-0.002	-0.004	-0.019
GT	-0.032	0.004	-0.008	0.107
MI	-0.007	0.060	-0.013	0.052
CYP	-0.032	0.004	-0.008	0.107
DTM	-0.009	0.060	-0.013	0.056
PTCA	-0.032	0.004	-0.008	0.107
CCP	-0.032	0.004	-0.008	0.107
ICD	-0.032	0.004	-0.008	0.107
HAD	-0.005	0.061	-0.013	0.045
OTDC	-0.032	0.004	-0.008	0.107
OTDA	-0.032	0.004	-0.008	0.107
NNA	-0.032	0.004	-0.008	0.107
DCA	-0.032	0.004	-0.008	0.107
TCH	-0.032	0.004	-0.008	0.107
BZD	0.010	0.058	-0.009	-0.006
CH	0.010	0.058	-0.009	-0.006
BZAA	0.010	0.058	-0.009	-0.006
IDA	0.010	0.058	-0.009	-0.006
NHA	0.010	0.058	-0.009	-0.006
OA1	0.010	0.058	-0.009	-0.006
OA1	0.010	0.058	-0.009	-0.006
HPM	0.010	0.058	-0.009	-0.006
HXC	0.010	0.058	-0.009	-0.006
HXA	0.010	0.058	-0.009	-0.006
GS	0.007	0.015	-0.082	0.011
AA	0.073	-0.002	-0.004	-0.019
BT	0.010	0.058	-0.009	-0.006
DIT	0.010	0.058	-0.009	-0.006
BZA	0.005	0.006	-0.079	0.012
BZD	0.005	0.006	-0.079	0.012
PMT	0.005	0.006	-0.079	0.012
SS	0.005	0.006	-0.079	0.012
OA	0.005	0.006	-0.079	0.012
IME	0.005	0.006	-0.079	0.012
CS	0.005	0.006	-0.079	0.012

Factor scores after Varimax rotation:

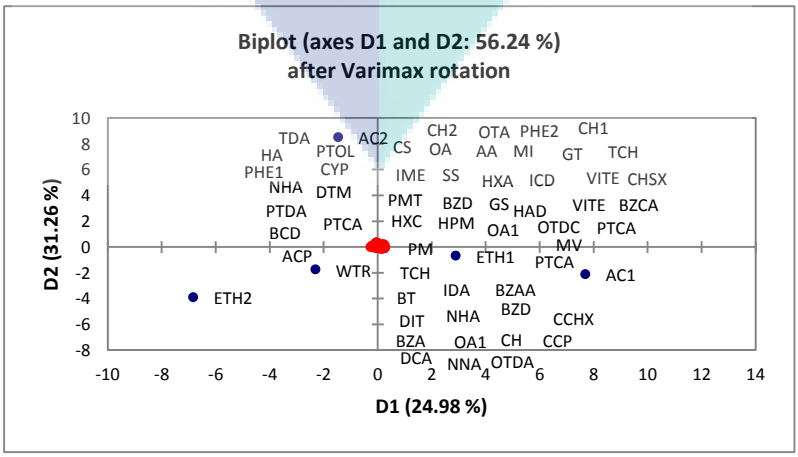
	D1	D2	D3	D4
ETH1	0.586	-0.159	0.216	1.893
ETH2	-1.384	-0.895	1.093	-0.299
AC1	1.561	-0.486	0.363	-1.094
AC2	-0.296	1.943	0.220	-0.299
WTR	-0.467	0.403	-1.892	-0.201



Squared cosines of the observations after Varimax rotation:

	D1	D2	D3	D4
ETH1	0.086	0.006	0.012	0.896
ETH2	0.479	0.200	0.298	0.022
AC1	0.609	0.059	0.033	0.299
AC2	0.022	0.944	0.012	0.022
WTR	0.055	0.041	0.895	0.010

Values in bold correspond for each observation to the factor for which the squared cosine is the largest



APPENDIX. A3

CHARACTERISTIC INFRARED ABSORPTION FREQUENCIES

Bond	Compound Type	Frequency range, cm^{-1}
C-H	Alkanes	2960-2850(s) stretch 1470-1350(v) scissoring and bending
	CH3 Umbrella Deformation	1380(m-w)-Doublet-isopropyl, *t*-butyl
C-H	Alkanes	3080-3020(m) stretch 1000-675(s) bend
C-H	Aromatic Rings	3100-3000(m) stretch
	Phenyl Rings Substitution Band	870-675(s) bend
	Phenyl Rings Subtitution Overtones	2000-1600(w) - fingerprint region
C-H	Alkynes	3333-3267(s) stretch 700-610(b) bend
C=C	Alkanes	1680-1640(m,w)) stretch
C-C	Alkynes	2260-2100(w,sh) stretch
C=C	Aromatic Rings	1600, 1500(w) stretch
C-O	Alcohol, Ether, Carboxylic acids, Ester	1260-1000(s) stretch
C=O	Aldehyde, Ketone, Carboxylix acid, Esters	1760-1670(s) stretch
O-H	Monomeric -- Alcohols, Phenols	3640-3160(s,br) stretch
	Hydrogen-bonded--Alcohols, Phenols	3600-3200(b) stretch
	Carboxylic acids	3000-2500(b) stretch
N-H	Amines	3500-3300(m) stretch 1650-1580 (m) bend
C-N	Amines	1340-1020(m) stretch
C□N	Nitriles	2260-2220(v) stretch
NO_2	Nitro Compound	1660-1500(s) asymmetrical stretch 1390-1260(s) symmetrical stretch

v: variable, m: medium, s: strong, br: broad, w: weak

APPENDIX. A4

ANOVA OUTPUT OF TOTAL PHENOLIC COMPOUND

Between-Subjects Factors

		N
TIME	10	18
	20	18
	30	18
	40	18
	50	18
	60	18
TEMP	50	18
	60	18
	70	18
	80	18
	90	18
	100	18

Tests of Between-Subjects Effects

Dependent Variable: total phenolic content (TPC)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	712540.355[a]	35	20358.296	39.574	.000
Intercept	4114041.056	1	4114041.056	7.997E3	.000
TIME	37437.276	5	7487.455	14.555	.000
TEMP	647249.911	5	129449.982	251.638	.000
TIME * TEMP	27853.168	25	1114.127	2.166	.006
Error	37038.957	72	514.430		
Total	4863620.368	108			
Corrected Total	749579.312	107			

a. R Squared = .951 (Adjusted R Squared = .927)

Levene's Test of Equality of Error Variances(a)

Dependent Variable: total phenolic content (TPC)

F	df1	df2	Sig.
2.663	35	72	.000

Tests the null hypothesis that the error variance of the dependent variable is equal across groups. a. Design: Intercept + TIME + TEMP + TIME * TEMP

Tests of Between-Subjects Effects

Dependent Variable: total phenolic content (TPC)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	712540.355[a]	35	20358.296	39.574	.000
Intercept	4114041.056	1	4114041.056	7.997E3	.000
TIME	37437.276	5	7487.455	14.555	.000
TEMP	647249.911	5	129449.982	251.638	.000
TIME * TEMP	27853.168	25	1114.127	2.166	.006
Error	37038.957	72	514.430		
Total	4863620.368	108			
Corrected Total	749579.312	107			

a. R Squared = .951 (Adjusted R Squared = .927)

Estimated Marginal Means

1. TIME

Dependent Variable:PHENOLIC

TIME	Mean	Std. Error	99% Confidence Interval	
			Lower Bound	Upper Bound
10	224.790	5.346	210.645	238.935
20	211.025	5.346	196.880	225.169
30	199.872	5.346	185.728	214.017
40	185.654	5.346	171.510	199.799
50	177.383	5.346	163.238	191.527
60	172.321	5.346	158.176	186.466

2. TEMP

Dependent Variable:PHENOLIC

TEMP	Mean	Std. Error	99% Confidence Interval	
			Lower Bound	Upper Bound
50	189.852	5.346	175.707	203.997
60	334.255	5.346	320.110	348.400
70	244.132	5.346	229.987	258.276
80	168.658	5.346	154.514	182.803
90	142.444	5.346	128.300	156.589
100	91.704	5.346	77.559	105.848

3. TEMP * TIME

Dependent Variable:PHENOLIC

TEMP	TIME	Mean	Std. Error	99% Confidence Interval	
				Lower Bound	Upper Bound
50	10	203.556	13.095	168.908	238.203
	20	197.506	13.095	162.859	232.153
	30	192.444	13.095	157.797	227.092
	40	183.802	13.095	149.155	218.450
	50	182.444	13.095	147.797	217.092
	60	179.358	13.095	144.711	214.005
60	10	428.370	13.095	393.723	463.018
	20	365.407	13.095	330.760	400.055
	30	344.543	13.095	309.896	379.190
	40	303.432	13.095	268.785	338.079
	50	286.889	13.095	252.242	321.536
	60	276.889	13.095	242.242	311.536
70	10	266.519	13.095	231.871	301.166
	20	260.099	13.095	225.452	294.746
	30	245.037	13.095	210.390	279.684
	40	239.235	13.095	204.587	273.882
	50	228.741	13.095	194.094	263.388
	60	225.160	13.095	190.513	259.808
80	10	175.531	13.095	140.884	210.178

	20	174.420	13.095	139.773	209.067
	30	171.827	13.095	137.180	206.474
	40	168.247	13.095	133.600	202.894
	50	164.790	13.095	130.143	199.437
	60	157.136	13.095	122.489	191.783
90	10	155.160	13.095	120.513	189.808
	20	153.309	13.095	118.661	187.956
	30	145.654	13.095	111.007	180.302
	40	139.728	13.095	105.081	174.376
	50	131.827	13.095	97.180	166.474
	60	128.988	13.095	94.340	163.635
100	10	119.605	13.095	84.958	154.252
	20	115.407	13.095	80.760	150.055
	30	99.728	13.095	65.081	134.376
	40	79.481	13.095	44.834	114.129
	50	69.605	13.095	34.958	104.252
	60	66.395	13.095	31.748	101.042

Post Hoc Tests

TIME

Multiple Comparisons

Dependent Variable: total phenolic content (TPC)

	(I) consentration (%)	(J) consentration (%)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	2.50	5.00	-81.9104(*)	10.19554	.000	-106.3830	-57.4378
		10.00	-240.9063(*)	10.19554	.000	-265.3789	-216.4336
	5.00	2.50	81.9104(*)	10.19554	.000	57.4378	106.3830
		10.00	-158.9958(*)	10.19554	.000	-183.4685	-134.5232
	10.00	2.50	240.9063(*)	10.19554	.000	216.4336	265.3789
		5.00	158.9958(*)	10.19554	.000	134.5232	183.4685

Based on observed means.

* The mean difference is significant at the .05 level.

Post Hoc Test

TIME

Multiple Comparisons
Dependent Variable:PHENOLIC

	(I) TIME	(J) TIME	Mean Difference (I-J)	Std. Error	Sig.	99% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	10	20	13.7654	7.56035	.459	-12.7034	40.2343
		30	24.9177	7.56035	.018	-1.5512	51.3866
		40	39.1358*	7.56035	.000	12.6669	65.6047
		50	47.4074*	7.56035	.000	20.9385	73.8763
		60	52.4691*	7.56035	.000	26.0003	78.9380
	20	10	-13.7654	7.56035	.459	-40.2343	12.7034
		30	11.1523	7.56035	.681	-15.3166	37.6211
		40	25.3704	7.56035	.015	-1.0985	51.8392
		50	33.6420*	7.56035	.000	7.1731	60.1108
		60	38.7037*	7.56035	.000	12.2348	65.1726
	30	10	-24.9177	7.56035	.018	-51.3866	1.5512
		20	-11.1523	7.56035	.681	-37.6211	15.3166
		40	14.2181	7.56035	.422	-12.2508	40.6870
		50	22.4897	7.56035	.044	-3.9792	48.9586
		60	27.5514*	7.56035	.006	1.0826	54.0203
	40	10	-39.1358*	7.56035	.000	-65.6047	-12.6669
		20	-25.3704	7.56035	.015	-51.8392	1.0985
		30	-14.2181	7.56035	.422	-40.6870	12.2508
		50	8.2716	7.56035	.882	-18.1973	34.7405
		60	13.3333	7.56035	.495	-13.1355	39.8022
	50	10	-47.4074*	7.56035	.000	-73.8763	-20.9385
		20	-33.6420*	7.56035	.000	-60.1108	-7.1731
		30	-22.4897	7.56035	.044	-48.9586	3.9792
		40	-8.2716	7.56035	.882	-34.7405	18.1973
		60	5.0617	7.56035	.985	-21.4071	31.5306
	60	10	-52.4691*	7.56035	.000	-78.9380	-26.0003
		20	-38.7037*	7.56035	.000	-65.1726	-12.2348
		30	-27.5514*	7.56035	.006	-54.0203	-1.0826
		40	-13.3333	7.56035	.495	-39.8022	13.1355
		50	-5.0617	7.56035	.985	-31.5306	21.4071

Based on observed means. The error term is Mean Square(Error) = 514.430.
*. The mean difference is significant at the .01 level.

Homogeneous Subsets
PHENOLIC

	TIME	N	Subset 1	2	3	4
Tukey HSDa	60	18	1.7232E2			
	50	18	1.7738E2	1.7738E2		
	40	18	1.8565E2	1.8565E2	1.8565E2	
	30	18		1.9987E2	1.9987E2	1.9987E2
	20	18			2.1102E2	2.1102E2
	10	18				2.2479E2
	Sig.		.495	.044	.015	.018
Duncana	60	18	1.7232E2			
	50	18	1.7738E2			
	40	18	1.8565E2	1.8565E2		
	30	18		1.9987E2	1.9987E2	
	20	18			2.1102E2	2.1102E2
	10	18				2.2479E2
	Sig.		.100	.064	.145	.073

Means for groups in homogeneous subsets are displayed. Based on observed means.
The error term is Mean Square(Error) = 514.430. a. Uses Harmonic Mean Sample Size = 18.000.

APPENDIX. A5

OPTIMIZATION OF TOTAL PHENOLIC COMPOUND

ANOVA for Response Surface Quadratic Model Analysis of variance table [Partial sum of squares]

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Model	30324.33	5	6064.87	1256.65	<0.0001	significant
A	*1918.31*	*1*	*14546.78*	*3014.11*	*<0.0001*	
A^2	*7.28*	*1*	*7.28*	*1.51*	*0.2589*	
B^2	*11884.93*	*1*	*11884.93*	*2462.57*	*<0.0001*	
AB	*210.42*	*1*	*10.42*	*43.60*	*0.0003*	
Residual	33.78	7	4.83			
Lack of Fit	*33.78*	*3*	*1.26*			
Pure Error	*0.000*	*4*			*0.000*	
Cor Total	30358.11	12				

The Model F-value of 1256.65 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise.

Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, B^2, AB are significant model terms.
Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Std. Dev.	2.20	R-Squared	0.9989
Mean	176.10	Adj R-Squared	0.9981
C.V.	1.25	Pred R-Squared	0.9895
PRESS	317.70	Adeq Precision	105.765

The "Pred R-Squared" of 0.9895 is in reasonable agreement with the "Adj R-Squared" of 0.9981.

"Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 105.765 indicates an adequate signal. This model can be used to navigate the design space.

Factor	Coefficient Estimate	DF	Standard Error	95% CI Low	95% CI High	VIF
Intercept	205.62	1	0.91	203.47	207.78	
A-TIME	-17.88	1	0.90	-20.00	-15.76	1.00
B-TEMP	-49.24	1	0.90	-51.36	-47.12	1.00
A^2	1.62	1	1.32	-1.50	4.75	1.17
B^2	-65.60	1	1.32	-68.72	-62.47	1.17
AB	-7.25	1	1.10	-9.85	-4.66	1.00

Final Equation in Terms of Coded Factors:

Concentration =
+205.62
-17.88 * A
-49.24 * B
+1.62 * A^2
-65.60 * B^2
-7.25 * A * B

Final Equation in Terms of Actual Factors:

Concentration = -239.29363
-0.026755 * TIME
+14.18025 * TEMPERATURE
+2.59840E-003 * $TIME^2$
-0.10496 * $TEMPERATURE^2$
-0.011605 * TIME * TEMPERATURE

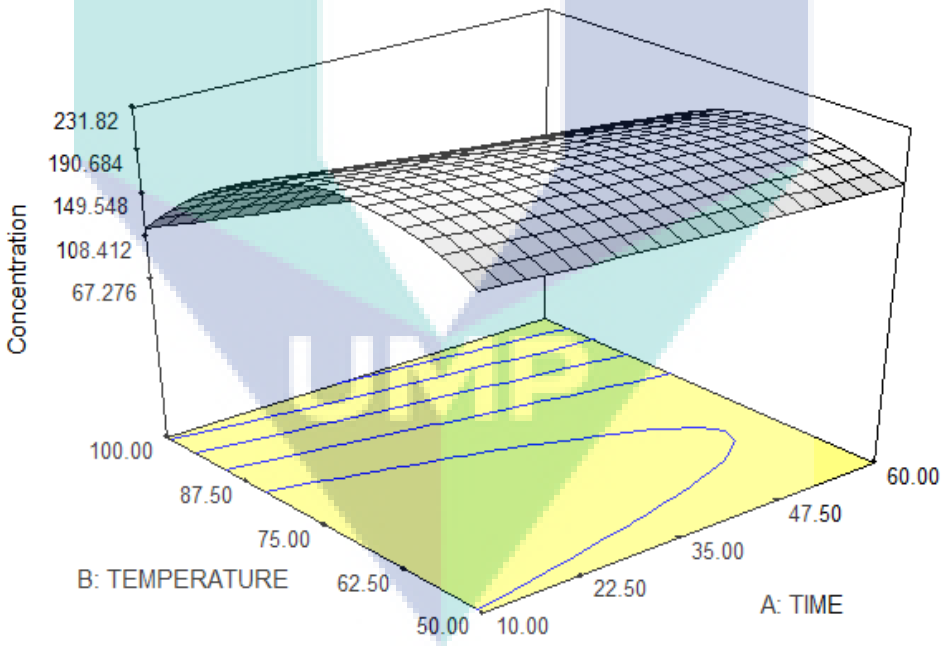
Diagnostics Case Statistics

Standard Order	Actual Value	Predicted Value	Residual	Student Leverage	Cook's Residual	Outlier Distance	t	
1	203.56	201.52	2.04	0.790	2.028	2.583	2.925	
2	179.36	180.26	-0.90	0.790	-0.896	0.504	-0.882	
3	119.61	117.54	2.06	0.790	2.049	2.636	2.999	
4	66.39	67.28	-0.88	0.790	-0.876	0.481	-0.859	
5	221.03	225.13	-4.10	0.494	-2.626	1.123	-19.936	*
6	191.15	189.37	1.78	0.494	1.141	0.212	1.171	
7	188.12	189.26	-1.14	0.494	-0.729	0.087	-0.702	
8	89.61	90.79	-1.18	0.494	-0.756	0.093	-0.730	
9	206.09	205.62	0.46	0.172	0.232	0.002	0.216	
10	206.09	205.62	0.46	0.172	0.232	0.002	0.216	
11	206.09	205.62	0.46	0.172	0.232	0.002	0.216	
12	206.09	205.62	0.46	0.172	0.232	0.002	0.216	
13	206.09	205.62	0.46	0.172	0.232	0.002	0.216	

* Case(s) with |Outlier T| > 3.50

Optimization result of total phenolic compound in Torbangun leave extract.

Std	Run	Book	Factor 1 Time (Minutes)	Factor 2 Temperature (^{O} C)	Response 1 Concentration (mg/L)
6	1	Block 1	60.00	75.00	191.149
2	2	Block 1	60.00	50.00	179.358
10	3	Block 1	35.00	75.00	206.087
11	4	Block 1	35.00	75.00	206.087
4	5	Block 1	60.00	100.00	66.395
13	6	Block 1	35.00	75.00	206.087
8	7	Block 1	35.00	100.00	89.605
12	8	Block 1	35.00	75.00	206.087
1	9	Block 1	10.00	50.00	203.556
9	10	Block 1	35.00	75.00	206.087
5	11	Block 1	10.00	75.00	221.025
3	12	Block 1	10.00	100.00	119.605
7	13	Block 1	35.00	50.00	188.124



Response surface showing the effect of (minute) and extraction temperature (^{O}C) on Total Phenolic Compound (mg GAE/100 g).

APPENDIX. A6

ANOVA OUTPUT OF TOTAL FLAVONOID COMPOUND

Between-Subjects Factors

		N
TIME	10	18
	20	18
	30	18
	40	18
	50	18
	60	18
TEMP	50	18
	60	18
	70	18
	80	18
	90	18
	100	18

Tests of Between-Subjects Effects

Dependent Variable:FLAVONOID

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	190409.023[a]	35	5440.258	31.700	.000
Intercept	396755.606	1	396755.606	2.312E3	.000
TIME	11641.038	5	2328.208	13.566	.000
TEMP	169513.391	5	33902.678	197.551	.000
TIME * TEMP	9254.595	25	370.184	2.157	.006
Error	12356.255	72	171.615		
Total	599520.884	108			
Corrected Total	202765.278	107			

a. R Squared = .939 (Adjusted R Squared = .909)

Levene's Test of Equality of Error Variances[a]

Dependent Variable:FLAVONOID

F	df1	df2	Sig.
4.069	35	72	.000

Tests the null hypothesis that the error variance of the dependent variable is equal across groups. a. Design: Intercept + TIME + TEMP + TIME * TEMP

Estimated Marginal Means

1. TIME

Dependent Variable:FLAVONOID

TIME	Mean	Std. Error	99% Confidence Interval	
			Lower Bound	Upper Bound
10	78.749	3.088	70.580	86.919
20	66.803	3.088	58.634	74.973
30	62.018	3.088	53.848	70.188
40	58.160	3.088	49.990	66.330
50	48.629	3.088	40.459	56.799
60	49.305	3.088	41.135	57.475

2. TEMP

Dependent Variable:FLAVONOID

TEMP	Mean	Std. Error	99% Confidence Interval	
			Lower Bound	Upper Bound
50	65.876	3.088	57.706	74.045
60	128.951	3.088	120.781	137.121
70	88.476	3.088	80.307	96.646
80	43.181	3.088	35.011	51.350
90	25.925	3.088	17.755	34.095
100	11.256	3.088	3.086	19.425

3. TEMP * TIME

Dependent Variable:FLAVONOID

TEMP	TIME	Mean	Std. Error	99% Confidence Interval	
				Lower Bound	Upper Bound
50	10	82.814	7.563	62.802	102.825
	20	73.744	7.563	53.732	93.756
	30	68.008	7.563	47.996	88.019
	40	62.581	7.563	42.569	82.593
	50	57.387	7.563	37.376	77.399
	60	50.721	7.563	30.709	70.733
60	10	186.612	7.563	166.601	206.624
	20	136.457	7.563	116.445	156.469
	30	121.961	7.563	101.950	141.973
	40	115.139	7.563	95.128	135.151
	50	108.783	7.563	88.771	128.795
	60	104.752	7.563	84.740	124.764
70	10	102.349	7.563	82.337	122.360
	20	97.155	7.563	77.143	117.166
	30	96.069	7.563	76.058	116.081
	40	92.581	7.563	72.569	112.593
	50	59.426	7.563	39.414	79.438
	60	83.279	7.563	63.267	103.291
80	10	49.946	7.563	29.934	69.957
	20	47.388	7.563	27.376	67.399
	30	44.984	7.563	24.973	64.996
	40	43.589	7.563	23.577	63.601
	50	39.248	7.563	19.236	59.260
	60	33.930	7.563	13.918	53.942
90	10	33.744	7.563	13.732	53.756
	20	31.883	7.563	11.872	51.895
	30	27.853	7.563	7.841	47.864
	40	24.163	7.563	4.151	44.174
	50	19.325	7.563	-.686	39.337
	60	18.581	7.563	-1.430	38.593
100	10	17.031	7.563	-2.981	37.043

	20	14.194	7.563	-5.818	34.205
	30	13.233	7.563	-6.779	33.244
	40	10.907	7.563	-9.105	30.919
	50	7.604	7.563	-12.407	27.616
	60	4.566	7.563	-15.446	24.578

Post Hoc Tests

TIME

Multiple Comparisons

Dependent Variable:FLAVONOID

	(I) TIME	(J) TIME	Mean Difference (I-J)	Std. Error	Sig.	99% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	10	20	11.9458	4.36673	.081	-3.3421	27.2338
		30	16.7312*	4.36673	.004	1.4433	32.0192
		40	20.5892*	4.36673	.000	5.3013	35.8772
		50	30.1202*	4.36673	.000	14.8323	45.4082
		60	29.4443*	4.36673	.000	14.1564	44.7323
	20	10	-11.9458	4.36673	.081	-27.2338	3.3421
		30	4.7854	4.36673	.881	-10.5026	20.0733
		40	8.6434	4.36673	.364	-6.6446	23.9313
		50	18.1744*	4.36673	.001	2.8864	33.4623
		60	17.4985*	4.36673	.002	2.2105	32.7865
	30	10	-16.7312*	4.36673	.004	-32.0192	-1.4433
		20	-4.7854	4.36673	.881	-20.0733	10.5026
		40	3.8580	4.36673	.949	-11.4300	19.1460
		50	13.3890	4.36673	.035	-1.8990	28.6770
		60	12.7131	4.36673	.052	-2.5748	28.0011
	40	10	-20.5892*	4.36673	.000	-35.8772	-5.3013
		20	-8.6434	4.36673	.364	-23.9313	6.6446
		30	-3.8580	4.36673	.949	-19.1460	11.4300
		50	9.5310	4.36673	.259	-5.7570	24.8190
		60	8.8551	4.36673	.337	-6.4328	24.1431
	50	10	-30.1202*	4.36673	.000	-45.4082	-14.8323
		20	-18.1744*	4.36673	.001	-33.4623	-2.8864
		30	-13.3890	4.36673	.035	-28.6770	1.8990
		40	-9.5310	4.36673	.259	-24.8190	5.7570
		60	-.6759	4.36673	1.000	-15.9638	14.6121

60	10	-29.4443*	4.36673	.000	-44.7323	-14.1564
	20	-17.4985*	4.36673	.002	-32.7865	-2.2105
	30	-12.7131	4.36673	.052	-28.0011	2.5748
	40	-8.8551	4.36673	.337	-24.1431	6.4328
	50	.6759	4.36673	1.000	-14.6121	15.9638

Based on observed means. The error term is Mean Square(Error) = 171.615.
*. The mean difference is significant at the .01 level.

Homogeneous Subsets

FLAVONOID

	TIME	N	Subset 1	2	3
Tukey HSD[a]	50	18	48.6290		
	60	18	49.3049		
	40	18	58.1600	58.1600	
	30	18	62.0180	62.0180	
	20	18		66.8034	66.8034
	10	18			78.7492
	Sig.		.035	.364	.081
Duncan[a]	50	18	48.6290		
	60	18	49.3049		
	40	18	58.1600	58.1600	
	30	18		62.0180	
	20	18		66.8034	
	10	18			78.7492
	Sig.		.041	.064	1.000

Means for groups in homogeneous subsets are displayed. Based on observed means. The error term is Mean Square(Error) = 171.615. a. Uses Harmonic Mean Sample Size = 18.000.

TEMP

Multiple Comparisons

Dependent Variable:FLAVONOID

	(I) TEMP	(J) TEMP	Mean Difference (I-J)	Std. Error	Sig.	99% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	50	60	-63.0751*	4.36673	.000	-78.3630	-47.7871
		70	-22.6007*	4.36673	.000	-37.8886	-7.3127
		80	22.6950*	4.36673	.000	7.4070	37.9830
		90	39.9509*	4.36673	.000	24.6629	55.2388
		100	54.6200*	4.36673	.000	39.3320	69.9080
	60	50	63.0751*	4.36673	.000	47.7871	78.3630
		70	40.4744*	4.36673	.000	25.1864	55.7623
		80	85.7701*	4.36673	.000	70.4821	101.0580
		90	103.0259*	4.36673	.000	87.7380	118.3139
		100	117.6951*	4.36673	.000	102.4071	132.9830
	70	50	22.6007*	4.36673	.000	7.3127	37.8886
		60	-40.4744*	4.36673	.000	-55.7623	-25.1864
		80	45.2957*	4.36673	.000	30.0077	60.5836
		90	62.5516*	4.36673	.000	47.2636	77.8395
		100	77.2207*	4.36673	.000	61.9327	92.5086
	80	50	-22.6950*	4.36673	.000	-37.9830	-7.4070
		60	-85.7701*	4.36673	.000	-101.0580	-70.4821
		70	-45.2957*	4.36673	.000	-60.5836	-30.0077
		90	17.2559*	4.36673	.002	1.9679	32.5438
		100	31.9250*	4.36673	.000	16.6370	47.2130
	90	50	-39.9509*	4.36673	.000	-55.2388	-24.6629
		60	-103.0259*	4.36673	.000	-118.3139	-87.7380
		70	-62.5516*	4.36673	.000	-77.8395	-47.2636
		80	-17.2559*	4.36673	.002	-32.5438	-1.9679
		100	14.6691	4.36673	.015	-.6188	29.9571
	100	50	-54.6200*	4.36673	.000	-69.9080	-39.3320
		60	-117.6951*	4.36673	.000	-132.9830	-102.4071
		70	-77.2207*	4.36673	.000	-92.5086	-61.9327
		80	-31.9250*	4.36673	.000	-47.2130	-16.6370
		90	-14.6691	4.36673	.015	-29.9571	.6188

Based on observed means. The error term is Mean Square(Error) = 171.615.
*. The mean difference is significant at the .01 level.

Homogeneous Subsets

FLAVONOID

	TEMP	N	Subset 1	2	3	4	5	6
Tukey HSD[a]	100	18	11.2558					
	90	18	25.9249					
	80	18		43.1808				
	50	18			65.8758			
	70	18				88.4764		
	60	18					1.2895E2	
	Sig.		.015	1.000	1.000	1.000	1.000	
Duncan[a]	100	18	11.2558					
	90	18		25.9249				
	80	18			43.1808			
	50	18				65.8758		
	70	18					88.4764	
	60	18						1.2895E2
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed. Based on observed means. The error term is Mean Square(Error) = 171.615. a. Uses Harmonic Mean Sample Size = 18.000.

APPENDIX. A7

OPTIMIZATION OF TOTAL FLAVONOID COMPOUND

ANOVA for Response Surface Quadratic Model Analysis of variance table [Partial sum of squares]

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Model	8196.81	5	1639.36	894.56	< 0.0001	significant
A	*642.94*	*1*	*642.94*	*350.84*	*<0.0001*	
B	*4545.97*	*1*	*4545.97*	*2480.63*	*<0.0001*	
A^2	*2.50*	*1*	*2.50*	*1.36*	*0.2814*	
B^2	*2427.17*	*1*	*2427.17*	*1324.45*	*<0.0001*	
AB	*96.40*	*1*	*96.40*	*52.60*	*0.0002*	
Residual	12.83	7	1.83			
Lack of Fit	*12.83*	*3*	*4.28*			
Pure Error	*0.000*	*4*	*0.00*			
Cor Total	8209.64		12			

The Model F-value of 894.56 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise.

Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, B^2, AB are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Std. Dev.	1.35	R-Squared	0.9984
Mean	54.91	Adj R-Squared	0.9973
C.V.	2.47	Pred R-Squared	0.9853
PRESS	121.07	Adeq Precision	82.371

The "Pred R-Squared" of 0.9853 is in reasonable agreement with the "Adj R-Squared" of 0.9973.

"Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 82.371 indicates an adequate signal. This model can be used to navigate the design space.

Factor	Coefficient Estimate	DF	Standard Error	95% CI Low	95% CI High	VIF
Intercept	69.03	1	0.56	67.70	70.36	
A-TIME	-10.35	1	0.55	-11.66	-9.04	1.00
B-TEMP	-27.53	1	0.55	-28.83	-26.22	1.00
A^2	-0.95	1	0.81	-2.88	0.98	1.17
B^2	-29.64	1	0.81	-31.57	-27.72	1.17
AB	4.91	1	0.68	3.31	6.51	1.00

Final Equation in Terms of Coded Factors:

Concentration = +69.03
-10.35 * A
-27.53 * B
-0.95 * A^2
-29.64 * B^2
+4.91 * A * B

Final Equation in Terms of Actual Factors:

Concentration = -81.94993
-0.89671 * TIME
+5.73876 * TEMPERATURE
-1.52094E-003 * $TIME^2$
-0.047431 * $TEMPERATURE^2$
+7.85480E-003 * TIME * TEMPERATURE

Diagnostics Case Statistics

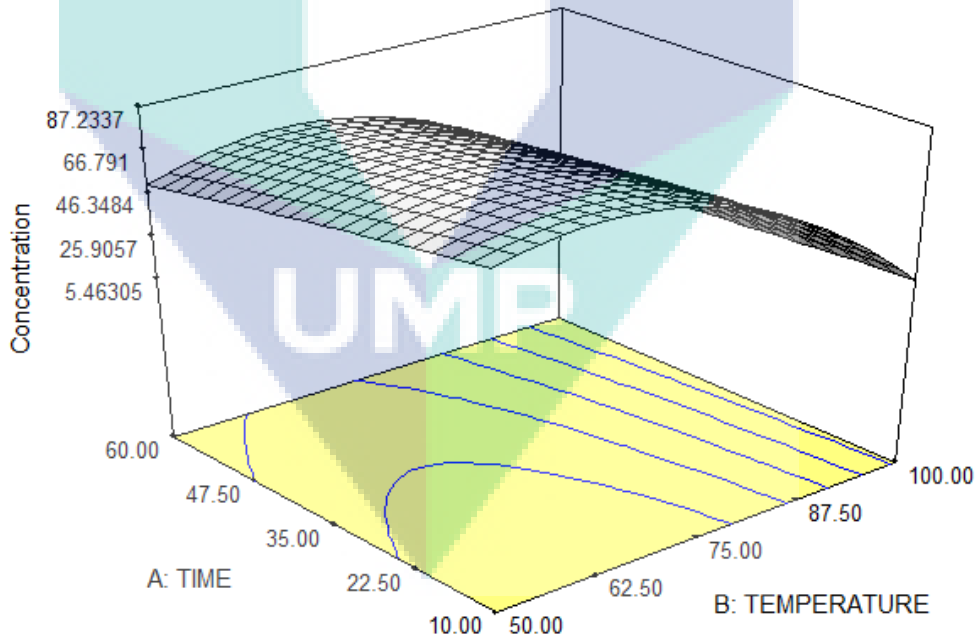
Standard Order	Actual Value	Predicted Value	Residual	Student Leverage	Cook's Residual	Outlier Distance	t	Run Order
1	82.81	81.22	1.60	0.790	2.575	4.162	10.345	*11
2	50.71	50.70	0.016	0.790	0.026	0.000	0.024	12
3	17.03	16.35	0.68	0.790	1.102	0.762	1.122	13
4	4.57	5.46	-0.90	0.790	-1.447	1.314	-1.600	10
5	76.15	78.43	-2.28	0.494	-2.368	0.913	-4.912	*6
6	58.60	57.72	0.88	0.494	0.915	0.136	0.903	8
7	65.30	66.91	-1.61	0.494	-1.675	0.457	-2.003	4
8	12.07	11.86	0.21	0.494	0.222	0.008	0.206	1
9	69.31	69.03	0.28	0.172	0.227	0.002	0.211	7
10	69.31	69.03	0.28	0.172	0.227	0.002	0.211	3
11	69.31	69.03	0.28	0.172	0.227	0.002	0.211	2
12	69.31	69.03	0.28	0.172	0.227	0.002	0.211	9
13	69.31	69.03	0.28	0.172	0.227	0.002	0.211	5

* Case(s) with |Outlier T| > 3.50

Optimization result of total flavonoid compound inTorbangun leave extract.

Std	Run	Book	Factor 1 Time (Minutes)	Factor 2 Temperature (O C)	Response 1 Concentration (mg/L)
8	1	Block 1	35.00	100.00	12.07
11	2	Block 1	35.00	75.00	69.306
10	3	Block 1	35.00	75.00	69.306
7	4	Block 1	35.00	50.00	65.295
13	5	Block 1	35.00	75.00	69.306
5	6	Block 1	10.00	75.00	76.148
9	7	Block 1	35.00	75.00	69.306
6	8	Block 1	60.00	75.00	58.605
12	9	Block 1	35.00	75.00	69.306
4	10	Block 1	60.00	100.00	4.566
1	11	Block 1	10.00	50.00	82.814
2	12	Block 1	60.00	50.00	50.712
3	13	Block 1	10.00	100.00	17.031

The Model F-value of 894.56 implies the model is significant.



Response surface showing the effect of (minute) and extraction temperature (^{O}C) on total flavonoid compound (mg catechin/100 g).

APPENDIX. A8

ANOVA OUTPUT OF ANTIOXIDANT ACTIVITY

Between-Subjects Factors

		N
Time	10	18
	20	18
	30	18
	40	18
	50	18
	60	18
Temperature	50	18
	60	18
	70	18
	80	18
	90	18
	100	18

Tests of Between-Subjects Effects

Dependent Variable:DPPH

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1127.659[a]	35	32.219	9.100	.000
Intercept	1888.549	1	1888.549	533.437	.000
Time	40.111	5	8.022	2.266	.057
Temperature	1052.609	5	210.522	59.464	.000
Time * Temperature	34.938	25	1.398	.395	.994
Error	254.905	72	3.540		
Total	3271.113	108			
Corrected Total	1382.564	107			

a. R Squared = .816 (Adjusted R Squared = .726)

Levene's Test of Equality of Error Variances[a]

Dependent Variable:DPPH

F	df1	df2	Sig.
3.971	35	72	.000

Tests the null hypothesis that the error variance of the dependent variable is equal across groups. a. Design: Intercept + Time + Temperature + Time * Temperature

Estimated Marginal Means

1. Time

Dependent Variable:DPPH

Time	Mean	Std. Error	99% Confidence Interval	
			Lower Bound	Upper Bound
10	4.977	.443	3.803	6.150
20	4.838	.443	3.664	6.011
30	4.433	.443	3.259	5.606
40	3.891	.443	2.717	5.064
50	3.584	.443	2.411	4.758
60	3.368	.443	2.195	4.542

2. Temperature

Dependent Variable:DPPH

Temperature	Mean	Std. Error	99% Confidence Interval	
			Lower Bound	Upper Bound
50	3.486	.443	2.312	4.659
60	9.817	.443	8.644	10.991
70	6.429	.443	5.256	7.602
80	3.270	.443	2.096	4.443
90	1.621	.443	.448	2.795
100	.467	.443	-.706	1.641

3. Temperature * Time

Dependent Variable:DPPH

Temp	Time	Mean	Std. Error	99% Confidence Interval	
				Lower Bound	Upper Bound
50	10	4.669	1.086	1.794	7.543
	20	3.855	1.086	.981	6.730
	30	3.373	1.086	.499	6.248
	40	3.253	1.086	.379	6.127
	50	2.982	1.086	.107	5.856
	60	2.781	1.086	-.093	5.656
60	10	8.482	1.086	5.608	11.356
	20	11.607	1.086	8.732	14.481
	30	10.964	1.086	8.090	13.838
	40	9.619	1.086	6.744	12.493
	50	9.367	1.086	6.493	12.242
	60	8.865	1.086	5.991	11.739
70	10	8.524	1.086	5.649	11.398
	20	7.580	1.086	4.706	10.455
	30	6.496	1.086	3.622	9.370
	40	5.713	1.086	2.838	8.587
	50	5.261	1.086	2.387	8.136
	60	5.000	1.086	2.125	7.874
80	10	4.799	1.086	1.925	7.674
	20	3.524	1.086	.650	6.398
	30	3.393	1.086	.519	6.268
	40	3.022	1.086	.148	5.897
	50	2.530	1.086	-.344	5.405
	60	2.349	1.086	-.525	5.223
90	10	2.339	1.086	-.535	5.214
	20	1.857	1.086	-1.017	4.732
	30	1.817	1.086	-1.057	4.691
	40	1.355	1.086	-1.519	4.229
	50	1.245	1.086	-1.629	4.120
	60	1.114	1.086	-1.760	3.989
100	10	1.047	1.086	-1.828	3.921
	20	.603	1.086	-2.272	3.477
	30	.552	1.086	-2.322	3.426
	40	.382	1.086	-2.493	3.256
	50	.120	1.086	-2.754	2.995
	60	.100	1.086	-2.774	2.975

Post Hoc Tests

Time

Multiple Comparisons
Dependent Variable:DPPH

	(I) Time	(J) Time	Mean Difference (I-J)	Std. Error	Sig.	99% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	10	20	.1389	.62719	1.000	-2.0569	2.3347
		30	.5440	.62719	.953	-1.6518	2.7398
		40	1.0861	.62719	.516	-1.1098	3.2819
		50	1.3922	.62719	.242	-.8036	3.5880
		60	1.6083	.62719	.120	-.5875	3.8041
	20	10	-.1389	.62719	1.000	-2.3347	2.0569
		30	.4051	.62719	.987	-1.7907	2.6009
		40	.9472	.62719	.659	-1.2486	3.1430
		50	1.2533	.62719	.353	-.9425	3.4491
		60	1.4694	.62719	.191	-.7264	3.6653
	30	10	-.5440	.62719	.953	-2.7398	1.6518
		20	-.4051	.62719	.987	-2.6009	1.7907
		40	.5421	.62719	.954	-1.6538	2.7379
		50	.8482	.62719	.755	-1.3476	3.0440
		60	1.0643	.62719	.538	-1.1315	3.2601
	40	10	-1.0861	.62719	.516	-3.2819	1.1098
		20	-.9472	.62719	.659	-3.1430	1.2486
		30	-.5421	.62719	.954	-2.7379	1.6538
		50	.3062	.62719	.996	-1.8896	2.5020
		60	.5223	.62719	.960	-1.6735	2.7181
	50	10	-1.3922	.62719	.242	-3.5880	.8036
		20	-1.2533	.62719	.353	-3.4491	.9425
		30	-.8482	.62719	.755	-3.0440	1.3476
		40	-.3062	.62719	.996	-2.5020	1.8896
		60	.2161	.62719	.999	-1.9797	2.4119
	60	10	-1.6083	.62719	.120	-3.8041	.5875
		20	-1.4694	.62719	.191	-3.6653	.7264
		30	-1.0643	.62719	.538	-3.2601	1.1315
		40	-.5223	.62719	.960	-2.7181	1.6735
		50	-.2161	.62719	.999	-2.4119	1.9797

Based on observed means. The error term is Mean Square(Error) = 3.540.

Homogeneous Subsets

DPPH

	Temperature	N	Subset 1	2	3	4	5
Tukey HSD[a]	100	18	.4673				
	90	18	1.6214	1.6214			
	80	18		3.2697			
	50	18		3.4856			
	70	18			6.4289		
	60	18				9.8173	
	Sig.		.447	.045	1.000	1.000	
Duncan[a]	100	18	.4673				
	90	18	1.6214	1.6214			
	80	18		3.2697	3.2697		
	50	18			3.4856		
	70	18				6.4289	
	60	18					9.8173
	Sig.		.070	.010	.732	1.000	1.000

Means for groups in homogeneous subsets are displayed. Based on observed means. The error term is Mean Square(Error) = 3.540. a. Uses Harmonic Mean Sample Size = 18.000.

APPENDIX. A9

OPTIMIZATION OF ANTIOXIDANT ACTIVITY

ANOVA for Response Surface Quadratic Model Analysis of variance table [Partial sum of squares]

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Model	17.55	5	3.51	52.75	< 0.0001	significant
A	*3.54*	*1*	*3.54*	*53.24*	*0.0002*	
B	*8.10*	*1*	*8.10*	*121.69*	*<0.0001*	
A^2	*0.050*	*1*	*0.050*	*0.75*	*0.4155*	
B^2	*4.63*	*1*	*4.63*	*69.54*	*<0.0001*	
AB	*0.011*	*1*	*0.011*	*0.17*	*0.6961*	
Residual	0.47	7	0.067			
Lack of Fit	*0.47*	*3*	*0.16*			
Pure Error	*0.000*	*4*	*0.000*			
Cor Total	18.01	12				

The Model F-value of 52.75 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise.

Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, B^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Std. Dev.	0.26	R-Squared	0.9741
Mean	2.40	Adj R-Squared	0.9557
C.V.	10.76	Pred R-Squared	0.7851
PRESS	3.87	Adeq Precision	22.484

The "Pred R-Squared" of 0.7851 is in reasonable agreement with the "Adj R-Squared" of 0.9557.

"Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 22.484 indicates an adequate signal. This model can be sed to navigate the design space.

Factor	Coefficient Estimate	DF	Standard Error	95% CI Low	95% CI High	VIF
Intercept	3.06	1	0.11	2.80	3.31	
A-Time	-0.77	1	0.11	-1.02	-0.52	1.00
B-Temp	-1.16	1	0.11	-1.41	-0.91	1.00
A^2	-0.13	1	0.16	-0.50	0.23	1.17
B^2	-1.29	1	0.16	-1.66	-0.93	1.17
AB	0.053	1	0.13	-0.25	0.36	1.00

Final Equation in Terms of Coded Factors:

Antioxidant =
+3.06
-0.77 * A
-1.16 * B
-0.13 * A2
-1.29 * B2
+0.053 * A * B

Final Equation in Terms of Actual Factors:

Antioxidant =
-4.07536
-0.021991 * Time
+0.26123 * Temperature
-2.14897E-004 * $Time^2$
-2.07090E-003 * $Temperature^2$
+8.40000E-005 * Time * Temperature

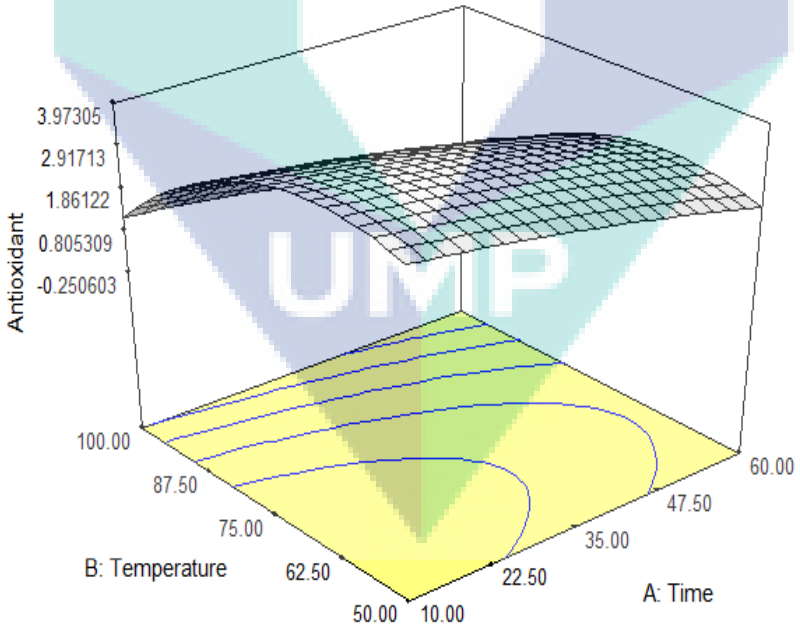
Diagnostics Case Statistics

Standard Run Order	Actual Value	Predicted Value	Residual	Student Leverage	Cook's Residual	Distance	Outlier t	Order
1	3.53	3.61	-0.079	0.790	-0.672	0.284	-0.643	1
2	2.09	1.97	0.12	0.790	1.035	0.673	1.041	13
3	1.27	1.18	0.089	0.790	0.753	0.356	0.727	9
4	0.040	-0.25	0.29	0.790	2.460	3.799	6.184 *	6
5	3.68	3.69	-9.540	E-003	0.494	-0.052	-0.048	10
6	1.74	2.15	-0.41	0.494	-2.251	0.825	-3.964 *	2
7	2.88	2.92	-0.043	0.494	-0.234	0.009	-0.217	5
8	0.22	0.60	-0.38	0.494	-2.069	0.697	-3.073	12
9	3.14	3.06	0.084	0.172	0.360	0.005	0.336	3
10	3.14	3.06	0.084	0.172	0.360	0.005	0.336	11
11	3.14	3.06	0.084	0.172	0.360	0.005	0.336	4
12	3.14	3.06	0.084	0.172	0.360	0.005	0.336	8
13	3.14	3.06	0.084	0.172	0.360	0.005	0.336	7

Optimization result of antioxidant activity in Torbangun leave extract.

Std	Run	Book	Factor 1 Time (Minutes)	Factor 2 Temperature (O C)	Response 1 Concentration (mg/L)
1	1	Block 1	10.00	50.00	3.53
6	2	Block 1	60.00	75.00	1.74
9	3	Block 1	35.00	75.00	3.14
11	4	Block 1	35.00	75.00	3.14
7	5	Block 1	35.00	50.00	2.88
4	6	Block 1	60.00	100.00	0.04
13	7	Block 1	35.00	75.00	3.14
12	8	Block 1	35.00	75.00	3.14
3	9	Block 1	10.00	100.00	1.27
5	10	Block 1	10.00	75.00	3.68
10	11	Block 1	35.00	75.00	3.14
8	12	Block 1	35.00	100.00	0.22
2	13	Block 1	60.00	50.00	2.09

The Model F-value of 52.75 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise.



Response surface showing the effect of (minute) and extraction temperature (^{O}C) on antioxidant activity (mg vitamin C/100 g).

APPENDIX. A10

RESULT OF ANTIBIOTIC BIOASSAY IN TORBANGUN PLANT

Halaman/ *Page* : 1 Dari/*of* : 1

No.	Asal Sampel	Kode Sampel	Nomor Analisis	Jenis Sampel	Jumlah Sampel	Hasil Pengujian Residu Antibiotika				Metoda Uji
						PC's	ML's	AG's	TC's	
01	02	03	04	05	06	07	08	09	10	11
11	Drh.Awalludin Risch	Freeze dry Ati stem : DW = 1:10	D.10.0223	Solution	1	Negatif	Negatif	Negatif	Negatif	SNI 7424;2008
12		Freeze dry Ati stem : MeOH= 1:10	D.10.0221	Solution	1	Negatif	Positif	Negatif	Negatif	
13		Freeze dry Ati leaf : DW = 1:10	D.10.0222	Solution	1	Negatif	Negatif	Negatif	Negatif	
14		Freeze dry Ati leaf : MeOH= 1:10	D.10.0225	Solution	1	Positif	Negatif	Positif	Positif	
15		Fresh Ati stem : DW = 1:10	D.10.0228	Solution	1	Negatif	Negatif	Negatif	Negatif	
16		Fresh Ati stem : MeOH = 1:10	D.10.0227	Solution	1	Positif	Negatif	Negatif	Positif	
17		Fresh Ati leaf : DW = 1:10	D.10.0224	Solution	1	Negatif	Positif	Negatif	Negatif	
18		Fresh Ati leaf : MeOH = 1:10	D.10.0226	Solution	1	Positif	Positif	Negatif	Positif	
19		Freeze dry Tor stem : DW= 1:10	D.10.0235	Solution	1	Negatif	Negatif	Negatif	Negatif	
20		Freeze dry Tor stem:MeOH=1:10	D.10.0219	Solution	1	Positif	Negatif	Negatif	Positif	
21		Freeze dry Tor leaf : DW= 1:10	D.10.0230	Solution	1	Negatif	Negatif	Negatif	Negatif	
22		Freeze dry Tor leaf:MeOH= 1:10	D.10.0229	Solution	1	Positif	Negatif	Positif	Positif	
23		Fresh Tor stem : DW = 1:10	D.10.0231	Solution	1	Negatif	Negatif	Negatif	Negatif	
24		Fresh Tor stem : MeOH = 1:10	D.10.0233	Solution	1	Negatif	Negatif	Negatif	Positif	
25		Fresh Tor leaf : DW = 1:10	D.10.0232	Solution	1	Negatif	Negatif	Negatif	Negatif	
26		Fresh Tor leaf : MeOH = 1:10	D.10.0234	Solution	1	Positif	Positif	Positif	Positif	

Ket : PC's (Penicillina Grup)
ML's (Makrolida Grup)
AG's (Aminoglikosida Grup)
TC's (Tetrasiklina Grup)

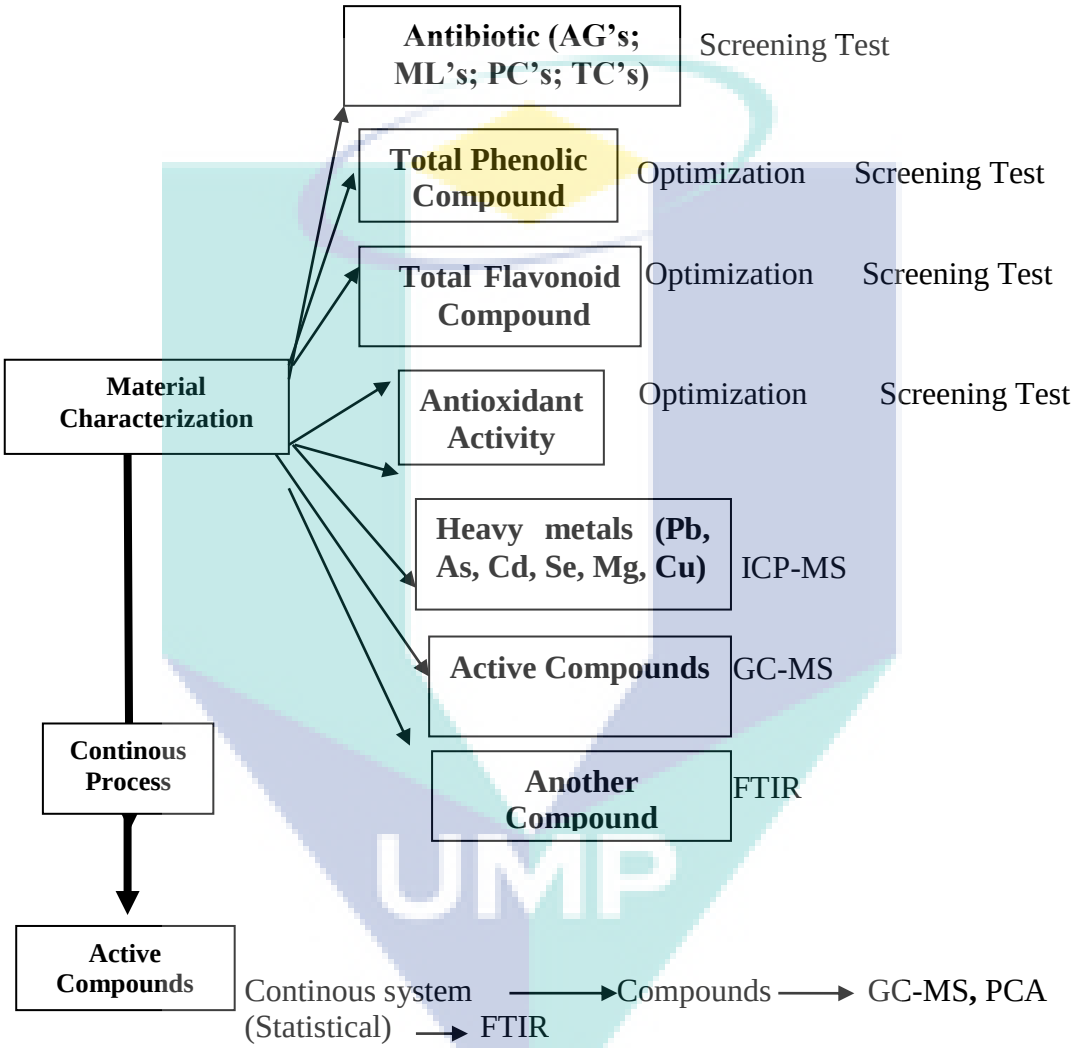
SUPLEMEN

Manajer Teknis,

Drh. Nuraini Triwijayanti
NIP. 19790921 200501 2 001

APPENDIX. A11

EXPERIMENTAL FLOWCHART



APPENDIX C

JOURNAL PUBLISH AND PAPER FOR INTERNATIONAL CONFERENCES

International Conferences

Title	Submition	Accepted	**Publish**
Active Substances of Torbangun Leaf (*Coleus amboinicus Lour*) that Promoted Women Milk Lactation		February 16th 2009	In proceeding
Histopathology Description of Mouse Lactation Mammary Gland After Administration of Torbangun Leaves (*Coleus amboinicus Lor*) by Clinical Test		21-22 July 2010	In proceeding
Analysis of Heavy Metals Toxicity with Determination of Arsenic, Lead, Cadmium, Selenium, Magnesium and Copper in Torbangun Plant (*Coleus amboinicus Lour*)		20-22 November 2012	In Proceeding
Lactagogue as Active Substances in Torbangun Plant (*Coleus amboinicus* Lor) can Increased Women Milk Lactation		20-22 November 2012	In Proceeding
Determination of Active Substances in Torbangun Plant (*Coleus amboinicus* Lour) by Using GC- MS and PCA for Increasing Women Milk Production		June 30th 2012	In Proceeding with ISBN