EXTRACTION OF ANTIOXIDANT, PHENOLIC CONTENT AND MINERALS OF COLEUS *BLUMEI* LEAVES BY BOILING

NORHIDAYAH BINTI HAJI SAAT

UNIVERSITI MALAYSIA PAHANG

UNIVERSITI MALAYSIA PAHANG

BORANG PENGESAHAN STATUS TESIS

JUDUL: **EXTRACTION OF ANTIOXIDANT,PHENOLIC CONTENT AND MINERALS OF COLEUS *BLUMEI* LEAVES BY BOILING**

SESI PENGAJIAN: **2008/2009**

Saya: **NORHIDAYAH BINTI HAJI SAAT**

(HURUF BESAR)

mengaku membenarkan kertas projek ini disimpan di Perpustakaan Universiti Malaysia Pahang dengan syarat-syarat kegunaan seperti berikut :

1. Hakmilik kertas projek adalah di bawah nama penulis melainkan penulisan sebagai projek bersama dan dibiayai oleh UMP, hakmiliknya adalah kepunyaan UMP.
2. Naskah salinan di dalam bentuk kertas atau mikro hanya boleh dibuat dengan kebenaran bertulis daripada penulis.
3. Perpustakaan Universiti Malaysia Pahang dibenarkan membuat salinan untuk tujuan pengajian mereka.
4. Kertas projek hanya boleh diterbitkan dengan kebenaran penulis. Bayaran royalti adalah mengikut kadar yang dipersetujui kelak.
5. *Saya membenarkan/tidak membenarkan Perpustakaan membuat salinan kertas projek ini sebagai bahan pertukaran di antara institusi pengajian tinggi.
6. ** Sila tandakan (✓)

☐	SULIT	(Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub di dalam AKTA RAHSIA RASMI 1972)
☐	TERHAD	(Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana penyelidikan dijalankan).
☐	TIDAK TERHAD	

Disahkan oleh

(TANDATANGAN PENULIS)

Alamat Tetap**:** **No 47,Jln Paya Rumput Jaya,76300 Sg.Udang Melaka**

Tarikh: **20 APRIL 2009**

(TANDATANGAN PENYELIA)

PROF IR DR JAILANI BIN SALIHON
Nama Penyelia

Tarikh: **20 APRIL 2009**

CATATAN: * Potong yang tidak berkenaan.

** Jika Kertas Projek ini SULIT atau TERHAD, sila lampirkan surat daripada pihak berkuasa/organisasi berkenaan dengan menyatakan sekali tempoh tesis ini perlu dikelaskan sebagai SULIT atau TERHAD.

♦ Tesis ini dimaksudkan sebagai tesis bagi Ijazah Doktor Falsafah dan Sarjana secara penyelidikan, atau disertasi bagi pengajian secara kerja kursus dan penyelidikan, atau Laporan Projek Sarjana Muda (PSM)

“Saya akui bahawa saya telah membaca karya ini dan pada pandangan saya karya ini adalah memadai dari segi skop dan kualiti untuk tujuan penganugerahan ijazah Sarjana Muda Kejuruteraan Kimia.”.

Tandatangan : .....

Nama Penyelia : ….....…...

Tarikh : .....

EXTRACTION OF ANTIOXIDANT, PHENOLIC CONTENT AND MINERALS OF COLEUS *BLUMEI* LEAVES BY BOILING

NORHIDAYAH BINTI HAJI SAAT
KA05032

Thesis submitted to the Faculty of Chemical and Natural Resources Engineering in Partial Fulfillment of the Requirement for the Degree of Bachelor Engineering in Chemical Engineering

Faculty of Chemical & Natural Resources Engineering
Universiti Malaysia Pahang

20 APRIL, 2009

I declare that this thesis entitled "*Extraction of antioxidant, phenolic content and minerals of Coleus blumei leaves by boiling*" is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature : .....
Name of Candidate: NORHIDAYAH BINTI HAJI SAAT
Date : 20 APRIL 2009

Special Dedication of This Grateful Feeling to My…

Beloved father and mother;
Mr. Haji Saat bin Haji Ahmad and Mrs. Maznah bt Jais

Loving brothers and sisters;
Mohd Nizam, Norzira, Nora'ain and Mohd Najib

Supportive families;
Uncles and Aunties

For Their Love, Support and Best Wishes.

ACKNOWLEDGEMENT

I would like to express my humble thanks to ALLAH S.W.T. for the strength, inspiration and encouragement given to me through out the completion of this thesis without any obstacles. A lot of experiences and knowledge were gained along the way.

I wished to express my sincere appreciation to my supervisors, Prof IR Dr Jailani bin Salihon for his critics, advices, motivation, friendship and input of ideas, relentless support, guidance and endless encouragement. I also like to express my heartfelt thanks to technical staff in laboratory Puan Nora, Encik Anuar, and Encik Razak, as helping to make my friends and I stay on the task concerning to the preparation and the thesis progress after all.

I am very thankful to my father, Haji Saat bin Haji Ahmad, my mother, Maznah Bt Jais, family members, and all my friends for their advice and motivation. Without their endless support and interest, this thesis would not have been same as presented here. I am also indebted to University Malaysia Pahang (UMP) for giving the facilities for my research.

Lastly, I am grateful to everybody that involved directly or indirectly in helping me completing this thesis.

ABSTRACT

Coleus leaves are commonly known as ati-ati leaves in Malaysia. Previous study has shown that the Coleus leaves have high antioxidant activity and nutritional value. The present work is to investigate whether antioxidant, minerals and phenolic content can be extracted by boiling the leaves in water. The antioxidant was determined by mixing the extract solution with DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) solution using different ratios. Acid ascorbic acid was used as standard in measurement by Uv-Vis Spectrophotometer. Phenolic content was measured by Uv-Vis Spectrophotometer using Gallic acid as standard. There is about 40.77 wt % of antioxidant activity, 6.256998 wt% of total phenolic content, and some minerals (magnesium, calcium, iron and zinc) existing in solution after the Coleus *blumei* leaves were removed. The wt% of the phenolic content is directly proportional to the wt% of antioxidant activity. The mineral concentration, antioxidant activity and phenolic content seemed to be highly correlated. As a conclusion, it is proven that the Coleus *blumei* leaves have high potential value for the nutritional purpose.

ABSTRAK

Daun *Coleus* dikenali sebagai daun ati-ati di Malaysia. Kajian sebelum ini menunjukkan bahawa daun Coleus menpunyai nilai antioksida dan nilai nutrisi yang tinggi. Kajian terkini ialah untuk mengkaji sama ada antioksida, kandungan fenol dan mineral boleh diekstrak dengan merebus daun ke dalam air. Antioksida dicari dengan mencampurkan larutan ekstrak dengan larutan *DPPH (2,2-Diphenyl-1-Picrylhydrazyl)* menggunakan nisbah yang berbeza. Asid askorbik digunakan dalam pengukuran menggunakan *Uv-Vis spectrophotometer*. Kandungan penol diukur menggunakan *Uv-Vis spectrophotometer* di mana acid galik digunakan sebagai larutan pengukur. Terdapat lebih kurang 40.77 wt% (peratus berat) bagi antioksida, 6.256998 wt% bagi kandungan fenol , dan beberapa mineral (magnesium, kalsium, zat besi , dan zink) wujud dalam larutan setelah daun *Coleus blumei* dibuang. Peratusan berat kandungan fenol berkadar langsung dengan kandungan antioksida. Kepekatan mineral, antioksida dan kandungan fenol sangat berkait rapat antara satu sama lain. Sebagai kesimpulan, daun *Coleus blumei* terbukti mempunyai potensi dan nilai nutrisi yang tinggi.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Introduction

With more health conscious society nowadays; the demand for healthier food product has been increasing. Fruits and vegetable provide important health benefits in our daily life. Nowadays, herbs are widely used to provide important health benefits in human diet. Herbs also believed have protective effects on high level exposure of free radicals that can cause the damage of cellular. Free radical can cause many diseases and can contribute to the aging process (Ames et. Al, 1993).The harmful action of the free radicals however can be blocked by antioxidant substances, which scavenge the free radicals and detoxify the organism. Current research has confirmed that food rich in antioxidants play an essential role in the prevention of cardiovascular diseases and cancer (Gerber et al., 2002; Kris-Etherton et al., 2002; Serafini, Bellocco, Wolk, & Ekstrom, 2002) as well as inflammation and problems caused by cell and coetaneous aging (Ames, Shigrenaga, & Hagen,1993). Antioxidants also can control the degenerative diseases where the oxidative damage has been implicated. Several plant extracts and different classes of phytochemicals have been shown to have antioxidant activity (Al Saikhan, Howard, & Miller, 1995; Bergman, Varshavsky, Gottlieb, & Grossman, 2001; Cao, Sofic, & Prior, 1996; Oomah & Mazza, 1994; Wang, Cao, & Prior, 1996; Yen & Duh, 1995). The search for newer natural antioxidants, especially of plant origin, has ever since increased. There are three important aspects that we focus

on this research which are determination of minerals, anti antioxidant activity and phenolic content.

1.1 Research Background / Problem Statement

In medical production, many researchers do a lot of efforts in order to improve the quality of supplementary food. Nowadays, mostly supplementary food is made by using chemical. This also brings out some effects for our health and bodies. So they try to find any other methods to produce better product like using the herbal and traditional methods. In fact of producing the traditional product gives a lot of advantages for our health using the traditional methods, there also have some weaknesses. The liquid solution form usually cannot be kept for a long time period. Using the preservative can damage the pureness of the herbs and lower the effectiveness and its quality. Thus, production of herbal supplementary food into solution form is not really suitable for commercial.

1.2 Objectives

The proposed research is aimed at determining the mineral profile, phenol content, and antioxidant activity in water that has been used to boil Coleus leaves.

1.3 Scopes of Study

To achieve the objectives, there are some scopes have been identified in this research:

i. Study on how to measure antioxidant and phenolic content in the solution of boiled coleus using UV/visible spectrophotometer
ii. Study on how to determine mineral profiles in the solution of boiled coleus using AAS

1.4 Rationale and Significance

Since the discovery of using Coleus in food supplement is not really well known yet, therefore it is expected that the information and knowledge gained from this research studies will increase the awareness of using this traditional plant hence provide much optional treatments to cure any diseases related.

CHAPTER II

LITERATURE REVIEW

2.1 Definition of Coleus

Coleus is a name which derives from an earlier classification under the genus name *Coleus*, species of which are currently included in either *Solenostemon* or another genus, *Plectranthus*. The word Coleus come from the Greek “koleus’, meaning sheath. It is believed that there are 150 species of Coleus .It is a genus of perennial plants, native to tropical Africa, Asia, Australia, the East Indies, the Malay Archipelago, and the Philippines. Many cultivars of the Southeast Asian species *Coleus* have been selected for their colorful variegated leaves, usually with sharp contrast between the colors where the leaves are green, pink, yellow, maroon, and red. Typically, in Malaysia this plant known as ati-ati. The plants need a well condition of in moist-drained soil to grow, and typically grow 0.5-1 m tall, though some may grow as tall as 2 meters. They are heat-tolerant, though they do less well in full sun in subtropical areas than in the shade. The leaves of the green type are often eaten raw with bread and butter. The chopped leaves are also used as a substitute for sage (Salvia officinalis Linn.) in stuffing. C. aromaticus is used for seasoning meat dishes and in food products (Uphof, 1959) while a decoction of its leaves is administered in cases of chronic cough and asthma (CSIR, 1992). It is considered to be an antispasmodic, stimulant and stomachic and is used for the treatment of headache, fever, epilepsy and dyspepsia (Khory &Katrak, 1999; Morton, 1992).

2.2 Antioxidant

An antioxidant in food is really important as it can protect human body from free radicals activity. It is also has capable of slowing or preventing the oxidation of other molecules. When electrons are transferred form a substance to an oxidizing agent, it called as oxidation reaction. Free radicals can be produced during the Oxidation reactions, where the start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols or polyphenols.

Although oxidation reactions are crucial for life, they can 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, causes 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 studied, particularly as treatments for stroke and neurodegenerative diseases. However, it is unknown whether oxidative stress is the cause or the consequence of disease. Antioxidants are also widely used as ingredients in dietary supplements in the hope of maintaining health and preventing diseases such as cancer and coronary heart disease.

Although some studies have suggested antioxidant supplements have health benefits, other large clinical trials did not detect any benefit for the formulations tested, and excess supplementation may be harmful In addition to these uses in medicine, antioxidants have many industrial uses, such as preservatives in food and cosmetics and preventing the degradation of rubber and gasoline.

Current research into free radicals has confirmed that foods rich in antioxidants play an essential role in the prevention of cardiovascular diseases and cancers. As far as our literature survey could ascertain, antioxidant activities of this plant have not previously been published.

Hence, the previous work investigated the possible antioxidative effects of freeze-dried powder obtained from aqueous extract of fresh leaves of *C. aromaticus*. In this study, they had examined the antioxidant activity of CAE (*C.aromaticus* hydroalcoholic extract) employing various in vitro assay systems, such as the β-carotene-linoleate model system, DPPH (2,2-Diphenyl-1-Picrylhydrazyl)/superoxide/ nitric oxide radical scavenging, reducing power and iron ion chelation, in order to understand the usefulness of this plant as a foodstuff as well as in medicine.

2.2.1 Antioxidant Assay using a *β*-carotene-linoleate Model System

On the previous experiment, the antioxidant activity of the extract was measured by the bleaching of β-carotene. By adding CAE and BHT (Butylated Hydroxytoluene) at various concentrations, it can prevent the bleaching of β-carotene to different degrees. β-Carotene in this model system undergoes rapid discoloration in the absence of an antioxidant. This is because of the coupled oxidation of β-carotene and linoleic acid, which generates free radicals. The linoleic acid free radical, formed upon the abstraction of a hydrogen atom from one of its diallylic methylene groups, which attacks the highly unsaturated β-carotene molecules. As a result, β-carotene will be oxidized and broken down in part; subsequently, the system looses its chromophore and characteristic orange colour, which can be monitored spectrophotometrically. The presence of different antioxidants can hinder the extent of β-carotene bleaching by neutralizing the linoleate-free radical and other free radicals formed in the system (Jayaprakasha, Singh, & Sakariah, 2001).

It also showed that the CAE was found to hinder the extent of β-carotene bleaching by neutralizing the linoleate-free radical and other free radicals formed in the system. In comparison, the CAE showed an appreciable antioxidant activity of 83.0% at 250 µg/ml, while BHT, a synthetic antioxidant had 89.6% antioxidant activity at 100 µg/ml.

Table 2.1: Antioxidant activity of aqueous extract of C. aromaticus in β-carotene-linoleate system

Sample	Concentration (µg/ml)	Antioxidant activity (%)
Aqueous extract	125	53.2 ± 1.04
	250	83.0 ± 1.33
	500	91.3 ± 1.41
BHT	50	64.2 ± 1.81
	100	89.6 ± 1.52
	200	95.3 ± 1.33

2.2.2 DPPH Radical-scavenging Activity

The CAE showed a concentration-dependent antiradical activity by inhibiting DPPH radical with an EC_{50} value of 210 µg/ml (Table 2). DPPH is usually used as a substrate to evaluate antioxidative activity of antioxidants (Oyaizu, 1986). The method is based on the reduction of methanolic DPPH solution in the presence of a hydrogen donating antioxidant, due to the formation of the non-radical form DPPH-H by the reaction. The extract was able to reduce the stable radical DPPH to the yellow-coloured diphenylpicrylhydrazine. It has been found that cysteine, glutathione, ascorbic acid, tocopherol, polyhydroxy aromatic compounds (e.g., hydroquinone, pyrogallol, gallic acid), and aromatic amines (e.g., *p*-phenylene diamine, *p*-aminophenol), reduce and decolorise 1,1-diphenyl-2-picrylhydrazyl by their hydrogen donating ability (Blois,

1958). It appears that the CAE possesses hydrogen donating capabilities and acts as an antioxidant. The scavenging effect increased with increasing concentration of the extract. However, scavenging activity of Gallic acid, a known antioxidant, used as positive control, was relatively more pronounced than that of CAE.

Table 2.2: Antiradical activity of aqueous extract of *C. aromaticus* observed with DPPH

Sample	Concentration (µg/ml)	% Inhibition	EC50 (µg/ml)
Aqueous extract	60	11.3 ± 0.22	210
	120	27.0 ± 0.41	
	180	42.0 ± 1.79	
	240	58.4 ± .050	
	300	72.7 ± 0.33	
Gallic acid			1.38

2.2.3 Assay of Superoxide Radical (O_2^-)-Scavenging Activity

The superoxide radical (O_2^-)-scavenging activity of the extract, were previously measured by the riboflavin-NBT-light system in vitro. Superoxide radical is known to be very harmful to cellular components as a precursor of more reactive oxygen species (Halliwell & Gutteridge, 1985). Photochemical reduction of flavins generates O_2^-, which reduces NBT, resulting in the formation of blue formazan (Beauchamp & Fridovich, 1971). The extract was found to be a moderate scavenger of superoxide radical generated in riboflavin-NBT-light system in vitro. The extract inhibited the formation of the blue formazan and the % inhibition was proportional to the concentration with an EC50 value of 73.9 µg/ml. These results indicated that the tested extract had a notable

effect on scavenging of superoxide when compared with ascorbic acid, which was used as positive control.

Table 2.3: Superoxide anion-scavenging activity of aqueous extract of *C. aromaticus* observed with the riboflavin-light-NBT system

Sample	**Concentration (µg/ml)**	**% Inhibition**	**EC_{50} (µg/ml)**
Aqueous extract	25	13.3 ± 1.89	73.9
	50	35.4 ± 1.14	
	75	52.5 ± 1.30	
	100	66.5 ± 1.05	
Ascorbic acid			17.4

2.2.4. Assay of Nitric Oxide-Scavenging Activity

The extract also showed a moderate nitric oxide-scavenging activity between 25 and 200 µg/ml in a dose-dependent manner (EC_{50} = 173 µg/ml) (Table 4). In addition to reactive oxygen species, nitric oxide is also implicated in inflammation, cancer and other pathological conditions (Moncada, Palmer, & Higgs, 1991). The plant/plant products may have the property to counteract the effect of NO formation and in turn may be of considerable interest in preventing the ill effects of excessive NO generation in the human body. Further, the scavenging activity may also help to arrest the chain of reactions initiated by excess generation of NO that are detrimental to human health. The extract showed a moderate nitric oxide-scavenging activity. The % inhibition was increased with increasing concentration of the extract. Curcumin, a natural antioxidant was used as a positive control for comparison (Sreejayan & Rao, 1997).

Table 2.4: In vitro NO-scavenging activity of aqueous extract of *C. aromaticus*

Sample	**Concentration (µg/ml)**	**% Inhibition**	**EC_{50} (µg/ml)**
Aqueous extract	25	14.4 ± 1.08	173
	50	20.2 ± 0.79	
	100	35.1 ± 0.77	
	200	55.6 ± 1.02	

DPPH radical-scavenging activities and amount of the isolated compounds of Coleus aromaticus are showed in Table 2.4 and 2.5. Values of tested material in Table 2.5 were determined from integration of HPLC signals and response factors calculated from standards. The results are from three separate experiments.

Table 2.5: Tested materials EC50 (lg/ml ± SD) Amounts (mg/ga ± SD)

Hexane extract >500 –

Ethyl acetate extracts 84.0 ± 0.35 –

Aqueous extract 348 ± 2.46 –

Chlorogenic acid 11.0 ± 0.57 1.33 ± 6.58

Rosmarinic acid 9.96 ± 0.94 44.8 ± 1.84

Caffeic acid 5.52 ± 0.35 2.42 ± 1.84

Gallic acid 1.38 ± 0.22 –

Figure 2.1 shows the extraction scheme for the isolation of antioxidant compounds from Coleus aromaticus while Figure 2.2 showed the structure of Compounds isolated from Coleus aromaticus leaves.

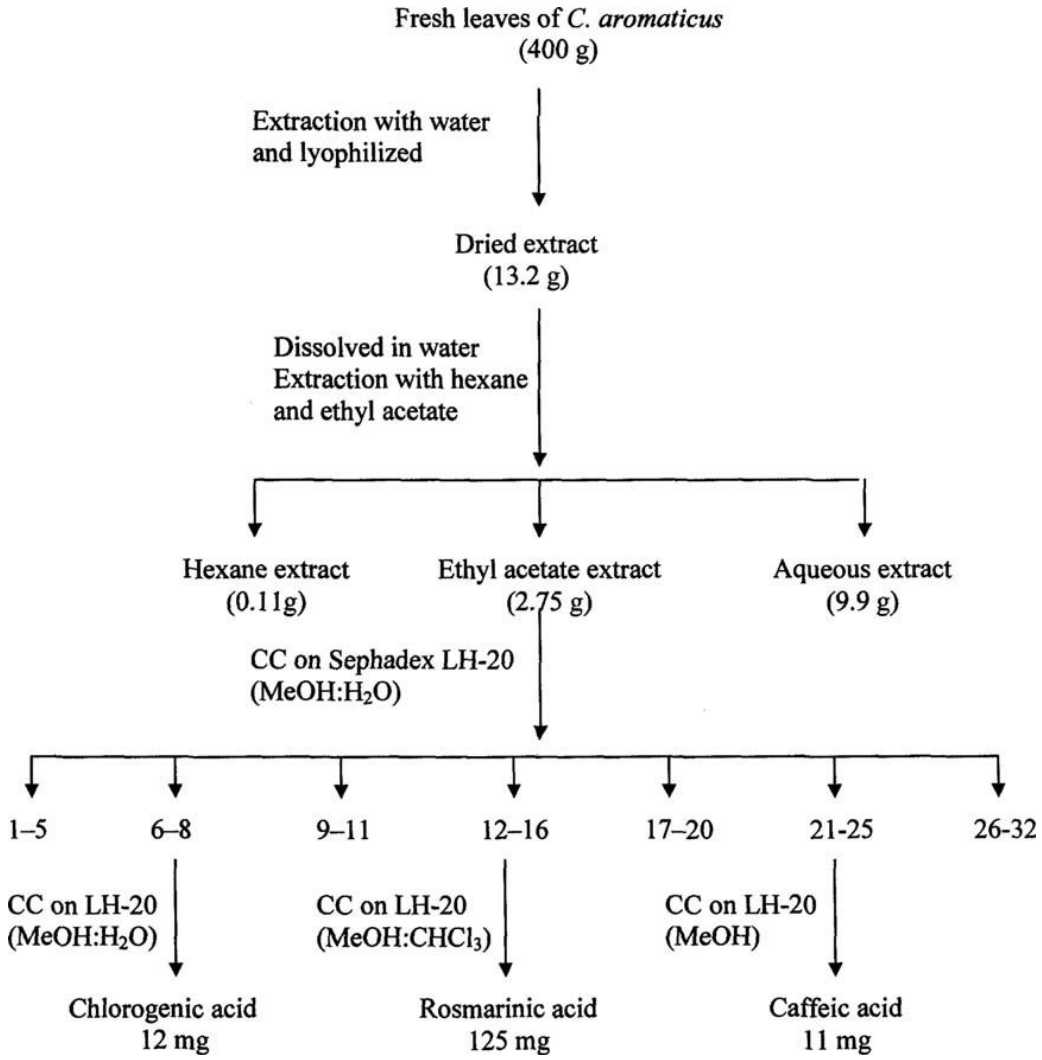


Figure 2.1: Extraction scheme for the isolation of antioxidant compounds from Coleus aromaticus

Table 2.6: Spectral data of the compounds isolated from Coleus *aromaticus*

Name of compounds	^{1}H NMR(270 MHz) TMS as int. standard	^{13}C NMR(68 MHz) TMS as int. standard	FAB MS (m/z)
(1) Chlorogenic acid	1.62–2.12 (m) 3.54–4.03 (m) 5.03–5.19 (m) 6.28 (d) 6.78 (d) 6.99 (dd) 7.06 (d) 7.57 (d)	36.56, 37.27, 68.34, 70.65, 70.94, 73.63, 114.33,114.86, 115.75, 121.31, 125.64, 144.94, 145.57, 165.82, 175.15,	355 [M + H]+, 377 [M + Na]+
(2) Rosmarinic acid	3.10 (2q) 5.24 (dd) 6.32 (d) 6.68 (dd) 6.77 (d) 6.87 (d) 6.88 (d) 7.05 (dd) 7.18 (d) 7.58 (d)	37.40, 73.73 114.77,115.16, 115.95,116.33, 117.28,120.39, 121.63, 122.78 127.37, 129.09, 144.71, 145.63, 146.21, 146.64, 148.90, 166.93, 171.36	361 [M + H]+, 383 [M + Na]+
(3) Caffeic acid	6.77 (d) 6.96 (dd) 7.05 (d) 6.28 (d) 7.42 (d)	114.64,115.24, 115.83,121.20, 125.80, 144.60, 145.63, 148.17, 168.00	181 [M + H]+, 203 [M + Na]+

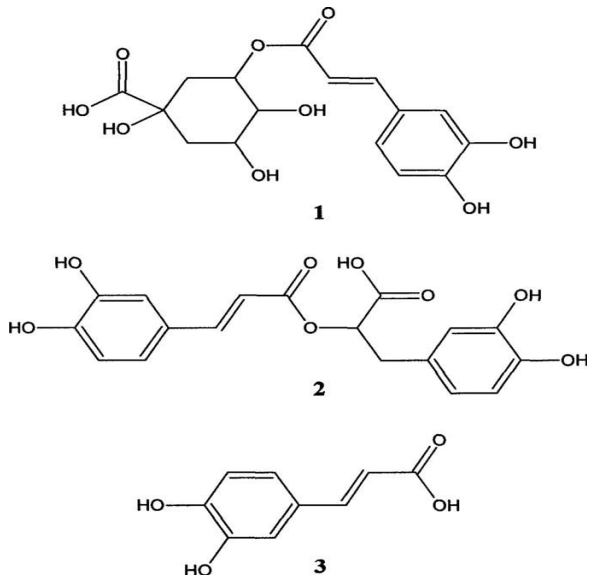


Figure 2 2: Compounds isolated from Coleus *aromaticus* leaves

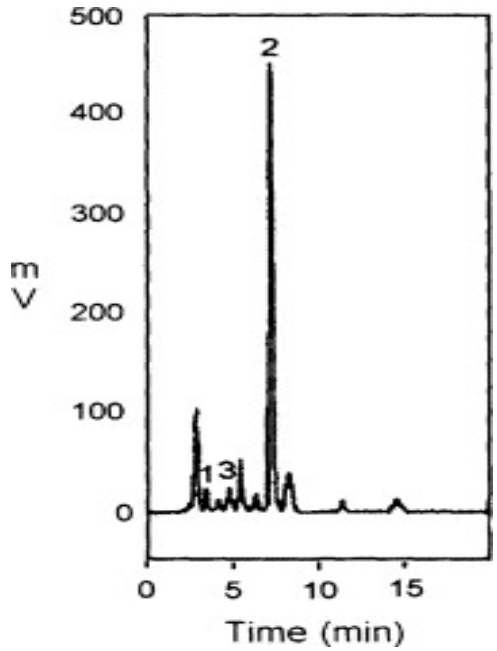


Figure 2.3: HPLC chromatogram of the ethyl acetate extract of *Coleus aromaticus* leaves. Chlorogenic acid (**1**), rosmarinic acid (**2**), and caffeic acid (**3**) were detected at 325 nm

The aqueous extract of *C. aromaticus* leaves exhibited different levels of antioxidant activity in all the models studied. The results from various free radical-scavenging systems revealed that the *C. aromaticus* had significant antioxidant activity and free

radical-scavenging activity which showed in Figure 2.3. The free radical-scavenging property may be one of the mechanisms by which this drug is useful as a foodstuff as well as a traditional medicine. Table 2.6 showed the spectral data of the compounds isolated from Coleus *aromaticus*. However, further investigation of individual compounds, their in vivo antioxidant activities and in different antioxidant mechanisms is warranted.

2.3 The UV/Vis Spectrophotometer

The UV/Vis spectrometer consists of a light source, a sample compartment, a diode-array detector, and a data acquisition computer. The sample compartment is between the light source and the detector. The spectrometer measures the amount of ultraviolet and visible light transmitted by a sample placed in the sample compartment. Typically liquid samples are used, contained in a transparent "cuvette" or "cell". A flow-through cell for the kinetics experiment is currently in the sample compartment, but another standard cuvette can easily be substituted for it. The sample compartment in our spectrometer is made for 1 cm cuvettes.

2.3.1 Theory of Absorption

Figure 2.4 shows the theory of adsorption of Uv/vis spectrometer while Figure 2.5 shows the Uv/vis spectrometer.When white light passes through or is reflected by a colored substance, a characteristic portion of the mixed wavelengths is absorbed. The remaining light will then assume the complementary color to the wavelength(s) absorbed. This relationship is demonstrated by the color wheel shown on the right. Here, complementary colors are diametrically opposite each other. Thus, absorption of 420-430 nm light renders a substance yellow, and absorption of 500-520 nm light makes it red. Green is unique in that it can be created by absorption close to 400 nm as well as absorption near 800 nm.

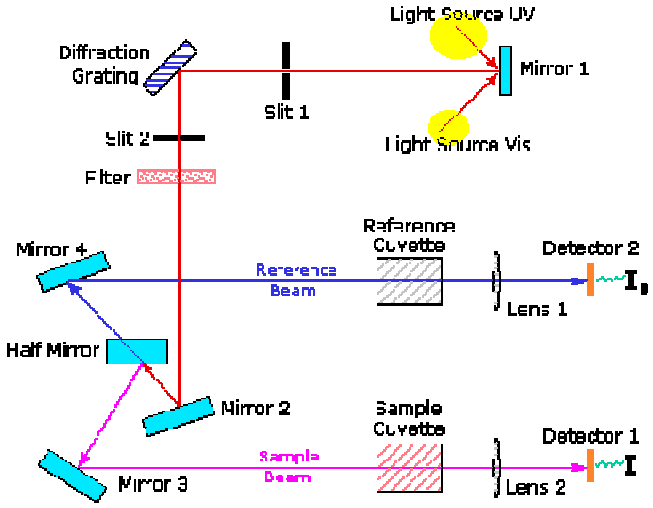


Figure 2.4: Theory of adsorption

Figure 2.5: Uv-vis spectrometer

2.4 Phenolic Content

Phenolic compound are reported to be active, quenching oxygen derived free radicals by donating hydrogen atom or an electron tothe free radical. Phenolic compounds are considered beneficial for human health, decreasing the risk of

degenerative diseases by reduction of oxidative stress and inhibition of macromolecular oxidation (Larson, 1988; Pereira et al., 2007; Pulido, Bravo, & Saura-Calixto, 2000; Silva et al., 2004; Velioglu, Mazza, Gao, &Oomah, 1998). These compounds have been reported to be well correlated with antioxidant potential (Katalinic, Milos, Modun,Music, & Boban, 2004). Total soluble phenolic compound content The Folin–Ciocalteu method measures the reduction of the reagent by phenolic compounds with the formation of a blue complex that can be measured at 750 nm against gallic acid as a standard (Imeh & Khokhar, 2002).

In addition to their delicious taste and refreshing flavor and aroma, fruits add important vitamins, minerals and other bioactive compounds to the human diet. It has been shown in epidemiological studies that a correlation exists between the consumption of fruits and reduced risk of chronic diseases (Block, Patterson, & Subar, 1992; Chun & Kim, 2004; He, Nowson, Lucas, & Macgregor, 2007; Kuskoski, Asuero, & Troncoso, 2005; Van't Veer, Janson,Klert, & Kok, 2000; Wu et al., 2004).

The combination of vitamins, minerals, phenolic antioxidants and fiber seem to be responsible for these effects (Ruxton, Gardner, & Walker, 2006). Parallel with this recognition, the consumption of tropical or ''exotic" fruits have increased all over the world.Different fruits differ markedly in the quantity and types of phenolic antioxidants and their conjugates (Macheix, Fleuriet, & Billot, 1990). The use of simple ''total antioxidant capacity" methods differing in their way of generating free radicals, the strategy to measure the end point of the inhibition reaction, and the sensitivity towards the different reducing molecules in the sample (Pellegrini et al., 2003; Roginsky & Lissi, 2005). Therefore, more than one method should be used to gain useful information about the total antioxidant capacity of phenolic compounds.

2.5 Folin-Ciocalteu Reagent

The Folin-Ciocalteu reagent (FCR) or Folin's phenol reagent or Folin-Denis reagent is a mixture of phosphomolybdate and phosphotungstate used for the colorimetric assay of phenolic antioxidants and polyphenolic antioxidants.It works by measuring the amount of the substance being tested needed to inhibit the oxidation of the reagent.

2.6 Mineral Profile in Plant Leaves

Leafy plants like herbs or vegetable hold an important place in well-balanced diets. The idea itself of a well-balanced diet changed in recent years and lesser amounts of red meat and more vegetable and fruits are advised ([Ames & Gold (1996)]; [Lucarini, Canali, Cappelloni, Di Lullo, & Lombardi-Boccia (1999)]; [Kratzer and Vohra (1986)]; [NRC (1989)]). On the other hand, with few exceptions, fruits and leafy plants are believed to occupy a modest place as a source of trace elements due to their high water content ([Gibson (1994)]).

The leafy plants most commonly consumed as salad greens in the Southern Brazil are lettuce, rucola and watercress. Butterhead lettuce (*Lactuca sativa*) is specially favored among lettuce varieties. Rucola (*Eruca sativa*), a vegetable with a piquant flavor belonging to the mustard family was brought to Brazil by Mediterranean immigrants. Watercress (*Nasturtium officinale*) follows closely the two first ones in consumers' preference. Other greens such as kale (*Brassica oleracea* var acephala), chicory (*Chicorium intybus*), Chinese cabbage (*Brassica chinensis*), cabbage (*B. oleracea* var capitata), and a spinach substitute (*Tetragonia expansa*) originally from New Zealand, are used primarily as cooked vegetables.

The previous research show that eight nutritionally important minerals (calcium, magnesium, iron, copper, manganese, zinc, sodium and potassium) in leafy green plants were found at the leaf part. Total minerals were also determined in cooked vegetables when they were generally consumed that way. The objective of the previous work was to examine the variability in the mineral content at plant leaves, in order to be able to reach the probable variation to be expected for the minerals in the studied leafy plants.

Kale, chicory, Chinese cabbage, and cabbage are most commonly served cooked in Southern Brazil and a brief cooking period with fat and seasonings is the most frequent preparation method. For this reason the mineral profile of these vegetables was also determined after cooking Table 2.7.

Table 2.7: Total K, Na, Ca, Mg, Fe, Mn, Cu, Zn (mg/100 g weight after cooking ±standard deviation b) in leafy vegetables cooked under dry heat

Mineral	Kale	Chicory	Chinese cabbage	Cabbage
K	816±517	718±659	256±110	275±103
Na	15±5	22±9	5±2	3±1
Ca	331±47	54±7	50±5	46±5
Mg	58±6	18±3	14±5	15±3
Fe	0.5±0.2	0.6±0.2	0.22±0.03	0.16±0.04
Mn	0,4±0,2	0,3±0,1	0,2±0,1	0,2±0,1
Cu	0,06±0,05	0,08±0,02	0,04±0,03	0,04±0,04
Zn	0,3±0,1	0,28±0,04	0,3±0,1	0,2±0,1

2.7 Basic Principle of AAS

The technique of flame atomic absorption spectrometer (AAS) requires a liquid sample to be aspirated, aerosolized, and mixed with combustible gases, such as acetylene and air or acetylene and nitrous oxide. The mixture is ignited in a flame whose temperature ranges from 2100 to 2800 ^{o}C. During combustion, atoms of the element of interest in the sample are reduced to free, unexcited ground state atoms, which absorb light at characteristic wavelengths, as shown in figure 2.6.

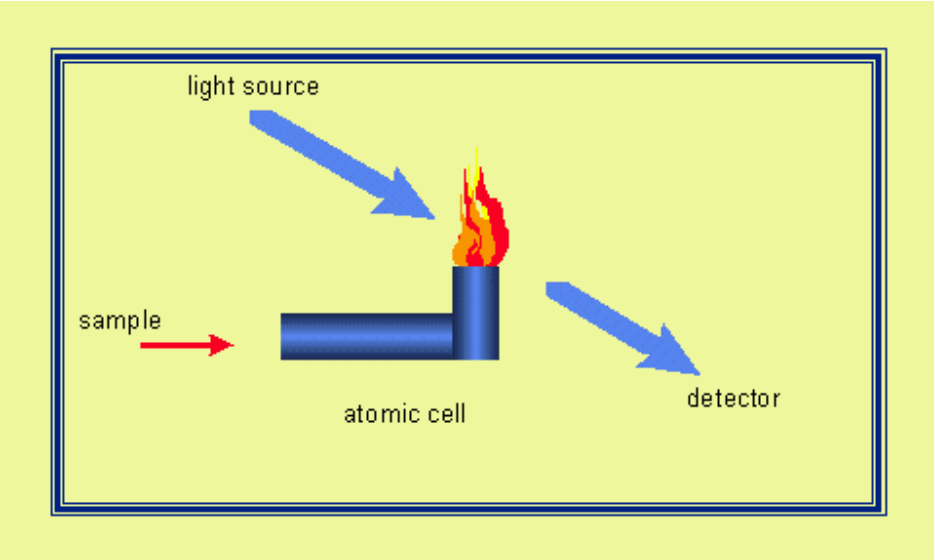


Figure 2.6: Operation principle of an atomic absorption spectrometer.

Figure 2.7 shows an atomic absorption spectrometer. This instrument in particular is designed to operate either with a flame or with a graphite furnace the graphite furnace is additionally equipped with an auto sampler.

Figure 2.7: Flame absorption spectrometer with attached graphite furnace

Flame atomic absorption hardware is divided into six fundamental groups that have two major functions: generating atomic signals and signal processing. Signal processing is a growing additional feature to be integrated or externally fitted to the instrument. The instrument parts are shown in Figure 2.8.

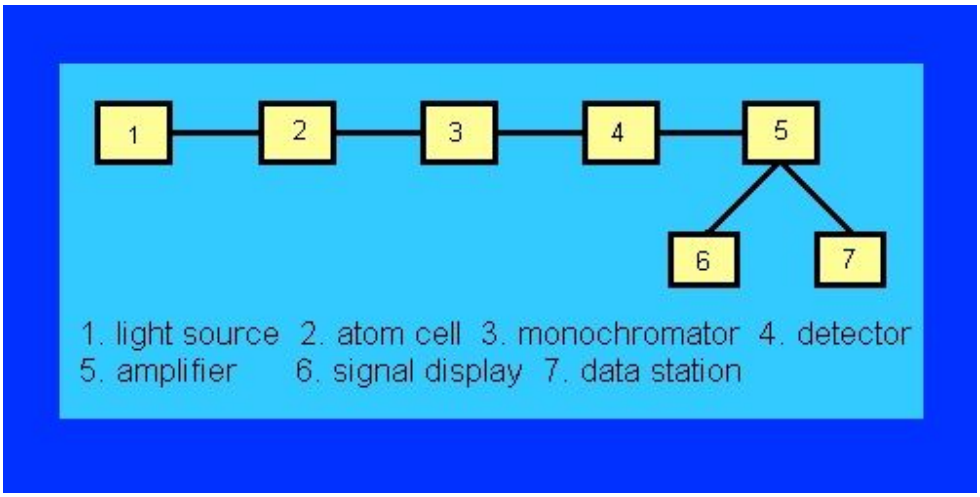


Figure 2.8: Schematic of basic instrumental parts of atomic absorption spectrometer

CHAPTER III

METHODOLOGY

3.1 The Overall Methodology

The overall methodology involved all the steps in determine and measuring those three aspect; antioxidant, mineral profile and phenol content. The whole study is divided into three major sections:

i. Sample preparation
ii. Analysis the sample using UV/visible to measure antioxidant and phenolic content, and using AAS to determine mineral profiles.
iii. Calculation and calibration curve preparation.

3.2 Sample Preparation

The extract solution was prepared by soaking 8.5g of Coleus *blumei* leaves in distilled water using the mass ratio of 1:10. Then it was boiled for an hour using the water bath at temperature of 35^0C. After removing the leaves, this solution was sonicated for about 30 minutes. Then it was filtrated using the Whatman paper.

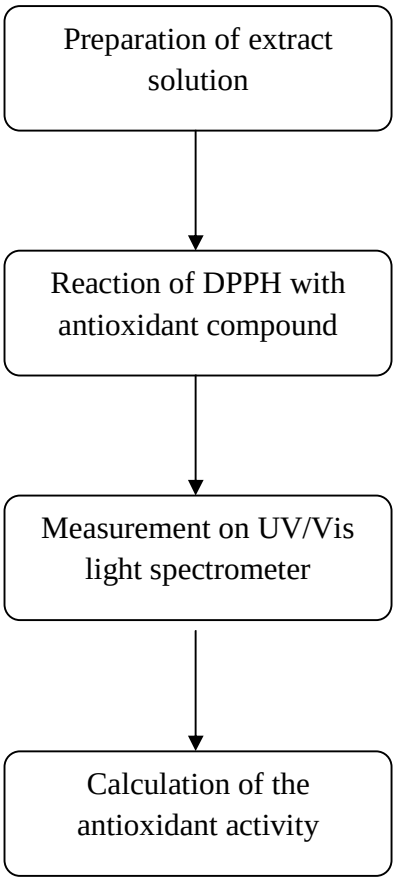


Figure 3.1: Flow diagram for measurement of antioxidant Using UV/Vis

3.3 Procedure Description on Measurement of Antioxidant Using UV/Vis

3.3.1 DPPH Solution Preparation

The solution of DPPH (2, 2-diphenyl-2-picrylhydrazyl hydrate) in methanol (0.002%) was prepared daily before the UV measurements. This solution of mixtures was kept in dark for 30minutes and optical density was measured at 517nm using spectrophotometer.

3.3.2 Mixture of DPPH Solution with Sample

Three ml of DPPH solution then mixed in the extract solution in 1cm path length disposable microcuvettes. The final mass ratio of extracts with DPPH was approximately 3; 1, 1.5; 1.0.75; 1. Ascorbic acid was used as standard and methanol (0.002%) used as blank. Measurement were performed at least triplicate.

When DPPH reacted with antioxidant compound which can donate hydrogen, it is reduced. Then it changed the color from deep to light violet then to yellow. After that, this solution was measured on 515nm on the UV/Vis light spectrophotometer. Antioxidant activity of the plant extracts was measured using formula given.

Antioxidant activity (%):

[(absorbance of control - absorbance of sample) / (absorbance of control)] x 100

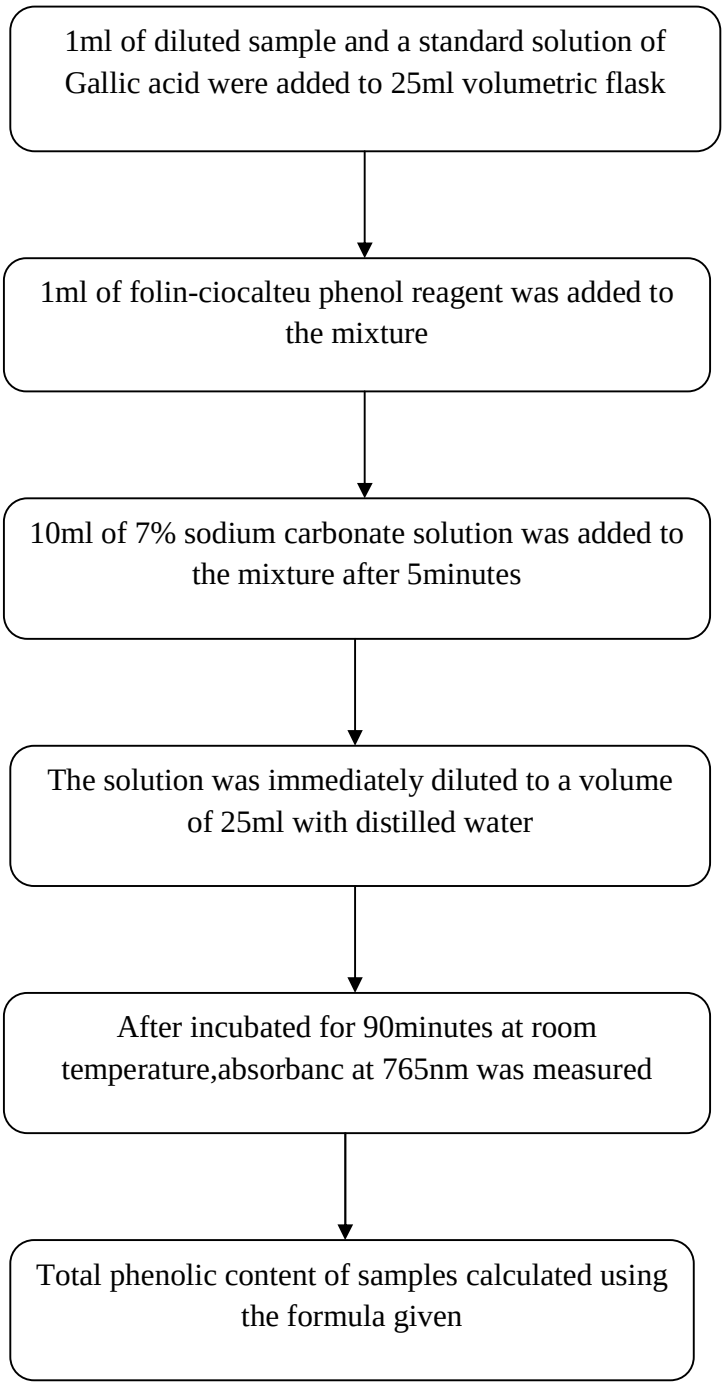


Figure 3.2: Flow diagram for measurement of phenolic content using UV/Vis

3.4 Procedure Description on Measurement of the Phenolic Content Using UV-vis

The total phenolic content was determined using the Folin–Ciocalteu method (Zheng & Wang, 2001). A calibration curve of Gallic acid was prepared, and the results were expressed as mg GAE (gallic acid equivalents)/cup. In this method the sample prepared same as previous method (3.2). Then 1ml of diluted sample and a standard solution of Gallic acid were added to 25ml volumetric flask containing nine ml of double distilled water. Then 1ml of folin-ciocalteu phenol reagent was added to the mixture and there is change of color of the reagent from yellow turned to green. After fives minutes, 10 ml of 7% sodium carbonate solution was added to the mixture then the solution was immediately diluted to a volume of 25 ml with distilled water. After incubated for 90 minutes at room temperature, absorbance at 765nm was measured. Lastly, total phenolic content of samples calculated using the formula given.

$$\% \text{ total phenol: } \frac{\text{extract of volume (ml)}}{1000} \times Y \times \frac{100}{(\text{sample weight (g)} \times 1000)} \times \text{dilution factor}$$

Where:

Y = concentration value from the graph of absorbance versus Gallic acid concentration

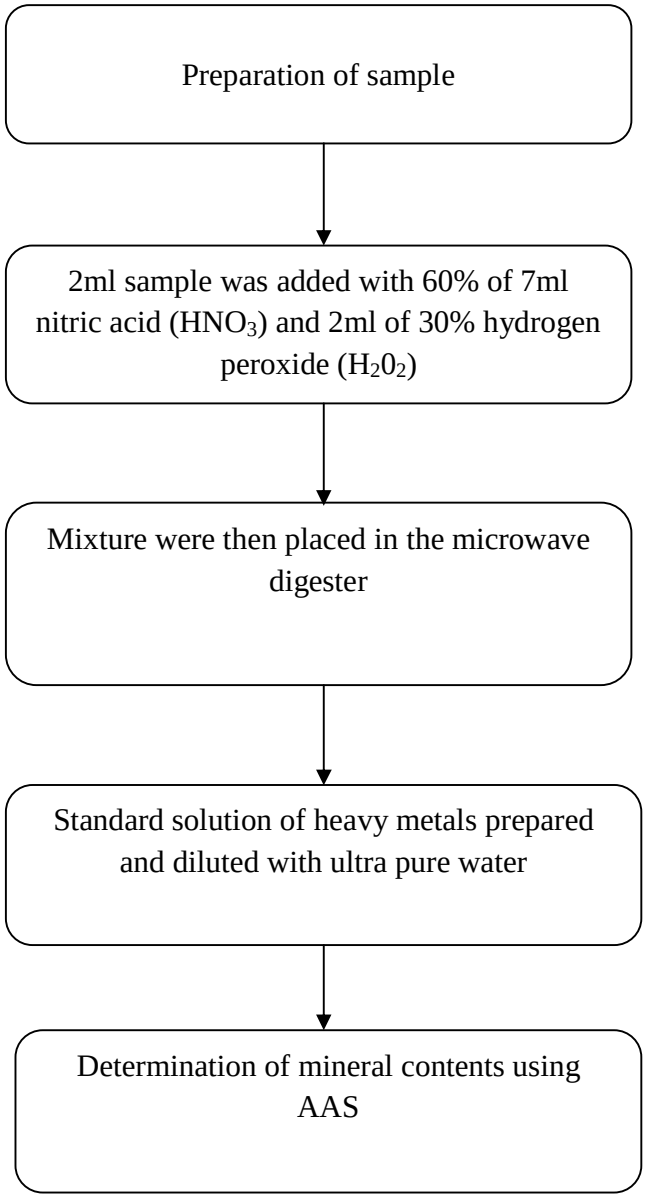


Figure 3.3: Flow diagram for measurement of mineral profiles using AAS

3.5 Procedure Description on the Determination of the Mineral Profile Using AAS

Total contents of each mineral were determined after digestion of the sample with nitric acid (65%, Suprapur grade, Merck, Germany). Ten grams of Coleus *blumei* were weighed and prepared same as the previous method. Then bout two of ml sample was added with 60% of 7ml nitric acid (HNO_3) and 2ml of 30% hydrogen peroxide (H_20_2) before it placed into digestion tube. The solution is allowed to be digested when contents of the solution were clear. The mixture digested in microwave digester for 20 minutes at temperature of 200 ^{0}C.

The standard solution of heavy metals prepared. The solution obtained then used to determine potassium, sodium, calcium, magnesium, iron, copper, magnesium, and manganese, zinc and lead by flame atomic absorption spectrometry (model 5100 PC with deuterium background correction lamp, microprocessor controlled Perkin–Elmer instrument, Überlingen, Germany). Standards for atomic absorption will be used for calibration from Carlo Erba (Italy), Merck (Germany) and J.T. Baker (USA). Then the Ultra-pure water (MilliQ plus, Millipore, USA) with resistivity of 18 MΩ will be used whenever water is needed as blank and for the dilution for standard solution. All glassware and polyethylene flasks will be soaked 24 h in nitric acid 10% and then rinsed with ultra-pure water before used

CHAPTER IV

RESULT AND DISCUSSION

4.1 Result and Discussion

From this experiment, it is shown that the minerals, antioxidant and phenolic contents of the Coleus *blumei* leaves can be extracted by boiling in water at 35^0C.The experiments of determining those three elements are completed. The procedure that we used in these experiments is followed step by step by counting on the precaution steps and several assumptions to ensure we get the maximum result from the experiment.

4.1.1 Antioxidant Activity

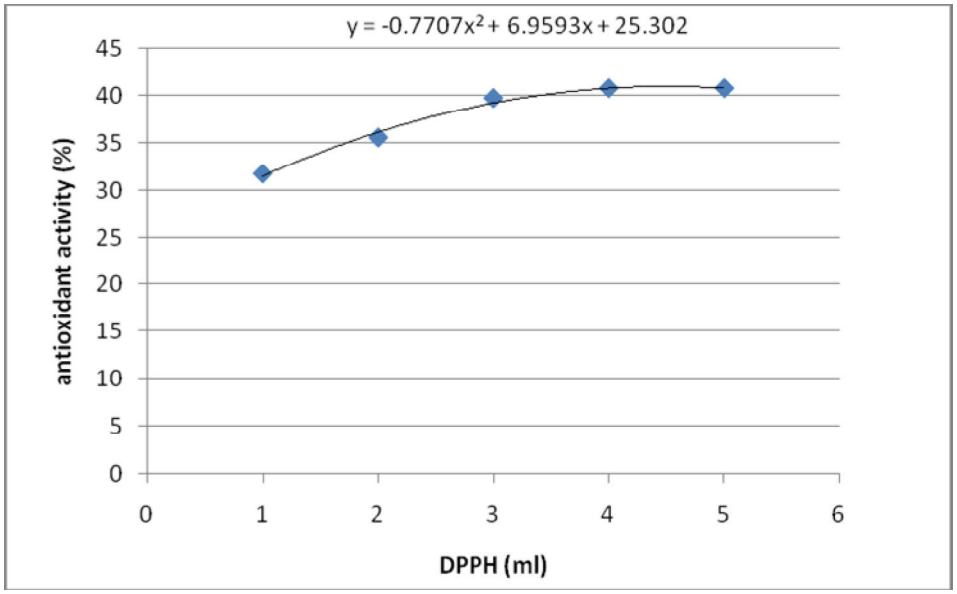


Figure 4.1: Antioxidant activity of sample solution

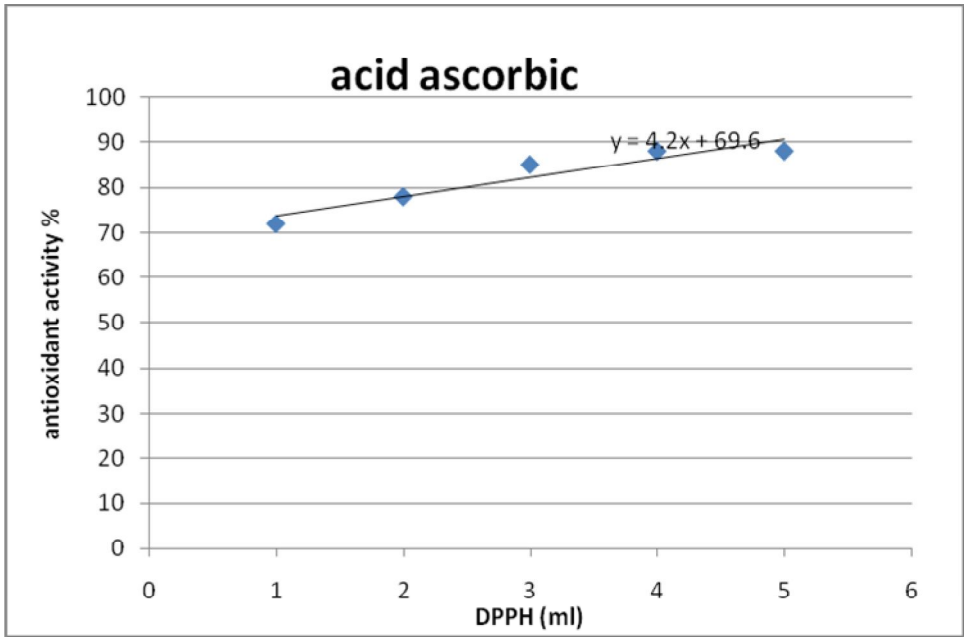


Figure 4.2: Antioxidant activity of standard solution

In order to prevent the free radicals implicated diseases which have serious effects on the cardiovascular system, the effective antioxidants from natural sources as alternatives to synthetic antioxidant become important nowadays. Many plants have

been investigated for their antioxidant activity. Polyphenol interact with phatogens, herbivores, and other plants to protect the plant from ultraviolet radiation and oxidants.

Therefore, in this study, the antioxidant properties of the methanol extracts of boiled coleus leaves solution is examined for DPPH radical scavenging activity according to the method described. The results of the experiment are shown in Table A-1 where ascorbic acid use as standard as shown in figure 4.2. From this both figure 4.1 and figure 4.2, there shows the weight percentage of antioxidant activity of boiled solution of Coleus leaves, and standard solution of ascorbic acid used different ratio of extract solution samples and DPPH solution. In the experiment of analyzing the antioxidant activity, we can see that the increase of DPPH volume in the same amount of sample increased the antioxidant activity. DPPH solution is oxidant agent contain of -OH ion and will contact with H ion from the sample solution. The data shows that the antioxidant activity increase slightly until four ml of DPPH solution added, it started to maintain as well as when five ml of DPPH solution added. At this level, we can say that it is the maximum reaction of H and OH ion between DPPH and sample solution where the percentage value of antioxidant activity is about 40.77 wt% .As well as the standard solution, but as expected acid ascorbic gives higher weight percentage of antioxidant activity than the extract sample which is bout 89.7 wt%. After another ml of DPPH solution added in both solutions, the absorbance value detected become negative and out of ranges as the solution too dilute as we can see the color of the solution become much clear. DPPH in this model system undergoes rapid discoloration in the absence of an antioxidant where it turn the color from deep violet to light violet then yellow in color because of the reduction of hydrogen ion.

4.1.2 Phenolic Content

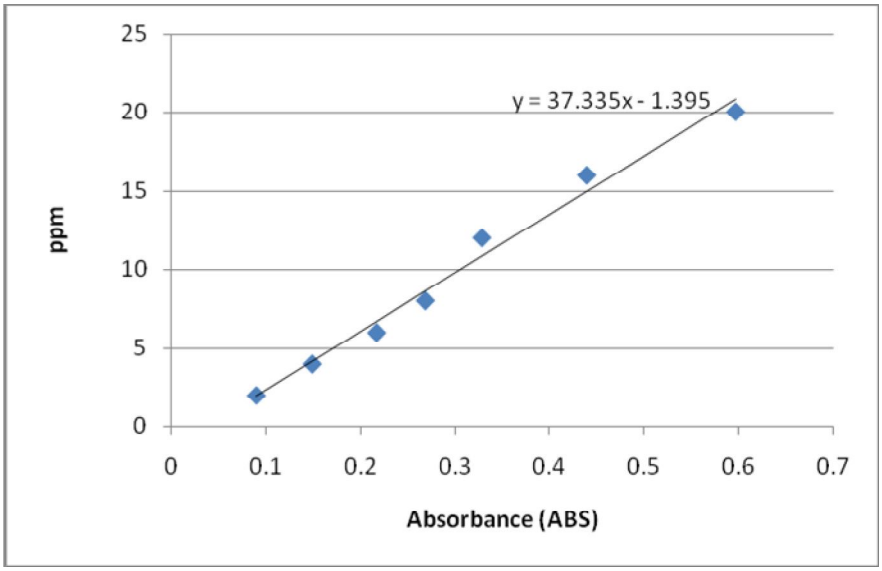


Figure 4.3: Concentration of Gallic acid versus absorbance

Studies on dietary and medicinal plants have shown that polyphenols inhibit oxidative stress (Manach et al., 2004;Rice-Evans et al., 1996). Antioxidant activity of leaf extracts from medicinal plants (Zainol et al., 2003) and fruits (Banerjee et al., 2005) found a direct linear relationship between the total phenolic content and total antioxidant activity, indicating that the phenolic compounds might be the major contributors to the antioxidant activities of these extracts (Martina et al., 2007) Phenolics are known as radical scavengers or radical-chain breakers, extinguishing the different oxidative free radicals.The antioxidative property of the phenolics has been predicted mainly due to their redox potential (Rice-Evans et al., 1995).

Table 4.1 shows that each analyze using the different amount of Gallic acid concentration with different volume of distilled water but using the same volume of sample solution,Na_2CO_3 and Folin-ciocalteau reagent. A calibration curve was prepared using Gallic acid standard at different concentration. The value of Gallic acid concentration is linear with the value of absorbance. Figure 4.3 shows the value of absorbance of each sample with different value of Gallic acid concentration. From the

linear graph of absorbance versus concentration of Gallic acid, we get the value of x regression. From the linear equation we can get the value of concentration, y then proceed with calculation of total phenol percent using the formula of:

$$\% \text{ total phenol: } \frac{\text{extract of volume (ml)}}{1000} \times y \times \frac{100}{(\text{sample weight (g)} \times 1000)} \times \text{dilution factor}$$

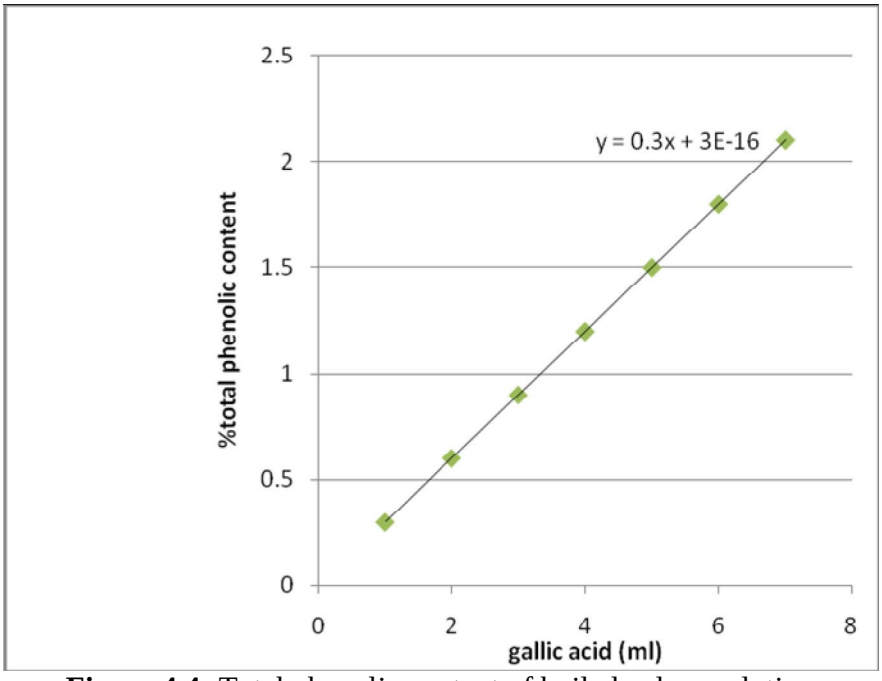


Figure 4.4: Total phenolic content of boiled coleus solution

From figure 4.4, we can say that increasing amount of Gallic acid increase the phenolic content in the sample solution. The value of phenolic content in the sample solution lower than the antioxidant activity as it is major contributors to the antioxidant activities. However, as we can see from the results, the phenolic content in the sample solution is so little. It might be happened because the sample was exposed to the light for a long time during the analyze process as we know the antioxidant is so sensitive to light. Studies on the phenolic compounds, flavonoids, tannins and their antioxidative

activities in the plant extracts of *Coleus* were not properly documented till now. The present study showed that the total phenolic content in different tissues of the three species of Coleus showed that *Blumei* type give lower of antioxidant activity and phenolic content compared with other coleus species.

4.1.3 Minerals

From the experiment of analyzing the minerals using the AAS, we can see that Magnesium offers the highest value of concentration among others which is 1.47 ppm (part per million). Then it followed by sodium with value of 0.63 ppm, calcium with 0.46 ppm, iron with 0.41 ppm and zinc with 0.03 ppm. The rest minerals which are potassium, manganese, copper, and lead undetected and give negative values.

For this analysis we get the Coleus leaves from Malacca where it takes about 4 hours for the journey .The coleus leaves not really fresh after arrived at Kuantan. Therefore the results bring out from the experiment maybe because of the nutrient had degrade by a long term sample storage and after exposed to heat when it is boiled (the nutrients transferred from the coleus leaves into the water). The other possibility of undetectable minerals (potassium, manganese, copper, and lead) because of the concentration of the sample is too dilute for those minerals as their amount in the Coleus solution too low compared with others that contain of higher concentration in the Coleus leaves. While the error from the AAS equipment also can be considered as the effects bring out by the experimental analysis. There is possibility of failure in complete burning of flame in AAS as well as not enough gas supplied.

CHAPTER V

CONCLUSION

5.1 Conclusion

Back to the objectives of this research, these experiments were carried out to see whether those antioxidant activity, phenolic content and some minerals exist in the solution After all the research have been done, we can conclude that :

i. In 8.5 gram of coleus leaves (after it boiled with water) it has about 40.77 wt% of antioxidant activity, 6.256998 wt% of total phenolic content, and only some mineral (magnesium, calcium, iron and zinc) existing in solution after the Coleus *blumei* leaves were removed.
ii. The wt% of the phenolic content is directly proportional to the wt% of antioxidant activity.
iii. The mineral concentration, antioxidant activity and phenolic content seemed to be highly correlated.

As a conclusion, it proven that the Coleus *blumei* leaves have high potential value for the nutritional purpose.

5.2 Recommendations

From this research, some recommendations can be made to improve the result of the analysis. The recommendations are:

i. Add more parameter to make sure the extraction process can produce better result for higher concentration of those nutrients in the boiled Coleus solution.
ii. Research must be carry out in clean condition. This reason is to avoid other derivatives interrupt the experiment especially in analysis process which will detect other compound that didn't have any related with the sample solution. All appliances must be clean up perfectly before running another experiment.
iii. Using some different methods, then compare which one of those method give the best result and more effective.
iv. For further study of Coleus's advantages, it is recommended to determine those nutrients after the boiled Coleus solution were spray dried into powder form.

REFERENCES

Ames & Gold (1996). B. Ames and L.S. Gold, Mom was right, at least about fruits and vegetables. *Chem. Health Safety* **3** (1996), pp. 17–21.

Gibson (1994). R.S. Gibson, Zinc nutrition in developing countries. *Nutrition Research Reviews* **7** (1994), pp. 151–173.

Hazell (1985). T. Hazell, Minerals in foods: Dietary sources, chemical forms, interactions, bioavailability. *World Review of Nutrition and Dietetics* **46** (1985), pp. 1–123.

Heaney, Weaver, Hinders, Martin, & Packard (1993). R.P. Heaney, C.M. Weaver, S.M. Hinders, B. Martin and P.T. Packard, Absorbability of calcium from *Brassica* vegetables: Broccoli, bok choy, and kale. *Journal of Food Science* **58** (1993), pp. 1378–1380.

Duh, P. D. (1998). Antioxidant activity of burdock (Arctium lappa Linne):its scavenging effect on free radical and active oxygen.

Journal of theAmerican Oil Chemist_s Society, 75, 455–461.Hermann, K. (1993). Antioxidant activity of phenolic acids in foods of plant origin. Gordian, 93(6), 92–96.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. Lebensmittel-Wissenschaft und Technologie, 28, 25–30.

CSIR (1992). The useful plants of India 136. New Delhi: Council of Scientific and Industrial Research.

Cuvelier, M.-E., Richard, H., & Berset, C. (1992). Comparison of the antioxidative activity of some acid-phenols: structure–activity relationship.Bioscience, Biotechnology, and Biochemistry, 56, 324–325.

Khory, N. R., & Katrak, N. N. (1999). Materia medica of India and their therapeutics 380. New Delhi: BDH Printers.

Kinsella, J. E., Frankel, E., German, B., & Kanner, J. (1993). Possible mechanism for the protective role of the antioxidant in wine and plant foods. Food Technology, 47, 85–89.

Kumaran, A., Karunakaran, R. J. (2005). Antioxidant and free radical scavenging activity of an aqueous extract of Coleus aromaticus. Food Chemistry, in press, doi:10.1016/j.foodchem.2005.03.032.

Lai, L. S., Chou, S. T., & Chao, W. W. (2001). Studies on the antioxidative activities of Hsian-tsao (Mesona procumbens Hemsl) leaf gum. Journal of Agricultural and Food Chemistry, 49, 963–968.

Ames, B., & Gold, L. S. (1996). Mom was right, at least about fruits and vegetables. Chem. Health Safety, 3, 17–21.

Gibson, R. S. (1994). Zinc nutrition in developing countries. Nutrition Research Reviews, 7, 151–173.

Hazell, T. (1985). Minerals in foods: Dietary sources, chemical forms, interactions, bioavailability. World Review of Nutrition and Dietetics, 46, 1–123.

Heaney, R. P., Weaver, C. M., Hinders, S. M., Martin, B., & Packard, P. T. (1993). Absorbability of calcium from Brassica vegetables: Broccoli, bok choy, and kale. Journal of Food Science, 58, 1378–1380.

Holland, B., Welch, A. A., Unwin, I. D., Buss, D. H., Paul, A. A., & Southgate, D. A. T. (1998). McCance and Widdowson's the composition of foods. London: Her Majesty's Stationer Office.

Kris-Etherton, P. M., Krummel, D., Russel, M. E., Dreon, D., Mackey, S., Borchers, J., & Wood, P. D. (1988). The effect of diet on plasma lipids, lipoprotein, and coronary heart disease. Journal of the American Dietetics Association,
88, 1373–1411.

Lucarini, M., Canali, R., Cappelloni, M., Di Lullo, G., & Lombardi-Boccia, G. (1999). In vitro calcium availability from Brassica vegetables (Brassica oleracea L.) and as consumed in composite dishes. Food Chemistry, 64, 519–523.

National Research Council (1996). Carcinogens and anticarcinogens in the human diet. Washington: National Research Press.National Research Council (1989). Recommended dietary allowances. Washington: National Research Press.Osler, M., Heitmann, B. L., Gerdes, L. U., Jorgensen, L. M., & Scroll, M. (2001). Dietary patterns and mortality in
Danish men and women: A perspective observational study. British Journal of Nutrition, 85, 219–225.

Razagui, I. B., & Barlow, P. J. (1992). A chemical clean-up procedure to reduce trace metal contamination from laboratory blenders. Food Chemistry, 44, 309–312.

Savage, G. P., Vanhanen, L., Mason, S. M., & Ross, A. B. (2000). Effect of cooking on the soluble and insoluble oxalate content of some New Zealand foods. Journal of Food Composition and Analysis, 13, 201–206.

Scherz, H., Senser, F. (1989). Food composition and nutrition tables. Deutsche Forschungsanstalt f .ur Lebensmittelchemie,Garching b. M.unchen.Weaver, C. M., & Plawecki, K. L. (1994). Dietary calcium: Adequacy of a vegetarian diet. American Journal of Clinical Nutrition, 59(suppl), 1238S–1241S.

APPENDIXES

Figure A-1: The boiled solution sonicated using ultrasonic bath

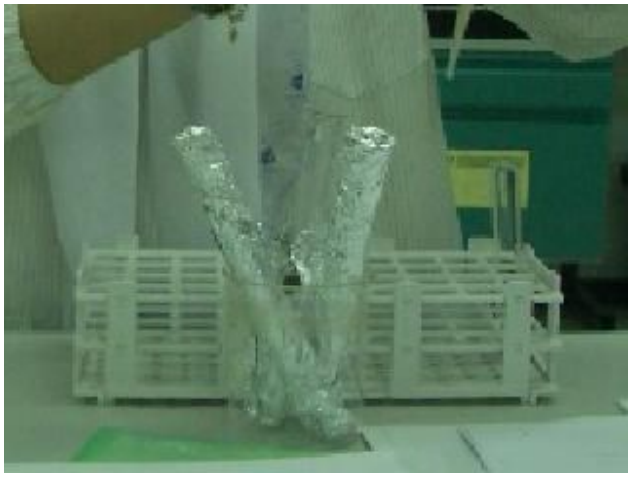


Figure A-2: DPPH solution preparation

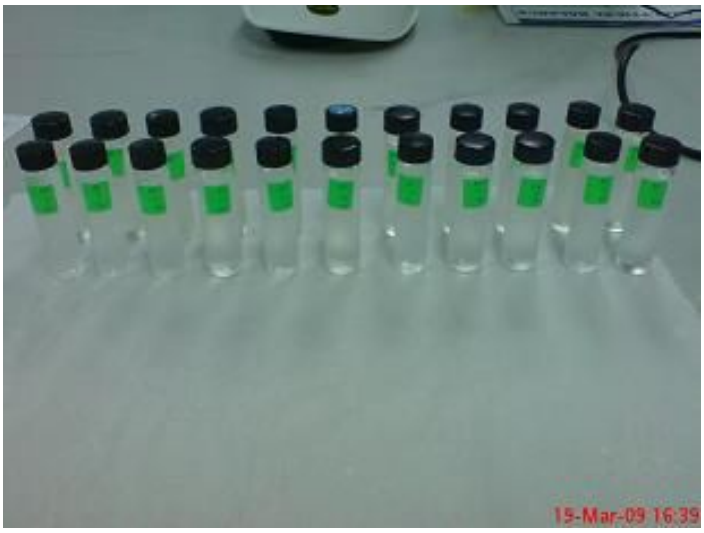


Figure A-3: Standard solution of heavy metals

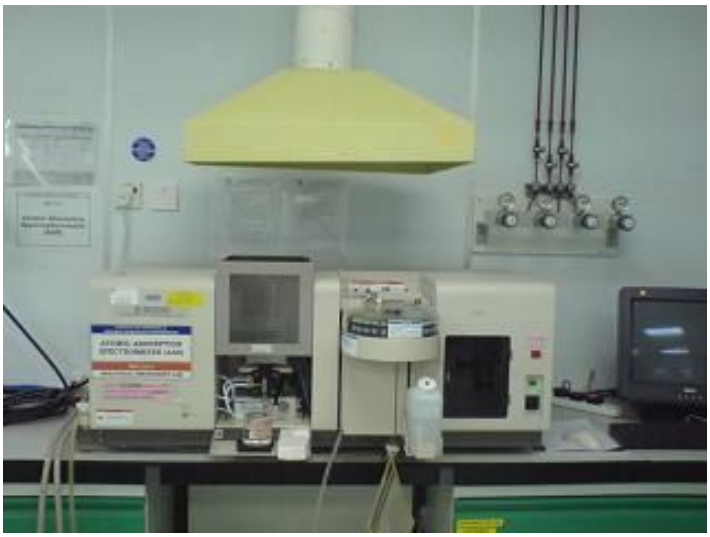


Figure A-4: AAS analyzing

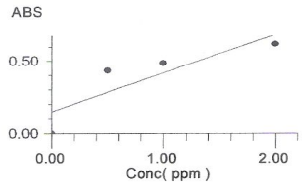
Table of Each Element 3/19/2009 4:45 PM

Analysis Mode : Flame/Manual
Analysis Name : Standard Analysis
Comment : Cd
Description :

Meas. Date : 3/19/2009 4:33 PM
Element : Na STD Unit : ppm UNK Unit :

Sample ID	Sample Name	Conc.	RSD(%)	ABS	REF
STD1		0.00	133.33	0.0003	-0.0181
STD2		0.50	0.00	0.4323	-0.0142
STD3		1.00	0.19	0.4849	-0.0151
STD4		2.00	0.06	0.6177	0.0042

Coefficient : K3=--
K2=--
K1=2.711543E-001
K0=1.465400E-001
Corr.Coef. : 0.8662

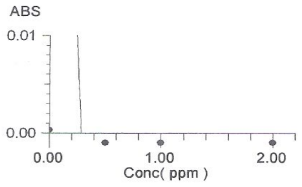


UNK-001		0.63	0.00	0.3184	-0.0505

Meas. Date : 3/19/2009 4:38 PM
Element : K STD Unit : ppm UNK Unit :

Sample ID	Sample Name	Conc.	RSD(%)	ABS	REF
STD1		0.00	633.33	0.0003	0.0048
STD2		0.50	-6.01	-0.0665	-0.1710
STD3		1.00	-3.38	-0.0917	-0.1467
STD4		2.00	-6.38	-0.7489	0.7900

Coefficient : K3=--
K2=--
K1=-3.791086E-001
K0=1.050200E-001
Corr.Coef. : -0.9242

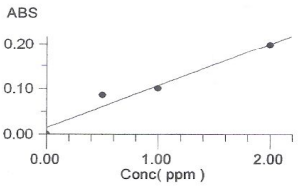


UNK-001		-1.94	0.00	0.8397	-1.0922

Meas. Date : 3/19/2009 4:42 PM
Element : Mn STD Unit : ppm UNK Unit :

Sample ID	Sample Name	Conc.	RSD(%)	ABS	REF
STD1		0.00	0.00	-0.0003	-0.0005
STD2		0.50	0.47	0.0856	0.0023
STD3		1.00	1.20	0.1001	0.0012
STD4		2.00	0.20	0.1966	0.0047

Coefficient : K3=--
K2=--
K1=9.227429E-002
K0=1.476000E-002
Corr.Coef. : 0.9769



UNK-001		-0.03	0.00	0.0124	-0.0065

2

Figure A-5: Result of sodium, kalium and manganese analysis

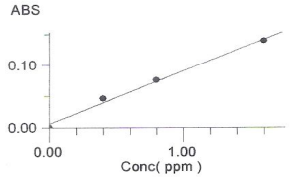
Table of Each Element 3/4/2009 5:08 PM

Analysis Mode : Flame/Manual
Analysis Name : Standard Analysis
Comment : Cd
Description :

Meas. Date : 3/4/2009 5:07 PM
Element : Pb STD Unit : ppm UNK Unit :

Sample ID	Sample Name	Conc.	RSD(%)	ABS	REF
STD1		0.00	-50.00	-0.0002	-0.0031
STD2		0.40	0.65	0.0463	-0.0036
STD3		0.80	0.79	0.0757	-0.0053
STD4		1.60	0.43	0.1395	-0.0032

Coefficient : K3=---
K2=---
K1=8.526429E-002
K0=5.639999E-003
Corr.Coef. : 0.9958



UNK-001	-0.06	0.00	0.0003	-0.0193
UNK-002	-0.07	0.00	0.0000	-0.0223
UNK-003	-0.06	0.00	0.0001	-0.0247

1

Figure A-6: Result of lead analysis

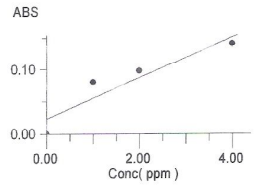
Table of Each Element 3/13/2009 4:30 PM

Analysis Mode : Flame/Manual
Analysis Name : Standard Analysis
Comment : Cd
Description :

Meas. Date : 3/13/2009 4:15 PM
Element : Cu STD Unit : ppm UNK Unit :

Sample ID	Sample Name	Conc.	RSD(%)	ABS	REF
STD1		0.00	0.00	-0.0002	-0.0022
STD2		1.00	0.00	0.0790	0.0629
STD3		2.00	0.00	0.0976	0.0756
STD4		4.00	0.00	0.1405	0.1090

Coefficient : K3=--
K2=--
K1=3.218571E-002
K0=2.290000E-002
Corr.Coef. : 0.9335

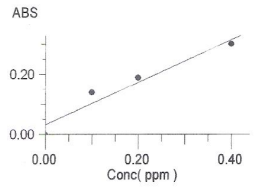


UNK-001	CL1	-0.66	0.00	0.0018	-0.0115
UNK-002	CL2	-0.70	0.00	0.0004	-0.0145
UNK-003	CL3	-0.70	0.00	0.0004	-0.0157

Meas. Date : 3/13/2009 4:20 PM
Element : Mg STD Unit : ppm UNK Unit :

Sample ID	Sample Name	Conc.	RSD(%)	ABS	REF
STD1		0.00	0.00	-0.0023	-0.0055
STD2		0.10	0.00	0.1391	0.0168
STD3		0.20	0.00	0.1880	0.0232
STD4		0.40	0.00	0.2983	0.0396

Coefficient : K3=--
K2=--
K1=7.061428E-001
K0=3.220000E-002
Corr.Coef. : 0.9674



UNK-001	CL1	1.44	0.00	1.0503	0.2261
UNK-002	CL2	1.50	0.00	1.0933	0.2285
UNK-003	CL3	1.47	0.00	1.0675	0.2266

Meas. Date : 3/13/2009 4:24 PM
Element : Fe STD Unit : ppm UNK Unit :

Sample ID	Sample Name	Conc.	RSD(%)	ABS	REF
STD1		0.00	0.00	-0.0004	0.0022
STD2		2.00	0.00	0.1231	0.0101
STD3		4.00	0.00	0.1680	0.0129

1

Figure A-7: Result of copper, magnesium and iron analysis

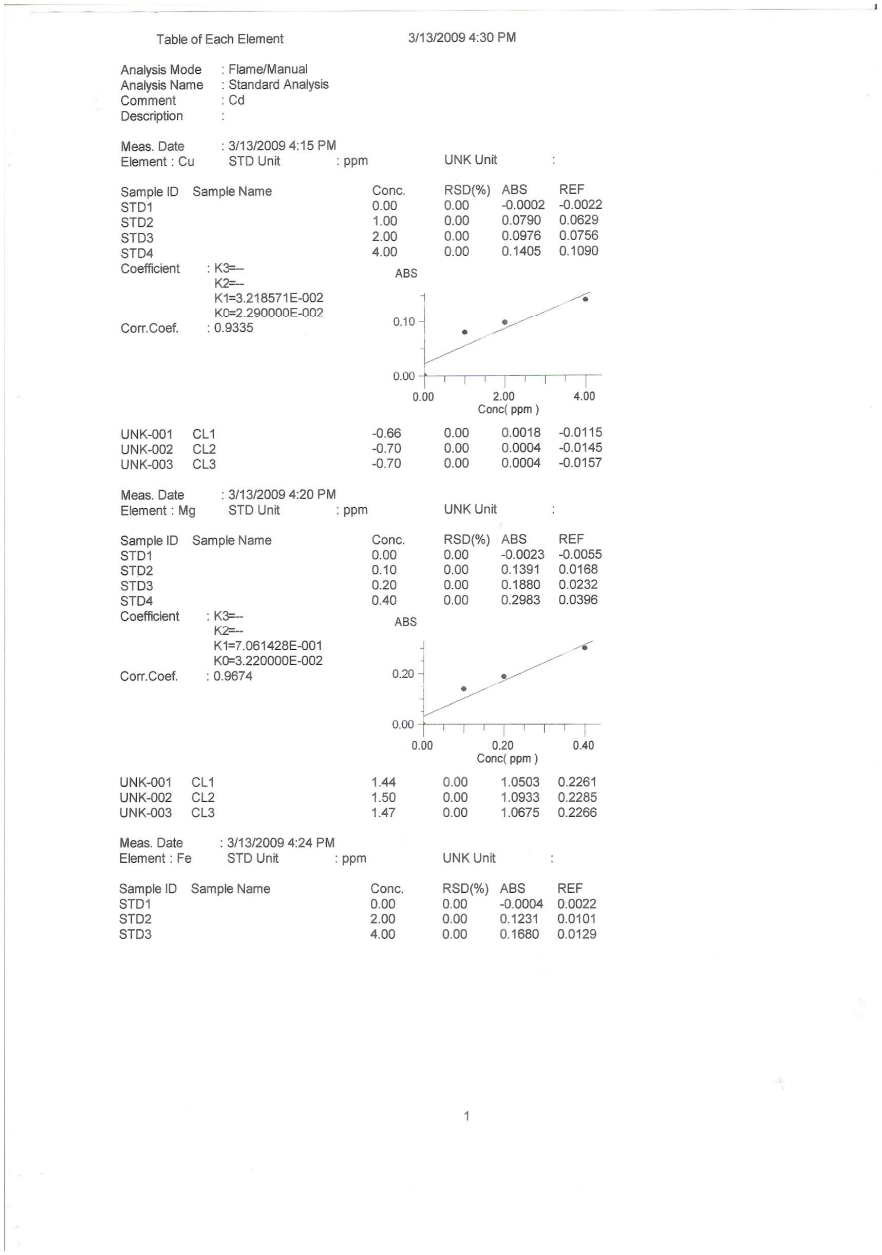


Table of Each Element 3/13/2009 4:30 PM

Analysis Mode : Flame/Manual
Analysis Name : Standard Analysis
Comment : Cd
Description :

Meas. Date : 3/13/2009 4:15 PM
Element : Cu STD Unit : ppm UNK Unit :

Sample ID	Sample Name	Conc.	RSD(%)	ABS	REF
STD1		0.00	0.00	-0.0002	-0.0022
STD2		1.00	0.00	0.0790	0.0629
STD3		2.00	0.00	0.0976	0.0756
STD4		4.00	0.00	0.1405	0.1090

Coefficient : K3=--
K2=--
K1=3.218571E-002
K0=2.290000E-002
Corr.Coef. : 0.9335

UNK-001	CL1	-0.66	0.00	0.0018	-0.0115
UNK-002	CL2	-0.70	0.00	0.0004	-0.0145
UNK-003	CL3	-0.70	0.00	0.0004	-0.0157

Meas. Date : 3/13/2009 4:20 PM
Element : Mg STD Unit : ppm UNK Unit :

Sample ID	Sample Name	Conc.	RSD(%)	ABS	REF
STD1		0.00	0.00	-0.0023	-0.0055
STD2		0.10	0.00	0.1391	0.0168
STD3		0.20	0.00	0.1880	0.0232
STD4		0.40	0.00	0.2983	0.0396

Coefficient : K3=--
K2=--
K1=7.061428E-001
K0=3.220000E-002
Corr.Coef. : 0.9674

UNK-001	CL1	1.44	0.00	1.0503	0.2261
UNK-002	CL2	1.50	0.00	1.0933	0.2285
UNK-003	CL3	1.47	0.00	1.0675	0.2266

Meas. Date : 3/13/2009 4:24 PM
Element : Fe STD Unit : ppm UNK Unit :

Sample ID	Sample Name	Conc.	RSD(%)	ABS	REF
STD1		0.00	0.00	-0.0004	0.0022
STD2		2.00	0.00	0.1231	0.0101
STD3		4.00	0.00	0.1680	0.0129

1

Figure A-8: Result of copper, magnesium and iron analysis

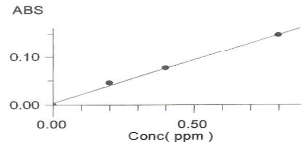
Table of Each Element 3/4/2009 5:29 PM

Analysis Mode : Flame/Manual
Analysis Name : Standard Analysis
Comment : Cd
Description :

Meas. Date : 3/4/2009 5:29 PM
Element : Zn STD Unit : ppm UNK Unit :

Sample ID	Sample Name	Conc.	RSD(%)	ABS	REF
STD1		0.00	-25.00	-0.0004	-0.0053
STD2		0.20	1.55	0.0453	-0.0133
STD3		0.40	0.53	0.0758	-0.0139
STD4		0.80	0.14	0.1467	-0.0040

Coefficient : K3=---
K2=---
K1=1.804286E-001
K0=3.700001E-003
Corr.Coef. : 0.9979



UNK-001	0.03	0.00	0.0087	-0.0087
UNK-002	0.04	0.00	0.0108	-0.0073
UNK-003	0.01	0.00	0.0062	-0.0070

1

Figure A-9: Result of zinc analysis

Table A-1: Antioxidant activity

Test tube	coleus(g)	DPPH(ml)	absorbance	Antioxidant activity (%)
1	0	1	0.287	control
2	0	1	0.287	control
3	0	1	0.287	control
4	8.5	1	0.196	31.70731707
5	8.5	1	0.196	31.70731707
6	8.5	1	0.196	31.70731707
7	8.5	2	0.185	35.54006969
8	8.5	2	0.185	35.54006969
9	8.5	2	0.185	35.54006969
10	8.5	3	0.173	39.72125436
11	8.5	3	0.173	39.72125436
12	8.5	3	0.173	39.72125436
13	8.5	4	0.17	40.76655052
14	8.5	4	0.17	40.76655052
15	8.5	4	0.17	40.76655052
16	8.5	5	0.17	40.76655052
17	8.5	5	0.17	40.76655052
18	8.5	5	0.17	40.76655052
19	8.5	6	-0.11	-
20	8.5	6	-0.11	-
21	8.5	6	-0.11	-