THE EXTRACTION OF ORTHOSIPHON STAMINEUS USING HYDRO DISTILLATION METHOD

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"I declare that this thesis is the result of my own research except as cited references. The thesis has not been accepted for any degree and is concurrently submitted in candidature of any degree"

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DEDICATION

Special dedication to my beloved father, mother,
brother and sisters……

ACKNOWLEDGEMENT

In preparing this thesis, I was in contact with many people, researchers, academicians and practitioners. They have contributed towards my understanding and thoughts. In particular, I wish to express my sincere appreciation to my main supervisor, Mr. Ahmad Ziad Bin Sulaiman for encouragement, guidance, critics and friendship. I am also indebted to FKKSA lectures for their guidance to complete this thesis. Without their continued support and interest, this thesis would not have been the same as presented here.

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Abstract

Orthosiphon Stamineus (Misai Kucing) produce essential oil which is important in medicine. Entrepreneur and researcher nowadays use steams distillation method to extract *Orthosiphon Stamineus* essential oil. The objective of this study is to obtain essential oil from *Orthosiphon Stamineus* plant source using hydro distillation technique. Rotary evaporator was used to extract the essential oil. Effects of time and particle size parameters were studied in order to have higher yield. The temperature and pressure for the extraction process were set at 100 ^{o}C and 1 bar respectively. The obtained result showed that optimum time was 12 hours. Longer time operation and smaller particles size proved that higher yield was obtained. The presence of either one these compositions, Eupatorin (EUP), Sinensetin (SEN), Rosmarinic Acid (RA) and 3-hydroxy-5,6,7,4-tetramethoxyflavone (TMF) should take as a characteristic for *Orthosiphon Stamineus* essential oil was studied as well. The essential oil was analyzed using High Performance Liquid Chromatography (HPLC). The analyzed using HPLC showed that the Rosmarinic Acid was obtained in *Orthosiphon Stamineus* extracted via hydro distillation method.

Abstrak

Daun Misai Kucing boleh menghasilkan minyak esen yang penting dalam industri makanan dan kesihatan. Bagi mendapatkan minyak esen ini, usahawan dan juga penyelidik pada masa kini menggunakan kaedah distilasi sama ada menggunakan air ataupun wap. Objektif kajian ini adalah bagi mendapatkan minyak esen daripada daun misai kucing menggunakan teknik pendidihan air. Oleh itu kajian ini dijalankan menggunakan *rotary evaporator* sebagai teknik bagi penghasilan minyak daun misai kucing yang bermutu tinggi. Pembolehubah yang digunakan di dalam experimen ini ialah masa yang diambil untuk memproses dan juga saiz daun misai kucing itu. Pemalar ini yang dijalankan pada suhu 100 ^{o}C dan tekanan pada 1 bar. Masa yang terbaik bagi mendapatkan jumlah minyak esen daun misai kucing yang paling maksimum ialah pada 12 jam. Kehadiran komponen Eupatorin (EUP), Sinensetin (SEN), Rosmarinic Acid (RA) and 3-hydroxy-5,6,7,4-tetramethoxyflavone (TMF) ini mengesahkan bahawa minyak ini daripada daun misai kucing. HPLC akan digunakan untuk menganalisis kehadiran komponen tersebut. Keputusan analisis HPLC menunjukkan kehadiran komponen Rosmarinic Acid yang diperolehi daripada minyak daun misai kucing menggunakan teknik pendidihan air.

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Symbols	Title
P	Pressure
m	Mass
T	Temperature
A	Surface area (m^2)
x	Distant (m)
ρ	Density
^oC	Degree Celsius
L	Liter
kg	Kilogram
^oK	Degree Kelvin
m	Meter
T_i	Temperature for species i

CHAPTER 1

INTRODUCTION

1.1 Extraction

Extraction is phenomenon that can be defined as the process of separating desired components from a material. There are many types of extraction like solid-liquid extraction and liquid-liquid extraction. Some of them are steam distillation, water distillation, solvent extraction, carbon dioxide extraction and cold pressing. The main objective in the extraction process is to extract the essential oil. However, more extraction on essential oil has been done in extraction field. The main resources of essential oils are plants.

1.2 Essential Oil

Essential oils, also known as Volatile oils, are the odorous principles found in various plant parts. It can be found in the bark of the plant, the flower of the plant or even in the leaves of the plant. Essential oils are oils that are found in bags inside plants cell. These oils can be free from the leaves and extracted using a few of methods like hydro distillation. The oil is also known as volatile oils, ethereal oils, or essential oils because of their properties easily evaporated at ambient temperature. However, essential oil is most preferable due to represent the "essences" or odor constituents of the plants.

Essential oils are usually colorless, particularly when fresh. Nevertheless with age essential oil may oxidize and resinify, which resulting the colour becomes darker. Therefore, essential oil need to be stored in a cool, dry place, tightly stoppered and preferably full in amber glass containers. These essential oils have many uses. Most essential oils are produced for the perfume industry and minute amounts are used for flavour prepackaged foods. Besides that some essential oils that are produced from plants have high medical values in them like Orthosiphon Stamineus leaves. The Orthosiphon Stamineus leaves are used in beverages in Malaysia to improve health and for the treatment of kidney, bladder inflammation, gout and diabetes. (Wangner, 1982)

1.3 Orthosiphon Stamineus

Orthosiphon Stamineus (Misai Kucing) is a medicinal herb found mainly throughout South East Asia. It is believed to have antiallergic, antihypertensive, antiinflammatory and diuretic properties. It is used as a remedy for arteriosclerosis (capillary and circulatory disorders), kidney stones and nephritis. (Anon, 2001)

It is trusted for many centuries in treating ailments of the kidney, bladder stone, urinary tract infection, liver and bladder problems, diabetes, rheumatism and gout. It is also used to reduce cholesterol and blood pressure. Misai Kucing has a mild diuretic action, useful for flushing the kidneys and urinary tract. It also relieves spasms of the smooth muscle in the walls of the internal organs, making it valuable for gallbladder problems. (Hegnauer, 1966)

(IDS Sabah, 1998)

Figure 1.1: Orthosiphon Stamineus

Researchers have found it to be mildly antiseptic as well. Misai Kucing *(misai kuching)* is also known as *Kumis Kucing* or *Remujung*. Other names for Misai Kucing are *Orthosiphon Stamineus Benth, Orthosiphon Aristatus, Orthosiphonblaetter, Indisher Nierentee, Feuilles de Barbiflore, Java Tea, Javatee, Kidney Tea,* and *Yaa Nuat Maeo*. The plant is from the family of *Lamiaceae / Labiatea*.

1.4 Water Distillation

This is the simplest and usually cheapest distillation method. The plant material is immersed in water and boiled. The steam and oil vapour is condensed and the oil is separated from the water. This method is suitable for flower blossoms and finely powdered plant material.

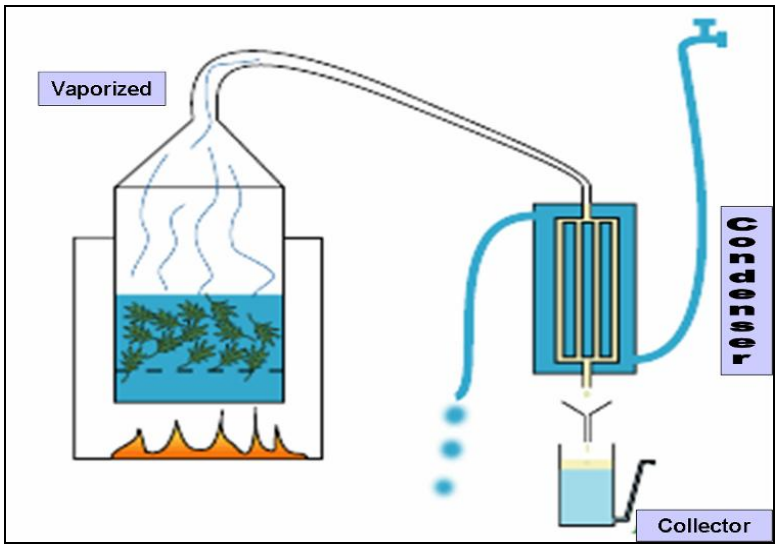


Figure 1.2: Simple Hydro Distillation Concept

The distillation temperature should be about 100°C. Care needs to be taken to prevent the plant material being damaged by contacting the overheated still walls. The pressure in the still should be atmospheric. The distillation time depends on the plant material being processed. Prolonged distillation produces only a small amount of extra oil, but does add unwanted high boiling compounds and oxidation products.

1.5 Steam Distillation

Essential oils are used in perfumes, handmade soaps, bath salts, lotions and other natural bath and body products. These volatile oils can be used individually or blended to create a special bouquet. One of the ways essential oils are extracted from plant material is through steam distillation. Deng-Kai Yang and Wang-Hsien Ding from Department of Chemistry, National Central University, Taiwan already use this method to determine alkylphenolic residues in fresh fruits and vegetables using steam distillation and gas chromatography–mass spectrometry. The following diagram provides an overview of the steam distillation process.

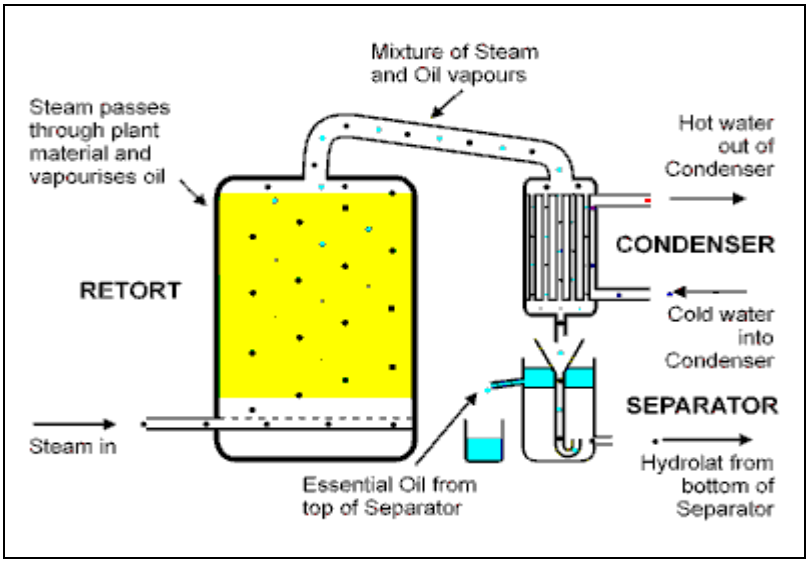


Figure 1.3: Simple Steam Distillation Unit

Steam distillation is a method where steam is flowed through the material. This steam functions as an agent that breaks up the pores of the leaves and releases the essential oil from the leaves. The system yields a mixture of vapor and desired essential oil. This vapor is then condensed further and the essential oil is collected. (R Rai and Suresh B, 2004)

1.6 Research Background / Problem Statement

The Government wants to nurture the Malaysian herbal industry to be a leading international player. The local herbal industry is growing at an annual rate of between 15% and 20% and has a market value estimated at RM2 billions. (Malaysian Industrial Development Authority, 2005)

Nowadays, in Malaysia, essential oil and oleoresin is gaining popularity as a herbal medication as it gave a lot of benefit to overcome the disease. It is because our people start to realize the important of healthy awareness. Hence, *Orthosiphon Stamineus* essential oil and oleoresin has a clear commercial value. Therefore the identification of the constituents of *Orthosiphon Stamineus* has been carried out extensively over the last 10 years. KUKTEM could further the research into producing and commercialize a new product based on the knowledge on the constituent of our local *Orthosiphon Stamineus* and the potential commercial value essential oil as a whole.

In this research, Hydro Distillation Unit is used to obtain *Orthosiphon Stamineus* essential oil. The factor of influences of rate of extraction is study to obtain a series of high quality essential oil extracts from *Orthosiphon Stamineus.*

Figure 1.4: The Traditional Hydro Distillation Unit

It is appropriate to improve the traditional hydro distillation unit due to the energy wasting when the operation. The extraction using traditional hydro distillation unit do not give the higher purity and quality of Orthosiphon Stamineus Essential Oil. It is hard to determine the exactly values of the feed and the solvent as well. However, rotary evaporator is seldom used for extracting essential oil. Rotary Evaporator is used to remove solvents from reaction mixtures and can accommodate large volumes of liquid. It is usually utilized to separate solvents such as n-hexane, acetone and ethanol from the essential oils produced in solvent extraction.

1.7 The Objective of the Research

To objectives of this research are:

- To study the effect of operating time and surface area on essential oil of Orthosiphon Stamnieus

1.8 The Scopes of the Research

To achieve the objective, scopes have been identified in this research. The scopes of this research are listed as below:-

- Prepare the Orthosiphon Stamineus leaves for the experiment. The leaves dry to remove the moisture from leaves. The leaves are divided into two sample which is non grinded sample and grinded sample (0.01 mm).

- The rotary evaporator is used for the hydro distillation process to extract the essential oils from Orthosiphon Stamineus leaves. The operating conditions are set at 100°C for temperature and 1 bar for pressure.

- The Orthosiphon Stamineus essential oil is subjected to qualitative and quantitative HPLC analysis. HPLC analysis is performed using an Agilent Technologies 1100 system equipped with an automatic injector, a column oven, and a UV detector. A LiChrosorb RP-18 column (250 x 4.6 i.d. mm, 5 µm particle size) (Merck Darmstadt, Germany) is used. The temperature is maintained at 25 ^{o}C, with injection volume of 20 µl and flow rate of 1 ml/min. All the markers is separate with methanol–water–tetrahydrofuran (45:50:5v/v) as mobile phase. The peak is detecting at 340 nm and identified by standard substances. The Eupatorin (EUP), Sinensetin (SEN), Rosmarinic Acid (RA) and 3-hydroxy-5,6,7,4-tetramethoxyflavone (TMF) compounds are use as markers.

CHAPTER II

LITERATURE REVIEW

2.0 Overview of Orthosiphon Stamineus

Nowadays, the consumption of herbal based products are getting a wide spread acceptance among consumers because of the numerous beneficial therapeutic impacts they could give to human body and indirectly helps us sustaining a healthy condition. Although artificial and synthetic drugs are common in the market to combat a lot of chronic diseases, but these medications usually have negative effects to human body. Therefore, products from herbs, especially the Misai Kucing (*Orthosiphon stamineus)* are the right choice in treating certain kinds of ailments or diseases without introducing side effects to human body if consumed accordingly based on scientific findings and research. The Misai Kucing (*Orthosiphon stamineus)* are confidently introduced to the public and backed by scientific research and findings from local and overseas scientist to prevent, reduce or to the extent in aiding the cure of certain types of chronic diseases or ailments.

According to Anon (2001), the scientific term of Orthosiphon stamineus have other synonyms such as Orthosiphon aristatus, Orthosiphon grandiflorum and Orthosiphon spicatus. This herb is also known by its vernacular names such as Java tea (English), Thé de Java (France), kumis kucing (Indonesia), kumis ucing (Sudanese), remuk jung (Javanese), kumis kucing or misai kucing (Malaysia), balbas-pusa and kabling-gubat (Philippines), kapen prey (Cambodia), hnwàd méew (Laos), yaa nuat maeo (Thailand) and r[aa]u m[ef]o in Vietnam. This herb is spread from India, Indo-

China and Thailand through Malaysia to tropical Australia. Through out Malaysia, it is abundantly be found but is apparently rare in Borneo, Sulawesi and the Moluccas. It is also now grown in South East Asia, Africa, Georgia and Cuba.

2.1 Characteristic of Orthosiphon Stamineus

Orthosiphon Stamineus is a herbaceous shrub, which grows to a height of 1.5m. The leaves are arranged in opposite pairs. There are simple, green,and glabrous with a lanceolate leaf blade and a serrate margin. The leaf apice is acuminate with an acute leaf base. The petiole is relatively short, about 0.3cm in length and reddish purple in color. The stem is quadrangle, reddish in color, erect and branches profusely.

Figure 2.1: Orthosiphon Stamineus Flower

The flowers are borne on verticils about 16cm in length. The terminal inflorescence is borne on a maroon pubescent. Bracts are green minute (1-2mm), caudiform in shape and two bracts normally holds a cluster of 5 flowers. The flowers are campanulate in shape, white bluish in color with long farexerted filaments, making the flowers look like cats whiskers.

Figure 2.2: Orthosiphon Stamineus Flower from Other Side

The flowers are hermaphrodite in nature, about 6.2m in length (including the stamens) with very irregular flower symmetry. There are two calyx lobes, which are greenish red in color, measuring about 6 mm in lengths and partially gamosepalous. One of the calyx margins is toothed and the other one entire, both covered with minute white hairs. There are two corolla lobes, which are partially gamopetalous and covered with minute hairs. The corollas are light violet in color with lobes much shorter than the corolla tube. The corollas are bilablade in shape with fringed margins.

Figure 2.3: Orthosiphon Stamineus Flower and Leaf

The labellum is light violet in color, hairy and pinkish on the under surface. There are 4 stamens which are inserted near the base of the corolla tube. The stamens are unequal in length, measuring from 4.7cm to 5.2cm. There is a single, central, terete style

with a clavate stigma. Orthosiphon Stamineus has been cultivated for a long time and is a popular garden plant. In the wild, it can be seen growing along the forest edges, roadsides and wastelands.

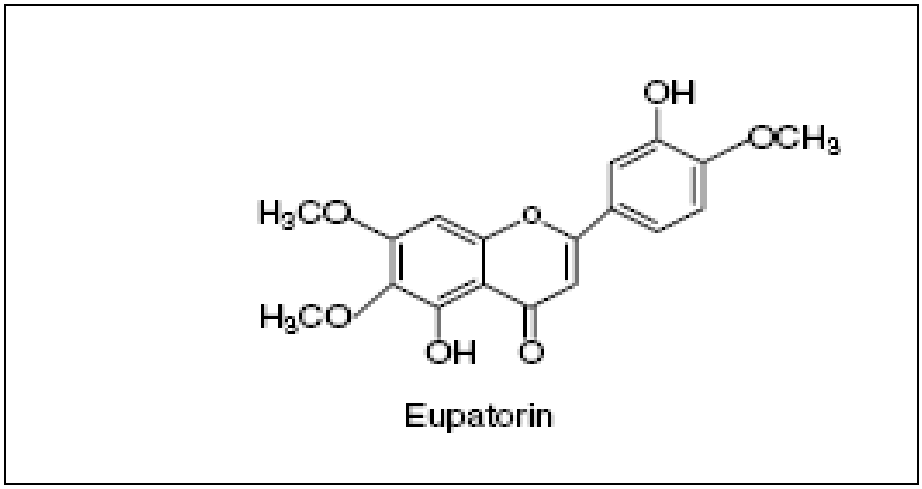
2.2 Chemical Composition of Orthosiphon Stamineus

Based on Hegnauer (1966) and Wangner (1982), the tea prepared from the leaves is used in beverages in Malaysia to improve health and for the treatment of kidney, bladder inflammation, gout and diabetes. Most land plants contain a chemical compound known as phenolics which are a class of low molecular weight secondary metabolites. Most of the protective effects of flavonoids (a class of phenolic compound) in land plants in biological systems is ascribed to their antioxidant abilities, capacity to transfer electrons, free radicals and chelating abilities (Hirano et al., 2001), activate antioxidant enzymes, reduce alpha-tocopherol radicals and inhibit oxidases (Elliott et al., 1992). According to Tezuka et al. (2000), this herb contains several active chemical compounds such as terpenoids (diterpenes and triterpenes), polyphenol (lipophilic flavonoids and phenolic acids) and sterols. The antioxidant capabilities of the phenolic compounds are important for the human body to destroy the free radicals that exists in our body. The existence of free radicals in a large quantity in our body could have the ability to destroy the structure and the inner part of our living cells, including genetic compounds (DNA) that could lead to cancer. Besides that, free radicals could also weaken the artery walls that will allow fat deposits to occur and leading to heart disease. In fact, the dried leaves and stem tips of Misai Kucing contains up to 12% minerals, inositol, phytosterols, saponins and up to 0.7% essential oils (Anon, 2001).

The therapeutic effects of Orthosiphon stamineus have been ascribed mainly to its polyphenol, the most dominant constituent in the leaf which has been reported by Hollman & Katan (1999) to be effective in reducing oxidative stress by inhibiting the formation of lipid peroxidation products in biological systems. It could lead to some of

the chronic diseases such as coronary heart disease and many more. This is proved by the research of Chung et al. (1999) and Venkatamuru et al. (1983) which among the different parts of plants studied, the leaves are reported to have the highest antioxidant properties whereby the phenolic fraction. It is the most active principle among the phytochemicals studied (Nakasugi & Komai (1998); Jung et al. (1999) & Pietta et al. (1998)). That is why the leaves of this herb is often used in traditional medicine and also in our product (Reeleaf tea and MissKuu tablets) compared to other parts of the plant because it contains much more active chemical components which contributes to the therapeutic benefits.

The specific polyphenol components that are dominant in the leaves of the Misai Kucing herb consists of four main polymethoxylated flavones, which are sinensetin (SEN), eupatorin (EUP), 3'-hydroxy-5,6,7,4'-tetramethoxyflavone (TMF) and rosmarinic acid (RA), which is the major phenolic acid (Schut and Zwaving, 1993). Based on the studies by Akowuah et al. (2004), the RA component is the main polyphenol compound in the leaves of Misai Kucing, which is the most polar (water soluble) component compared to the three polymethoxylated flavones studied. The extract of the leaves of Misai Kucing using polar extracting solvents gave the highest activity of free radicals scavenging which is possibly due to the high concentration of caffeic acid derivatives, especially RA (Akowuah et al., 2005). Sumaryono et al. (1991) found that, the derivatives of caffeic acid, including RA was reported to constitute 67% of total identified phenolics in aqueous methanol extract and about 94.6% in hot water extract. Therefore, by using the leaves of Misai Kucing in the form of tea drinking or tablets and capsules which contain the extract of the herb is beneficial for health, especially when the active chemical components from the leaves are extracted using polar solvents.



(Akowuah et al, 2004)

Figure 2.4: Molecules of Eupatorin

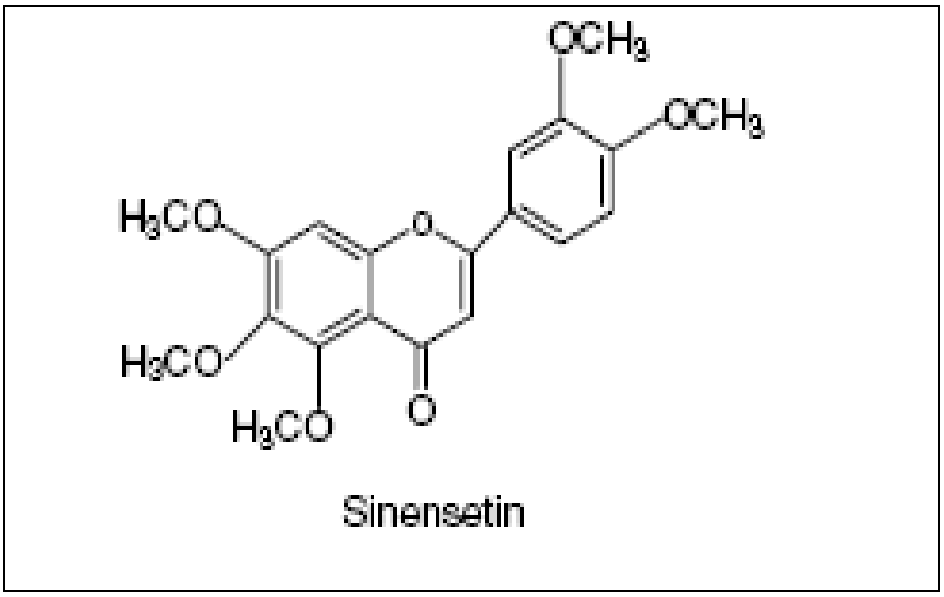


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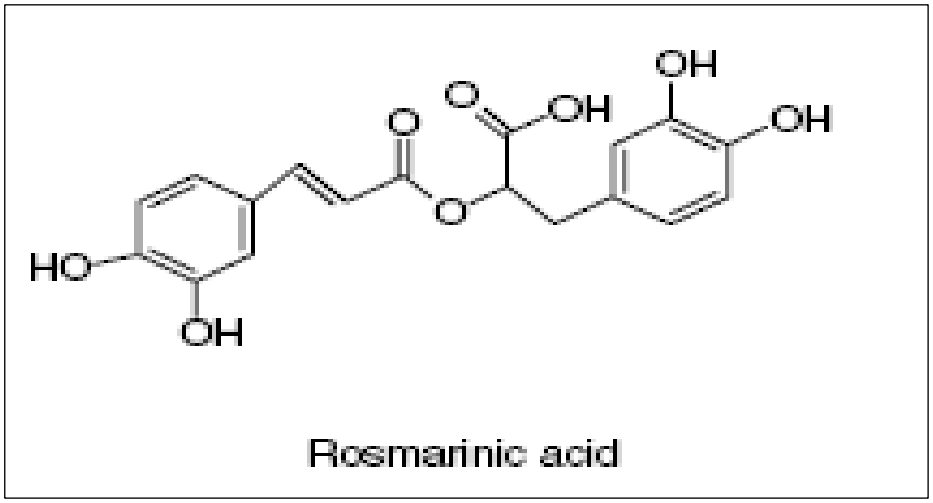
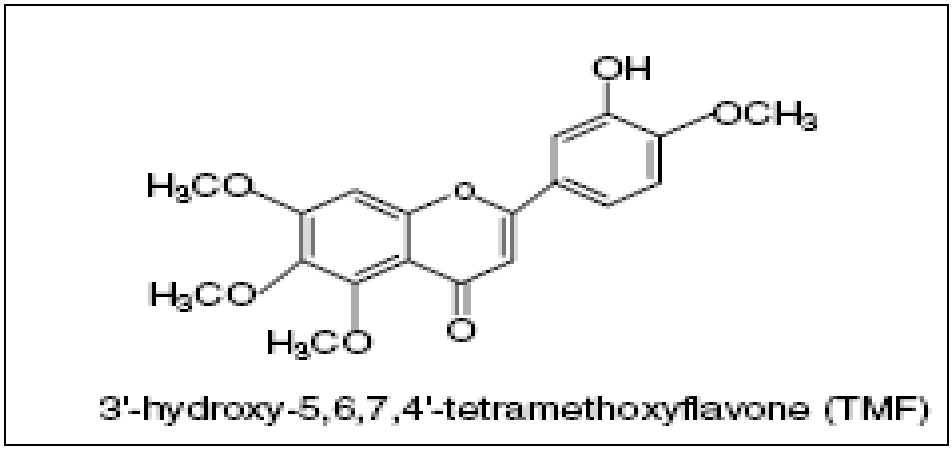
Figure 2.5: Molecules of Sinensetin



(Akowuah et al, 2004)

Figure 2.6: Molecules of Rosmarinic Acid



(Akowuah et al, 2004)

Figure 2.7: Molecules of 3-hydroxy-5,6,7,4 – tetramethoxyflavone (TMF)

Java tea has also been demonstrated to exhibit antimicrobial properties (Anon, 2001). Aqueous extracts of this herb had markedly inhibited the growth of both gram-positive and gram-negative bacteria during in vitro tests. It is concluded that saponins may play a part in bacteriostatic activity of this herb. Besides that, caffeic acid derivatives (which represent as much as 95% of the phenolic substances present in a hot water extract) may also be responsible for the antibiotic activity. Therefore, by consuming this herb in the form of hot water extract (tea drinking) or tablets and capsules which contain the same amount of active ingredients may be beneficial for the elimination of various kinds of pathogenic and harmful micro-organisms.

2.3 Application of Orthosiphon Stamineus

Orthosiphon stamineus is widely used in Malaysia as a traditional remedy for various ailments and diseases such as kidney stones, high blood pressure, diabetes, rheumatism, arthritis, gout and possibly other ailments as well. Besides of aiding in the cure of various types of illnesses, the extract of this herb if consumed can also increase the body's metabolism which translate into more calories burned and triggers the body to sweat more. The mode of action of the Misai Kucing extracts in reducing or to the extent of helping to cure the diseases mentioned are described as follows:

2.3.1 Kidney Stones

Kidney stones are referred to the crystals of minerals that are produced and somehow stored and stucked in the kidneys. Because of this, the people who suffer from this condition will feel pain and discomfort, especially in the area surrounding the kidneys. The lack of consuming water is one of the factors that may contribute in the forming of kidney stones. When water is consumed less, the output of urine will tend to

become more concentrate than usual and this situation will make the formation of kidney stones more possible to develop and the risk of getting it is increased if a person does not urinate often.

From the research by Dr. Sahabudin Raja Mohamed, senior consultant urologist at Hospital Kuala Lumpur, Orthosiphon stamineus extract inhibits calcium oxalate crystal aggregation by reducing crystal size and altering its surface. Crystal growth is inhibited by the reduction in retention time through diuresis with increasing potassium excretion. In addition, spontaneous voiding of stones less than five millimeters is also induced. The extract also reduces uric acid level by blocking its production. About 50% to 60% of kidney stones are calcium oxalate stones while 20% to 30% are uric acid stones. The inhibition of kidney stones is also made possible by the Misai Kucing extract due to the abundant levels of minerals and flavonoids in it. The minerals will form crystals of oxalate, uric or phosphate salts while the flavonoids acts as a chemical inhibitor to prevent the growth and aggregation of the crystals. Therefore, the existence of a promoter (minerals) as a former of the oxalate salt nuclei and the inhibitor (flavonoids) is very important and needed to control the growth of the referred salt crystals. Besides that, the diuretic effect of the extract of Misai Kucing also helps to prevent kidney stone development due to the increase in urine flow which makes static urine confinement in the kidneys and urine bladder lessen to occur which have been proven by Beaux et al. (1998) through rat study. The diuretic effect could be partially due to the high content of potassium (kalium) in the leaves and the presence of inositol (and possibly saponins), as well as to the isolated flavones sinensetin and 3’-hydroxy-5,6,7,4’-tetramethoxyflavone which exhibited a diuretic activity in rats after intravenous administration of 10mg / kg body weight of Misai Kucing extract (Anon, 2001).

From the research by Dr. Sahabudin Raja Mohamed, senior consultant urologist at Hospital Kuala Lumpur,, it is convincing that the extract from Misai Kucing does help inhibiting the development of kidney stones. Although the extract has a diuretic effect by nature, the potassium (kalium) level in the body will decrease to a depleting level

because of the high concentration of this mineral in Misai Kucing, that is around 600 – 700mg per 100g of fresh leaves (Anon, 2001).

2.3.2 High Blood Pressure

Blood pressure is defined as the force the blood exerts on arteries and veins as it circulates through the body. Blood pressure is measured as two numbers. Systolic pressure (the top number in a reading) denotes when the heart contracts and forces blood through the arteries while diastolic pressure (the bottom number) reflects when the heart relaxes. Normal blood pressure is 120 (systolic) over 80 (diastolic) or lower. High blood pressure is also synonym or most commonly known as hypertension in which the systolic and diastolic reading are 140 over 90 or higher respectively in at least three separate measurements.

The extract of Misai Kucing is very well known for its ability to decrease blood pressure in hypertensive patients and it has been practiced for generations by traditional medicine practitioners. Ohashi et al. (2000) concluded that , from the water decoction of leaves of Kumis Kucing that was partitioned into a mixture of chloroform and water, it was found that the chloroform-soluble portion showed an inhibitory effect on the contractile responses on rat thoracic aorta smooth muscle stimulated with potassium chloride (KCl) beforehand. The chloroform-soluble portion produces 13 chemical compounds. Among these compounds, it was found that a major constituent in the water decoction of leaves, methylripariochromene A (5), exhibited a continuous decrease in systolic blood pressure after subcutaneous administration in conscious stroke-prone spontaneously hypertensive rats (SHRSP). This proves that the extract of Orthosiphon stamineus or in this research can aid in reducing the blood pressure of rats in this case and certainly can be applied in humans as well.

The factor that might possibly reduce the blood pressure in hypertensive patients is from the antioxidant ability of the Misai Kucing extract. Through the research from Lopes et al. (2002), they found that the high antioxidant diet lowered blood pressure in obese hypertensive but not in lean normotensive respondents (humans). The increase of Total Antioxidant Capacity (TAC) of plasma in obese hypertensives during the High Antioxidant (HAO) diet correlated with the decline in blood pressure.

Changes of dietary cations measured in the urine did not change significantly in respondents with risk factor cluster (OHT). These findings raise the possibility that the Dietary Approaches to Stop Hypertension (DASH) diet reduces blood pressure in high-risk subjects in significant part through an antioxidant action. According to Akowuah et al. (2005), from the results obtained through the research, they found that the extracts of Orthosiphon stamineus are free radical inhibitors and primary antioxidants that react with free radicals and probably due to the higher concentration of caffeic acid derivatives, especially rosmarinic acid (RA).

According to the research of Beaux et al. (1998) found that, by feeding rats with the extract of Orthosiphon stamineus and Sambucus nigra, the urinary excretion of sodium (natrium / Na) increased. Hypertension is related to the high intake of salt (natrium chloride / NaCl). Therefore, by excreting excess sodium through the urine definitely reduce the blood pressure in hypertensive patients.

Therefore, the extract of Misai Kucing is a very good herb hypertension treatment. In addition, Lopes et al. (2002) realized lowering the ability of the blood pressure from the extract is not attributed by its diuretic ability but mainly from its antioxidant content. This is because the herb is rich in polyphenols which are a great source of antioxidants.

2.3.3 Diabetes

A person with diabetes does not produce enough of the hormone insulin or is unable to use it effectively, which causes high blood sugar (glucose) levels. Over time, this imbalance can lead to heart disease, nerve damage, kidney disease, vision loss and various other complications. There are two types of diabetes. Less common is insulin-dependent diabetes (type 1) which usually develops before the age of 30. Non-insulin-dependent diabetes (type 2) accounts for 90% of cases which it usually appears after the age of 40.

Based on a local research done by Mariam et al. (1996) study hypoglycaemic effect in normal rats treated orally with 1.0g / kg of body weight of the Orthosiphon stamineus extract. They found that, the hyperglycaemic effect induced by streptozotocin was also inhibited by the same dose of the same extract. It can be concluded that the aqueous extract of local Misai Kucing possessed some hypoglycaemic activities in both normal and streptozotocin-induced diabetic rats in lowering the blood sugar level. From a couple of testimonials obtained from Nusa Herba Sdn. Bhd. who consume this herb in the form of tea drinking or tablets said that they are prone to sweat more because of the heat generated inside the body. Some of them even had loss weight through consumption of this herb. Somehow there’s a chemical compound in the extract which increases the body’s metabolism and uses the glucose in the body to generate energy which indirectly will help diabetic patients. Therefore, this herb is potential in lowering blood glucose level in diabetic patients.

2.4 Overview of Separation Process

Separations are extremely important in Chemical manufacture. Separation processes are any set of operation that separate solutions of two or more components into two or more product that differ in composition. These may either removed a single components from a mixture or separate a solution into its almost pure components. This can be done by exploiting chemical and physical property differences between the substances through the used of a separating agent. There are three type of separation processes. 1st is gas –liquid separation, 2nd is liquid –liquid separation and 3rd is solid –liquid separation.

2.5 Essential Oil Extraction Process

There are a lot of methods to extract the essential oils .It can be extracted by steam distillation, hydro distillation, cold pressing technique, vapor-cracking, turbo-extractor and many other solvent extractors.

2.5.1 Hydro distillation

Hydro distillation is used in the manufacture and extraction of essential oils. The botanical material is immersed in the water then being boiled with the water. The hot water helps to release the aromatic molecules from the plant material since the hot water forces to break the pockets in which the oils are kept in the plant material. The molecules of these volatile oils then escape from the plant material and evaporate into the steam.

The temperature of the process needs to be carefully controlled - just enough to force the plant material to let go of the essential oil, yet not too hot as to burn the plant material or the essential oil. The steam which then contains the essential oil is passed through a cooling system to condense the steam, which form a liquid from which the essential oil and water is then separated.

During distillation, only very tiny molecules can evaporate, so they are the only ones, which leave the plant. These extremely small molecules make up an essential oil. Oils containing more of the smallest, and therefore most volatile of these tiny molecules, are termed ‘top notes’ in the perfumery world; those containing more of the heaviest and least volatile of the tiny molecules are called ‘base notes’. Those in between are known as middle notes.

2.5.2 Steam Distillation

Steam distillation is used in the manufacture and extraction of essential oils. The botanical material is placed in a still and steam is forced over the material. The hot steam helps to release the aromatic molecules from the plant material since the steam forces open the pockets in which the oils are kept in the plant material. The molecules of these volatile oils then escape from the plant material and evaporate into the steam.

The temperature of the steam needs to be carefully controlled - just enough to force the plant material to let go of the essential oil, yet not too hot as to burn the plant material or the essential oil. The steam which then contains the essential oil is passed through a cooling system to condense the steam, which form a liquid from which the essential oil and water is then separated.

During distillation, only very tiny molecules can evaporate, so they are the only ones, which leave the plant. These extremely small molecules make up an essential oil. Oils containing more of the smallest, and therefore most volatile of these tiny molecules, are termed ‘top notes’ in the perfumery world; those containing more of the heaviest and least volatile of the tiny molecules are called ‘base notes’. Those in between are known as middle notes.

Deng-Kai Yang and Wang-Hsien Ding from Department of Chemistry, National Central University, Taiwan already use this method to determine alkylphenolic residues in fresh fruits and vegetables using steam distillation and gas chromatography–mass spectrometry. The following diagram provides an overview of the steam distillation process.

2.6 Orthosiphon Stamineus Essential Oil Processing

2.6.1 Introduction to Rotary Evaporator

Rotary evaporators commonly found in organic laboratories. They are used to remove solvents from reaction mixtures and can accommodate large volumes of liquid. It is usually utilized to separate solvents such as n-hexane, acetone and ethanol from the essential oils produced in solvent extraction. Figure 2.8 below shows the technical specifications of rotary evaporator.

Table 2.8: Technical specifications of a rotary evaporator

Rotary Evaporator	Water Bath
Speed range:20-190 rpm	Temperature range: ambient to 95°C
Vacuum: <1 mmHg	Capacity: 3.5 Liters
Lift distance: 150 mm	Heater power: 1300W
Dimension: (w x d x h) – 385 x 335 x 470 - 610mm, excluding glassware	Dimension: (w x d x h) – 260 x 280 x 200mm

Rotary evaporator has several parts. The main parts of a rotary evaporator include a water bath, a speed motor, a condenser and a vacuum supply. A typical rotary evaporator has a water bath that can be heated in either a metal container or crystallization dish to keep the solvent from freezing during the evaporation process. Water or silicon oil is used as the heating medium. Besides that, the evaporator normally uses a variable speed sparkles induction motor that spins at 0- 220 rpm and provides high constant torque (R. Toreki, 2005).This enables the flask containing solution to rotate continuously according to the speed set as well as enhances the evaporation of solvent. Vacuum is used to evaporate the solvent while the condenser condenses the vapor trapped to liquid that is later collected for easy reuse or disposal. Rotary