THE EXTRACTION OF *ASYSTASIA GANGETICA* USING ETHYL LACTATE SOLVENT

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ABSTRACT

This paper presents the study of the leaves of *Asystasia Gangetica*. The leaves of *Asystasia Gangetica* are found to have a number of advantages to produce essential oil which is important in medicine, cosmetic and others. *Asystasia Gangetica* is a straggling herb usually found among short grasses and along pathways. The objective of this research is to obtain the essential oil from *Asystasia Gangetica* plant by using maceration method. The solvent used in this study is ethyl lactate. Prior to that, the leaves of *Asystasia Gangetica* need to be dried first at temperature 70°C to 90°C using oven. After drying, separately, the leaves and stems are cut into pieces. The small pieces of fresh samples (200 g) are extracted using the maceration and rotary evaporator methods are used to obtain the essential oil. The obtained result showed that optimum time is 7 days. Longer time operation and smaller particles size proved that higher yield is obtained. The presence of these components in the essential oil from leaves of *Asystasia Gangetica* is studied well. The essential oil is analysed using Gas Chromatography-Mass Spectrometer (GCMS). The result by GCMS showed that the essential oil contains hexadeconoic acid, n-hexadecanoic acid, phytol and 9, 12, 15-octadecatrienoic acid.

ABSTRAK

Kertas kerja ini membentangkan kajian daun *Asystasia Gangetica*. Daun *Asystasia Gangetica* didapati mempunyai kelebihan untuk menghasilkan minyak esen yang mana penting dalam bidang perubatan, kosmetik dan lain-lain. *Asystasia Gangetica* adalah herba terurai, bisanya ditemui dalam kalangan rumput pendek di sepanjang laluan. Objektif penyelidikan ini adalah untuk mendapatkan minyak esen dari tumbuhan *Asystasia Gangetica* dengan menggunakankaedah pemaseratan. Pelarut yang digunakan dalm kajian ini ialah etil laktat. Sebelum itu, daun *Asystasia Gangetica* perlu dikeringkan pada suhu 70 °C- 90 °C dengan menggunakan ketuhar. Pengeringan susulan, secara berasingan, daun dan batang dipotong kepingan. Kepingan-kepingan kecil sampel baru (200 gram) dikeluarkan menggunakan kaedah pemaseratan dan *rotary evaporator* digunakan untuk mendapatka minyak esen. Keputusan diperolehi menunjukkan bahawa masa optimum ialah 7 hari. Operasi masa yang lebih lama dan saiz zarah yang lebih kecil membuktikan bahawa hasil yang lebih tinggi lama dan saiz zarah yang lebih kecil membuktikan bahawa hasil yang lebih tinggi diperolehi. Kehadiran komponen ini dalam minyak esen dari daun Asystasia Gangetica dikaji juga. Minyak esen dianalisis menggunakan spectrometer kromatografi gas-jisim. Analisa menggunakan spectrometer kromatografi gas-jisim menunjukkan bahawa minyak esen mngandungi *hexadeconoic acid, n-hexadecanoic acid, phytol and 9, 12, 15-octadecatrienoic acid.*

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LIST OF SYMBOL

%	Percentages
α	Alpha
° C	Degree Celsius
°F	Fahrenheit
g	Gram

LIST OF ABBREVIATIONS

SFE	Supercritical Fluid Extraction
GC-MS	Gas Chromatography-Mass Spectrometer
NIST	National Institute of Standards Technology
rpm	Revolution per minute

CHAPTER 1

INTRODUCTION

1.1 EXTRACTION

Extraction is can be defined as a process of separating desired components from a solid material. There are many type of extraction such as solid-liquid extraction and liquid-liquid extraction. Some of them are steam distillation, water distillation, solvent extraction, carbon dioxide extraction and cold processing. The main objective in the extraction process is to extract the essential oil from *Asystasia Gangetica*.

1.2 ASYSTASIA GANGETICA

Asyatasia Gangetica is a straggling herb usually found among short grasses and along pathways. The plant is recognized as a potential food source because the leaves contain high amount of proteins, amino acids, minerals, sugars, lipids and fiber (Yeoh, 1993). It is also used traditionally for many ailments and diseases. The habitat of *Asystasia Gangetica* is easily found in Malaysia and this habitat gives more benefits to human. *Asystasia Gangetica* is an ornamental plant and has been used as a source of nutrition in times of food scarcity.

Asystasia Gangetica is also known as ‘rumput Israel’ or ‘rumput bunga putih’. Other names for *Asystasia Gangetica* are *Asystasia coromandeline Nees, Justicia gangetica Linn, Asystasia gangetica (L) T.Anders and Ruelia coromandeliana Nees. Asystasia Gangetica (Linn) T.Anderson* belonging to the family Acanthaceae is an ornamental plant and used as a vegetable in times of food scarcity. It has many medical properties and is used in folklore medicine for various ailments like asthma, rheumatism, swelling etc. The whole plant of *Asystasia Gangetica* had many benefits to human being (Akah PA, 2003).

1.3 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 had a market value estimated at RM 2 billion (Malaysian Industrial Development Authority, 2005). Nowadays, in Malaysia, essential oil is gaining popularity as an herbal medication as it gives a lot of benefits to overcome the disease. It is because people start to realize the important of healthy awareness. Hence, one of the potential wild and herb plant *Asystasia Gangetica* could be benefit as a nutraceutical and pharmaceutical product.

A characterisation to the useful chemical components presence in *Asystasia Gangetica* essential oil should be carried out to suggest as an alternative raw material for the neutraceutical and pharmaceutical products potential.

1.4 OBJECTIVE

The objectives of this research are:-

1. To obtain the essential oil from *Asystasia Gangetica* plant by using maceration method.

2. To calculate the percentages of yielded from *Asystasia Gangetica* by using gas chromatography-mass spectrometer (GC-MS).

1.5 SCOPES OF STUDY

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

1. Preparation of the *Asystasia Gangetica* leaves for the experiment. The leaves are dried into oven to remove the moisture from leaves. The leaves and stem were cut into pieces and separated.
2. In this experiment, ethyl lactate is used as a solvent to extract the *Asyatasia Gangetica* plant. Next, the rotary evaporator is used for the maceration process to extract the essential oil from *Asystasia Gangetica* leaves.
3. The *Asystasia Gangetica* essential oil is subjected to qualitative and quantitative GC-MS analysis. GC-MS analysis is performed using an Agilent 6890 gas chromatography instrument coupled to an Agilent 5973 mass spectrometer. Agilent Chem.
4. The GC-MS is fit with a capillary column 30 mm x 250 µm x 0.25 µm with temperatures of the injector and detector set at 250 °C. Each sample is diluted in dichloromethane and 1 µL of oil sample are injected using helium as a carrier gas (1 ml/min). Temperature is programmed from 40 °C to 260 °C with final hold time of 3 minute. The resulting mass spectra will be compared with internal mass spectra library to identify the individual compounds. Confirmation of identify is done by comparing the retention indices with those in the National Institute of Standards Technology (NIST) library.

1.6 CHAPTER SUMMARY

In this chapter, detail explanations of the early step in the process of designing the research have been discussed. This explanation will be helpful in supporting this research work. Therefore, the objectives of the research will be on the right path and can be achieved. The literature review will be explored in the next chapter.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

In this chapter, the finding of related articles from website and I-portal is needed in order to do the literature review. Literature reviews are research done in the past by other researchers and they are needed to support our research objectives. This chapter also presents a brief introduction about *Asystasia Gangetica* and also the review of previous studies on the extraction of essential oil using maceration method with solvent.

2.2 *ASYSTASIA GANGETICA*

2.2.1 Introduction

Asystasia gangetica is a species of plant in the acanthaceae family. It is commonly known as the Chinese Violet, Coromandel. In South Africa, this plant may simply be called asystasia while in Malaysia known as ‘rumput Israel’, ‘rumput bunga putih’, ‘rumput hantu or ‘rumput nyonya’. ‘Asystasia’ means inconsistency and it relates to the fact that the corolla is slightly regular which is an unusual characteristic in the Acanthacease family. The word ‘gangetica’ is derived from Ganges River and it is

locally used a potherb and leafy vegetable in times of food scarcity. Besides, the leaf of Asystasia Gangetica also uses in many ways such as as an attraction of bees to orchards. It has high nutrition and used as forage growth for cattle, goats and sheep in south East Asia and also used as an ornamental plant. Table 2.1 and Table 2.2 show the names of the habitat of Asystasia Gangetica and the Vernacular names of Asystasia Gangetica.

Table 2.1: Names of the habitat of Asystasia Gangetica

Scientific names	Common names
Asystasia coromandeline Nees	Asistasia (Tag.)
Justicia gangetica Linn	Zamboangenita (Tag.)
Asystasia gangetica (L) T.Anders	Chinese violet (Engl.)
Ruelia coromandeliana Nees	Coromandel (Engl.)
	Creeping foxglove (Engl.)
	Pruple Primrose (Engl.)

(Source: www.stuartxchange.org/Asistasia.html)

Table 2.2: Vernacular names of Asystasia Gangetica

Country	Name
Malaysia	Rumput Isreal, Ara Songsang, Rumput Bunga Putih, Rumput Hantu, Rumput Nyonya
English	Tropical Primrose, Chinese Violet, Senegal Tea Tree, Orange Hawkweed
Thailand	Baya, Yaya
Philippines	Asistasia, Zamboangenita (Tagalog), Bulak-bulak (Subanun)
India	Upputhali
French	Herba le Rail, Mange-tout, Herba Pistache, Pistache Marron
South Africa	Asystasia

(Source: www.globinmed.com)

2.2.2 Properties of Asystasia Gangetica

Asystasia Gangetica (L) T. Anderson (Acanthaceace) is a fast growing, spreading, perennial herb, branched, quadrangular stem up to 2 meter long, often rooting at the lower nodes (HN, 1958). It is herbaceous groundcover that grows from 300-600 mm in height. The leaves are green, oval-shaped with rounded base, very slightly saw-edged and smooth (Saunders, 1985). The *Asystasia Gangetica* leaves contain a lot of nutrition composition such as water, protein, fat, carbohydrate and so on. The leaves have a lot of benefits to human being. Figure 2.1 shows the leaves of *Asystasia Gangetica*.

Figure 2.1: The leaves of *Asystasia Gangetica*

(Source: www.stuartxchange.org/Asistasia.html)

2.2.3 Usage of Asystasia Gangetica

The leaves of *Asystasia Gangetica* give more advantages to human being in the world. *Asystasia Gangetica* is used as food and also as medicine. In Malaysia, *Asystasia Gangetica* are very famous among people because this plant gives more benefits to them. Commonly, in Malaysia, people use the young leaves and shoots as vegetables while in Kenya and Uganda, this plant is popular and it is cooked with beans and ground nuts (Odugbemi, 2008). Besides, this plant is also famous in other countries like in some parts of Africa and Nigeria. In Africa, the leaves are eaten as a vegetable and used as an herbal remedy in traditional African medicine. The leaves of *Asystasia Gangetica* also can be used as a medicine. The leaves are included as an ingredient in a number of pot herbs in the African communities. The extraction of the whole plant is given to women in labor as an aid to ease the pain during childbirth (Odugbemi, 2008). Other than that, the leaves of *Asystasia Gangetica* are used in many parts of Nigeria for the management of asthma because the leaves of *Asystasia Gangetica* are highly effective in the local treatment of asthma (Kakwaro, 1976). *Asystasia Gangetiga* gives a lot of benefits to others whether in medicine or food.

2.3 ESSENTIAL OIL

2.3.1 Introduction

Essential oil is used in a wide variety of consumer goods such as detergents, soaps, toilet products, cosmetics, pharmaceuticals, perfumes, confectionery food products, soft drinks, distilled alcoholic beverages (hard drinks) and insecticides. The world production and consumption of essential oils and perfumes are increasing very fast. Production technology is an essential element to improve the overall yield and quality of essential oil.

Essential oil is volatile that is distilled from aromatic plant materials. The odour and flavour of the oils are usually depending upon these oxygenated compounds. Many oils are terpeneoids, few are benzene derivatives. Table 2.3 shows the major constituents of some common essential oils (Naik, et al., 1989).

Table 2.3: Essential oils from some natural plant

Name	Part of plant used	Botanical name	Important	Uses
Lemongrass and citronella	Leaf	*Cymbopogon spp*	Citral Citronella terpenes	Perfumery Disinfectant
Eucalyptus	Leaf	*Eucalyptus globules Eucalyptus citriodora Eucalyptus dives*	Cineale Citronella terpenes	Not mention
Clove	Bud	*Eugenia caryophyllus*	Eugenol	Dentistry Flavoring
Lavender	Flower	*Lavendula intermedia*	Linalol	Perfumery
Sandalwood	Wood	*Santaium album*	Sanatols	Perfumery
Nutmeg	Nut	*Myristica fragrans*	Myristicin	Not mention
Almond	Nut	*Prunis communis*	Benzeldehyde	Not mention
Coriander	Seed	*Coriandrum sativum*	Linalool Terpenes	Not mention

(Source: Naik, et al., 1989)

Essential oils can be divided into two broad categories:

1. Large volume oils which are usually distilled from leafy material (e.g. lemon grass, citronella and cinnamon leaves).
2. Small volume oils which are usually distilled from fruit, seed, buds and to a lesser extent, flowers, (e.g. cloves, nutmeg, and coriander).

Due to the large quantity of plant material required, pure essential oils are expensive but they are also highly effective which is only a few drops at a time are required to achieve the desired effect. Essential oil has an immediate impact on our sense of smell, also known as ‘alfaction’. When essential oil is inhaled, olfactory receptor cells are simulated and the impulse is transmitted to the emotional center of the brain or ‘limbic system’. The limbic system is connected to areas of the brain linked to memory, breathing, and blood circulations as well as the endocrine glands which regulate hormone levels in the body. The properties of the oil, the fragrance and its effects, determine stimulation of these systems.

When used in massage, essential oils are not only inhaled but absorbed through the skin as well. They penetrate the tissue and find their way into the bloodstream where they are transported to the organs and systems of the body. Essential oils work quickly on the body and mind. Through our sense of smell to the olfactory nerves and our brain starts to react to the vapor from an essential oil in less than four seconds. The essential oil ingredients of oil when applied to the body are also absorbed quickly into the skin via the hair follicles, some almost instantly, depending on the essential oil.

Essential oils may be used singly or in combination to bring about curative and restorative processes in the mind and body, offering a gentle alternative to modern drugs. They can assist the treatment of physical, emotional and mental changes, skin care and therapeutic massage. Even when used solely for sensual pleasure, they can positively enhance and enrich our daily life.

2.3.2 Properties of essential oil

Each essential oil had its own properties and uses which can classify and identified accordingly to the type of plant it was derived. Table 2.4 shows the properties and uses of the top essential oil. From Table 2.4, it can be concluded that the significant use of the essential oil is mainly in pharmaceuticals industry where most of it have the

anti-depressant properties. There are also some other ways to enjoy the magnificent scent of these natural ingredients. A few drops of essential oil in radiator fragrance or light bulb ring will fill the room with a wonderful fragrance and ambience. One interesting use of this oil is to fresh the shoes by only dropping a few drops of Geranium oil directly into shoes or place a cotton ball dabbed with a few drops of lemon oil.

Table 2.4: Properties and uses of the top essential oil

Essential oil	Biological name	Properties	Uses
Clory Sage	*Salvia Sclarea*	Warning, soothing, antiseptic, anticonvulsive, astringent, antichloristic, digestive, deodorant, tonic, uterine, bactericidal, antidepressant	Menstrual problems, anxiety, depression, high blood pressure, acne boils, oily skin and hair, cramp, migraine, the genitor-urinary system disorders such as ulcers and wrinkles
Eucalyptus	*Eucalyptus Globulus*	Antiseptic, analgesic, antineuralgic, antirheaumatic, antispasmodic, diuretic, expectorant, antiviral, hypoglycemic, febrifuge, vulnerary, depurative, stimulant.	Muscular aches and pains, poor circulation, rheumatoid arthritis, asthma, bronchitis, flu, cold, epidemics, chicken pox, headaches, neuralgia, throat infections, skin disorders such as burn, cuts, heroes and insect bites.
Geranium	*Pelargonium Graveolens*	Soothing, refreshing, relaxing, antidepressant, astringent, antiseptic, antihaemarrhagis, deodorant, diuretic, fungicidal, anti-inflammatory.	Anxiety, adrenocortical glands and menopausal problems, sore throat, tonsillitis, cellulites, engorgement of breast, broken capillaries, eczema and mature skin.

Jasmine	*Jasminum Officinale*	Analgesic (mild), antidepressant, anti-inflammatory, antiseptic, antispasmodic, aphrodisiac, carminative, cicatrizing, expectorant, galactagogue, sedative, tonic (uterine)	Depression, nervous, exhaustion and stress related conditions, jasmine is said to produce the feeling of optimism, confidence, euphoria. Jasmine is also used for catarrh, coughs, labor paints and skin problem.
Lavender	*Lavendula Vera Officinals*	Analgesic, anti-conclusive, antidepressant, antimicrobial, anti-rheumatic, antiseptic, antispasmodic, antitoxic, deodorant, diuretic	Excellent first aid oil. It soothes cuts, bruises and insect bites. One of the most versatile therapeutic essences. For nervous system disorders such as depression, headache, hypertension, insomnia, migraine and shock. Useful in treating skin conditions such as acne, allergies, boils, sunburn. Treatment of disorders such as flu, throat infections and asthma.
Lemon	*Citrus Limonum*	Refreshing, antiseptic, stimulating, anti-anemic, anti-rheumatic, anti-sclerotic, antitoxic, hypertensive	Warts, depression, acne and indigestion, arthritis, cellulites, high blood pressure, obesity, poor circulation, asthma, throat infections, bronchitis, cold, fever, flu. Treatment of anemia, brittle, nails, corns, mouth ulcers, greasy skin, cuts, spots and varicose veins.

(Sources: www.earthchildpendants.co.uk/essential oils)

2.3.3 Sources of essential oil

Essential oil is generally derived from one or more plant parts, such as flowers (e.g. rose, jasmine, carnation, clove, mimosa, rosemary, lavander), leaves (e.g. mint, *Ocimum* spp., lemongrass, jamrosa), leaves and stems (e.g. geranium, patchouli, petitgrain, verbena, cinnamon), bark (e.g. cinnamon, cassia, canella), wood (e.g. cedar, sandal, pine), roots (e.g. angelica, sassafras, vetiver, saussurea, valerian), seeds (e.g fennel, coriander, caraway, dill, nutmeg), fruits (bergamot, orange, lemon, juniper), rhizomes (e.g. ginger, calamus, curcuma, orris) and gums or oleoresin exudations (e.g. balsam of Peru, *Myroxylon balsamum*, storax, myrrh, benzoin).

2.4 TYPE OF EXTRACTION METHOD

2.4.1 Soxhlet Extraction

Solvent extraction of solid samples is commonly known as solid-liquid extraction. Solid-liquid extraction (leaching) is the process of removing a solute or solutes from a solid by using liquid solvent. Soxhlet is one of the leaching techniques mostly used for a long time (Luque de Castro, et al., 1998). A soxhlet extractor is a piece of laboratory apparatus invented in 1879 by Franz von Soxhlet. It is originally designed for the extraction of a lipid from a solid material. The concept of the Soxhlet extraction is organic compounds that are extracted by repeated washing with an organic solvent under reflux in special glassware. In general, to setup this method may consist of round bottom flask containing the solvent, an extraction chamber and a condenser (Brill, et al., 2006). It is stated in several extraction studies. The raw materials are dried and grounded before the extraction can be preceded.

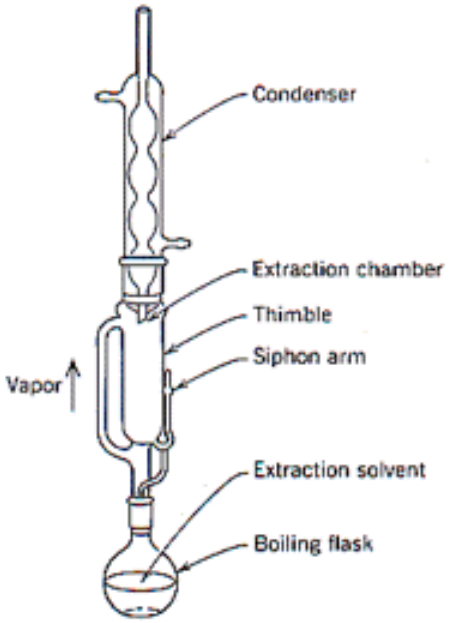

Figure 2.2: Soxhlet extraction apparatus

(Source: Luque de Castro, et al., 1998)

In general operation of the soxhlet extraction as a Figure 2.2, the sample is placed in a thimble-holder and filled with condensate fresh solvent that is from the heating of the boiling flask. When the liquid reaches the overflow level, a siphon aspirates the solute of the thimble-holder and back into boiling flask. This operation is repeated until complete extraction is achieved.

2.4.1.1 Advantages and Disadvantages of Soxhlet Extraction

According to previous studies on soxhlet extraction method, there are many advantages of this conventional extraction method. In the soxhlet extraction, there is displacement of transfer equilibrium by repeatedly bringing fresh solvent into contact with the solid matrix and it enables maintaining a relatively high extraction temperature with heat from the boiling flask. Besides, by using soxhlet extraction, it does not require filtration after the leaching step and also this method is a very simple method to run the sample (Luque de Castro, et al., 1998). Besides the advantages, these methods also have disadvantages such as soxhlet extraction method takes long time to be completed and