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EXTRACTION OF ESSENTIAL OIL FROM MURRAYA KOENIGII LEAVES: POTENTIAL STUDY FOR APPLICATION AS NATURAL-BASED INSECT REPELLENT

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ABSTRACT

Murraya Koenigii leaves contain many useful chemical constituents. The leaves which known as *daun kari* in Malaysia is belong to family Rutaceace and can be found in Malaysia, India, Bangladesh, Nepal, Sri Lanka and Burma. The aim of this study is to characterize the components of essential oil of the leaves and finding its potential to be applied as insect repellent. The essential oil was extracted using steam distillation and hydro-distillation. The amount of leaves and water used were 200 gram and 2 mL. The time of extraction for both methods were between 3 to 9 hours. For every hour of extraction time, the yield between these two methods were compared. The results shows that the percentage yield for steam distillation in method is higher compared to conventional method which is hydro-distillation method. The highest yield obtained from 9 hours steam distillation is 0.25% (w/w) whereby 9 hours hydro-distillation method can only collected 0.09% (w/w) of yield. Components identification of the essential oil performed by GC-MS detected the presence of 30 different components, majorly hydrocarbons. The analysis reported the existence of α-pinene and Caryophyllene which are the active ingredients for insect repellent. Based on this study, the present of these two repellent activity compounds in the extracted essential oil proves its potential to be used as active ingredients in natural-based insect repellent. The repellency test towards Blattaria shows 100% repellency.

Keywords: murraya koenigi, essential oil, hydrodistillation, steam distillation, insect repellent.

INTRODUCTION

Murraya Koenigii leaf is important leafy vegetable with many uses. It is belong to family Rutaceace based on semi-evergreen aromatic tree found throughout Malaysia, India, Bangladesh, Nepal, Sri Lanka and Burma. It is commonly known as curry patta or *daun kari* in Malaysia. Murraya Koenigii is widely use in Indian cookery for centuries and have a versatile role to play in traditional medicine (Jain, 2012). Murraya Koenigii trees grow up to 1650 m altitude with a wide and cultivate form (Verma, 2012). The leaves of Murraya Koenigii contain protein, carbohydrate, fiber, minerals, carotene, and nicotinic acid. It also contains crystalline glycoside, carbazole alkaloids, koenigin, girinimbin, iso-mahanimbine, koenine, koenidine and koenimbine (Bhandari, 2012). The leaves have slightly pungent, bitter and feebly acidic taste. They also will retain the flavor and other quantity even after drying (Sinha Parul, 2012). The essential oil of Murraya Koenigii leaves is also utilized for soap and cosmetic aromatherapy industry.

Essential oil (EO) is complex mixtures of volatile organic compounds produces as secondary metabolites in plants and they are constituted by hydrocarbons (terpenes and sesquiterpenes) and oxygenated compounds (alcohols, esters, ethers, aldehydes, ketones, lactones, phenols and phenols ethers (Guenther, 1972). About 3000 essential oil are known and 10% of them have commercial importance in the cosmetics, food and pharmaceutical (FAO, 1995). The oils normally bear the name of the plant species from which they are derived. Essential oils are so termed as they are believed to represent the very essence of odour and flavour (Sinha Parul, 2012). Essential oil has a great benefit to help protect our bodies and homes from any diseases. It also can be used as an insect repellent if the active ingredient of insect repellent is present inside the essential oil. A large number of essential oil extracted from different families has been shown to have high repellence against anthropod species (Luz Stella, 2010). Repellent activities of essential oil appear to be associated with the presence of some monoterpenes such as α-pinene, cineole, eugenol, limonene terpinolene, citronellol, citronellal, camphor, and tymol. They are common constituents described in the literature presenting mosquito repellent activity (Ibrahim, 1998).

The percentage of malaria and diarrhoea in Malaysia is increasing over the year. Malaria is a disease that cause by mosquitoes while diarrhoea is cause by house flies. Plant-based repellents were used in traditional way throughout the communities because for many of the poor communities this was the cheapest way to protect themself from mosquito bites (Maia, 2011). In Malaysian communities, Murraya Koenigii was used traditionally as a plant that will repel the house fly.

Chemical based insect repellent that contained DEET is the most effective repellent in the market but the percentage of toxicology that is very high was deemed unsafe for human use especially for children, pregnant woman and lactating woman (Maia, 2011). Moreover, DEET produced human toxicity reaction after its application. With the current awareness about the harm that contributed by the use of DEET, some countries have discontinued the used of DEET as a result of health concerns (Nkomo, 2012). Thus having a natural-based

insect repellent was a good alternative to substitute the chemical based insect repellent in the market. With its known traditional application as insect repellent in rural community, the essential oil of Murraya Koenigii was further studied.

In this research work, the yield of Murraya Koenigii's essential oil using two methods which are hydro-distillation and steam distillation was compared. The other scope of this research work is to investigate the active ingredients in the essential oil to prove its potential to be used as an insect repellent and to observe the repellent activity of insect towards the essential oil using Y-shaped testing equipment.

MATERIAL AND METHODS

Plant Material

Fresh leaves of Murraya Koenigii were collected around Kuantan, Pahang area. Only fresh leaves with the finest quality were used as a raw material in this research work.

Essential Oil Extraction

- Pre-treatment Methods

The collected fresh leaves of Murraya Koenigii were washed to remove the dirt on the surface of the leaves. It is to make sure that no any other impurities stick to the Murraya Koenigii leaves. The excess water moisture on the leaves' surfaces were then adsorbed using paper towel. The extraction was carried out using only fresh leaves.

- Steam Distillation (SD)

200g of fresh leaves of Murraya Koenigii were steamed in the upper round flask with 1000mL of distilled water until oil was distillate. The operating temperature was 130°C. Each extraction was performed within the period of extraction time of 3, 4, 5, 6, 7, 8 and 9 hours. The essential oil was then separated from the hydrosol by using diethyl ether as a solvent (Verma, 2012).

- Hydro-Distillation (HD)

200g of washed and fresh leaves of Murraya Koenigii was mixed together with 1000 mL of distilled water in a round flask. The operating temperature was 100°C as the boiling point of water. The extraction was done between 3 to 9 hours in the interval of 3, 4, 5, 6, 7, 8 and 9 hours. The essential oil was then separated from its hydrosol by using diethyl ether as a solvent (Verma, 2012).

Analysis of Essential Oil

- Gas Chromatography-Mass Spectrometry (GC-MS).

For the determination of the chemical constituents contained in the essential oil of Murraya Koenigii leaves, Gas Chromatography-Mass Spectrometry (GC-MS) machine was used. This machine, model Agilent 7890A GC coupled to a 5973C VL MSD Triple-Axis selective Detector is available in Laboratory of University Malaysia Pahang. This GC-MS is using Helium gas as the carrier gas at contant pressure.

Repellency Test

The repellence of the volatile oil was evaluated using the instrument that has been modified from Y-tube Olfactometer. This instrument was made from a transparent prospex and has been fabricated with a shape of Y. The test has been done by using Blattaria (5 in number). The observation during the test was recorded. The percentage of repellency was calculated based on the formula: % Repellence = [(C-T)/Cx100] where C is the total of Blattaria that landed on the control and T is the number of Blattaria that landed on the treated area of the essential oil (Nkomo, 2012).

RESULTS AND DISCUSSION

Effect of Extraction Time on the Percentage Yield of Steam and Hydro-Distillation

The essential oil was extracted by using steam distillation and hydro distillation with the operating temperature of 130 °C and 100 °C. The operating temperature of 130 °C must be higher than the boiling point of water which is 100 °C in steam distillation method in order to produce steam. As shown in Figure-1 and Figure-2, the trend of the graph was increasing over the time starting at 3 hours extraction and the highest yield was obtained at 9 hours extraction for both methods. 9 hours of extraction was found to be the time for the highest yield of essential oil by steam and hydro-distillation methods. Previous study by Ismail, 2014 showed that after 9 hours of extraction, the percentage yield would decrease because of the overexposed of the Murraya Koennigii leaves towards the heat that may allowed the plant cell started to degrade. The highest yield obtained in this research from steam distillation method at 9 hours extraction was 0.25% whereby for hydro-distillation at 9 hours extraction is 0.09%.

Figure-3 shows a complete comparison of the yield obtained by both methods. The total yield obtained from steam distillation is 0.745% while for hydro-distillation is 0.170% from 3 to 9 hours extraction. This result shows that since hydro-distillation is the simplest method compared to steam-distillation, thus the extraction of essential oils is less efficient. In steam distillation method, there were no contacts between biomass (Murraya Koenigii leaves) with the boiled water so there would be no loss of essential oil when it flows to the condenser. 99% of essential oil may flow with the steam to the condenser. In hydro-distillation, the surface contact between biomass and boiled water will cause less effectiveness to obtain high yield because some of the oil is not condense (Mulvaney, 2012). The contact between the leaves and the water may cause some of the essential oil to be trapped inside the water and does not leave as steam towards condenser.

From the previuos study of curry leaves originate from Vietnam and Malaysia, the method that used was conventional hydro-distillation and the highest yield obtained was 0.83% (Vietnam) and 0.22% (Malaysia) (Nguyen, 2012; Ismail, 2014). Therefore, it showed that

the essential oil obtained by the previous study is higher compared to this research work which is only 0.09%. This probably cause by the type of the leaves used in this study that varied.

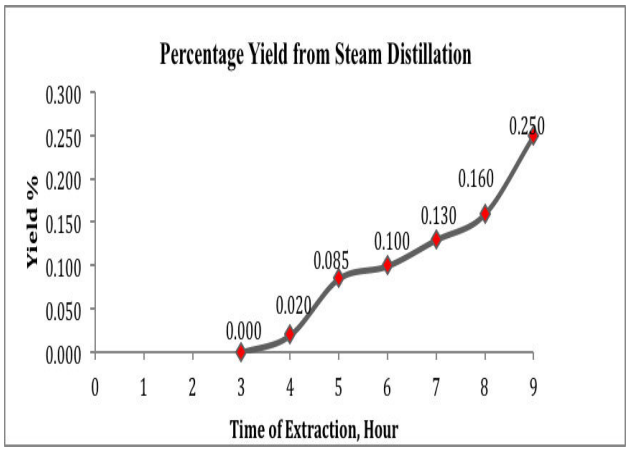

Figure-1. Percentage yield of essential oil using steam distillation.

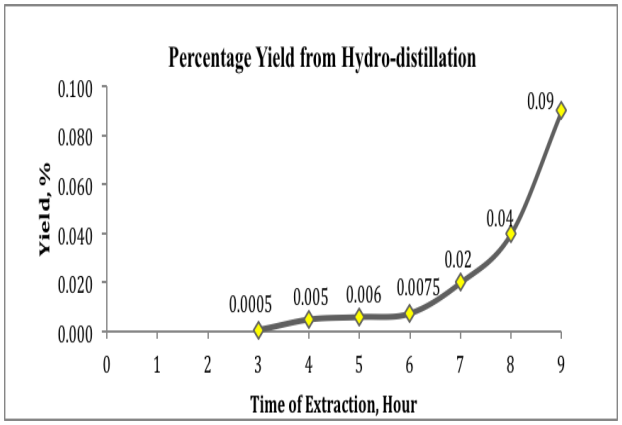

Figure-2. Percentage yield of essential oil using hydro-distillation.

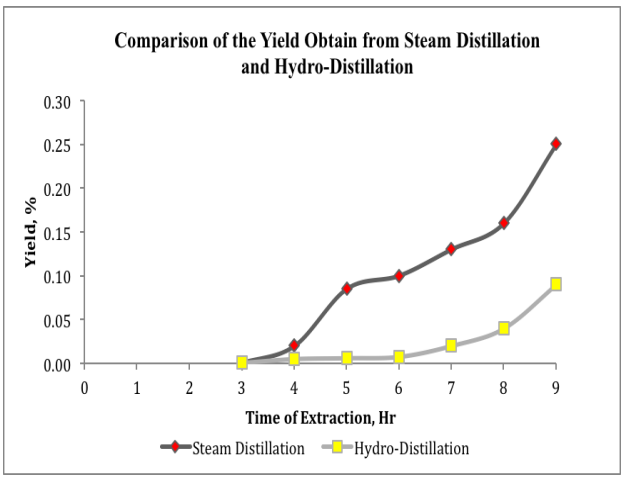

Figure-3. The comparison of the yield from steam distillation and hydro-distillation.

Analysis of Chemical Constituents of Essential Oil from Steam and Hydro-Distillation.

In order to know the chemical composition of Murraya Koenigii esssential oil, the analysis using Gas Chromathography-Mass Spectrometry (GC-MS) was performed. Table-1 and 2 shows the result of the chemical constituents inside the essential oil. Different compostion has been obtained from the two different methods. The components were dominated by the terpene hydrocarbon. The major component that obtained in Sample 1 by using hydro-distillation was β-Phellandere (21.21%), Caryophyllene (20.06%), β-Piperdinopropiophenone (15.71%), α-Caryophyllene (12.59%), and Oleic acid (12.44%).

In sample 2, which is the essential oil from steam distillation method, contained different chemical constituents. Table-2 shows the result for chemical constituents inside the essential oil of the leaves for sample 2. The major chemical constituent presence in the sample 2 was Caryophyllene (37.98%), Napthalene (16.30%), Azulene (9.69%), Cyclopentadecanone, 2-hydroxy (8.46%) and α-Pinene (6.51%). Interestingly, there was a presence of active ingredients for insect repellent that has been detected in the essential oil using steam distillation method which are α-pinene and Caryophyllene.

The previous study of essential oil of curry leaves in Vietnam by Nguyen (2012) had found about 76 components of curry leaves and it is apparently represented by hydrocarbons up over 90%. Further analysis shows that the major constituents obtained in this study were monoterpene hydrocarbon as α-pinene (19.03%), β-pinene (4.03%), and sesquiterpene hydrocarbon were β-Phellandrene (18.22%), trans-β-Caryophyllene (27.24%), β-Caryophyllene (4.87%), and bicyclogermacrene (5.23%). However, the result is different from the previous study of curry leaves in Malaysia that found only 30 different component in essential oil with the major constituents were α-pinene (3.44%), Caryophellene (32.19%), Naphthalene (11.39%), Globulol (10.95%), α-caryophyllene (7.92%), pentasiloxane (6.35%), cyclohexasiloxane (4.11%) and 4-cyclohexylidene-n-butanol (4.39%). These previous result is slightly dissimilar from the result that has obtained from this research work because of the different equipment and type of leaves used. Even though the method is the same which is hydro-distillation but different efficiency for different equipment and also the source of raw materials that grows in various environments will gave inconsistent properties that have contributed to the results obtained in the research.

Table-1. Chemical constituent, percentage area and retention time of Murraya Koenigii Leaves essential oil by using hydro-distillation (Sample 1).

Compound	Area %	Retention Time, RT
Cyclohexane	0.51	11.351
Caryophyllene	20.06	11.752
α-Caryophyllene	12.59	12.153
β-Phellandere	21.21	6.114
1,3,6-Octatriene	2.33	6.382
Nephthalene	1.04	12.549
Decahydro-4a-methyl-1-methylene	2.78	12.661
Globulol	0.46	13.683
Azulene	0.30	13.998
n-Hexadecanoic	0.95	14.763
9-Octadecenoic acid	3.52	17.988
Oleic Acid	12.44	18.737
Phytol	3.46	19.037
β – Piperidinopropiophenone	15.71	20.031
Triacontane	2.46	20.743

Repellent Activity

From Table-1 and Table-2, the present of small amount of α-pinene and Caryophyllene indicates that the essential oil of Murraya Koenigii leaves has a potential to be used as the active ingredients for natural based insect repellent.

The repellency test was conducted using 5 Blattaria (in number) to prove feasibility of the essential oil of Murraya Koenigii to repel insect. The test was performed using Y-shaped transparent prospex as shown in Figure-4 that was a modification from Y-tube Olfactometer (Ndungu *et al.* 1999). The percentage of the repellency was calculated as follows:

$$Repellency\ (100\%) = \frac{(5-0) \times 100}{5}$$
$$= 100\%$$

Table-2. Chemical constituent, percentage area and retention time of Murraya Koenigii leaves essential oil by using steam distillation (Sample 2).

Compound	Area %	Retention Time, RT
α-Pinene	6.51	4.708
β-Phellandere	1.70	10.308
Caryophyllene	37.98	11.672
Naphthalene	16.30	12.575
Azulene	9.69	38.636
1,4-Dimethyl-8-isopropylidenetricyclo	5.57	40.046
Oxalic Acid	0.12	44.853
n-Hexadecanoic acid	0.67	51.384
1-Eicosene	0.23	52.993
Phytol	9.21	55.706
Oleic Acid	0.74	59.691
Palmitic Acid	2.82	60.460
Cyclopentadecanone, 2-hydroxy	8.46	62.158

A complete repellency was showed by the test using Blattaria. The observation of the repellency throughout the test was summarized in Table-3 below:

Table-3. Observation of the repellency test.

Parameter	Observation
Coackroach Only	All the coackroaches stay at different position.
Coackroach with Biscuit	Some of the coackroach go to the biscuit site.
Coackroach with Repellent	The coackroach move away from the repellent site (with essential oil).

Figure-4. Y-shape instrument to test the repellency of essential oil.

CONCLUSIONS

The essential oil of Murraya Koenigii leaves was extracted successfully. The active ingredient for insect repellent contained by the essential oil of Murraya Koenigii leaves was found to be α-pinene and Caryophyllene. The percentage yield of essential oil was compared between the two methods; steam distillation and hydro-distillation. Steam distillation produce more yield 0.25% (w/w) compared to hydro-distillation which only 0.09% (w/w). The present of small amount of active ingredients in the essential oil proves that the essential oil of Murraya Koenigii leaves has a potential to be implemented as natural-based insect repellent. Repellency test of the essential oil towards Blattaria was successfully done in order to provide the strong evidence to prove that the essential oil has a potential to be a natural-based insect repellent.

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