

Screening of Plant Species Suitable for Insect Repellent and Attractant

(Penyaringan Spesies Tumbuhan sebagai Penghalau dan Penarik Serangga)

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Abstract

Five plants species; *Cymbopogon nardus* (serai wangi), *Cymbopogon citratus* (serai makan), *Citrus hystrix* (limau purut), *Citrus limon* (limau nipis) and *Polygonum sp.* (daun kesum) have been selected based on their aromatic properties. Air-dried samples were extracted using three different organic solvents; (hexane, chloroform, and ethanol). Extracts then were dried using the rotary evaporator. Aromatic samples were hydrodistilled for their essential oil and analyzed for the determination of chemical profile such as alkaloids, terpene and flavonoid using the gas chromatography (GC). The essential oil was tested on their repellent or attractant properties using guinea pigs and mosquitoes. Most extracts showed repellent properties.

Keywords: Screening, insect repellent, insect attractant, alkaloid, flavonoid, terpene.

Abstrak

Lima tumbuhan; *Cymbopogon nardus* (serai wangi), *Cymbopogon citratus* (serai makan), *Citrus hystrix* (limau purut), *Citrus limon* (limau nipis) dan *Polygonum sp.* (daun kesum) telah dipilih berdasarkan sifat-sifat aromatik tumbuhan berkenaan. Sampel kering disediakan untuk diekstrak menggunakan tiga pelarut organik; (heksana, klorofom, dan etanol). Ekstrak tumbuhan dikering menggunakan pengering berputar. Ekstrak disuling untuk mendapatkan minyak pati dan dianalisis untuk menentukan profil kimia seperti alkaloid, terpena dan flavonoid menggunakan kromatografi gas (GC). Minyak pati tumbuhan berkenaan diuji sama ada mempunyai sifat-sifat penghalau atau penarik menggunakan tikus putih dan nyamuk. Kesemua ekstrak menunjukkan sifat penghalau.

Kata kunci: Penyaringan, penghalau serangga, penarik serangga, alkaloid, flavonoid, terpena.

Introduction

Based on the report published by The Malaysian Science and Technology Information Centre (MASTIC)(2006) only a small number of plants are reported having medicinal values, spices and herbs. It has been widely known that the tropics including Malaysia is the home for large number of plant species (estimated as high as 25,000 species). Every single species of these plants contain specific chemical compounds used as one of their defensive mechanism against bacterial, fungal, or insects attacks to ensure distributions domination. This study aims to extract chemical compounds from selected plants species using suitable method. The extracts were tested for their suitability as attractant or repellent to insect. Insect's repellent is the chemical which repel or make the insect avoid the chemicals. While, the attractant is the chemical which attract the insects. Insect attractant chemicals are of important values in agricultural industries as pollinator.

Methodology

Selection of Plants Species and Extraction

Five plants species have been selected based on their aromatic properties, and dominance such as density at the natural habitat. Plants (10 kg each) were collected from various places in Kepong, Selangor and air-dried before chopped into small pieces. The samples were soaked in organic solvents (hexane, chloroform and ethanol). All extract were dried using the rotary evaporator and were hydrodistilled to yield essential oil (Figure 1). The essential oil was analyzed using the gas chromatography to determine the chemical profile such as alkaloids, terpenes or flavanoids.

Bioassay Experiment

The essential oils then were studied on their effect to insect, whether the oil repels or attracts the insect. A guinea-pig was used in the experiment. Parts of guinea-pig body was cleaned shaved exposing the skin, and the animal then placed in the experimental box. The box consisted of two compartments, one for the guinea-pig and the other for the mosquitoes, and between them separated by a door. First, the essential oils were applied to the guinea-pig skin; then, the door separating the two compartments was opened, exposing the guinea-pig to the mosquitoes.

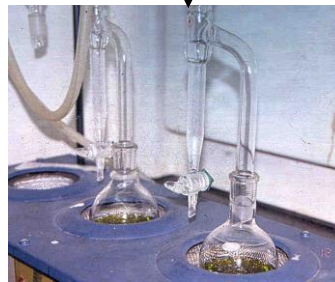
The reaction of the mosquitoes to the essential oils were observed, whether the essential oil attracted or repelled the mosquitoes



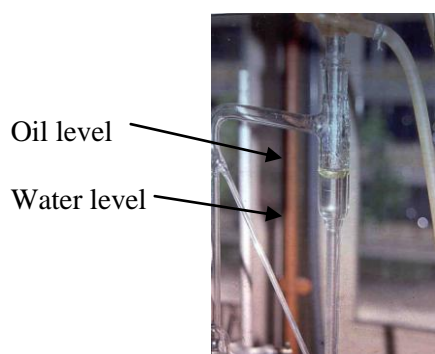
Cymbopogon nardus
(Serai wangi)



Sample collection



Extraction and distillation



Essential oil

Figure 1 Extraction and hydrodistillation of samples

Results And Discussion

The content of essential oil from *Cymbopogon nardus* (serai wangi), *Cymbopogon citratus* (serai makan), *C. hystrix* (limau purut), *C. limon* (limau nipis) and *Polygonum sp.* (daun kesum) using distillation procedure were shown in Table 1.

Table 1 Content (%) of Essential Oil Extracted from Various Plants

Sampel	Product (g)	Weight of Sample (g)	Essential Oil Yield (%)
<i>Cymbopogon nardus</i>	5.0	384	1.30
<i>Cymbopogon citratus</i>	5.0	625	0.80
<i>Citrus limon</i>	5.0	1667	0.30
<i>Citrus hystrix</i>	5.0	208	2.40
<i>Polygonum sp.</i>	2.0	1000	0.20

It showed that all plants studied contained very small percentage of essential oils (< 3%). *Citrus hystrix* (limau purut) contained highest amount of essential oils (2.4%), whilst *Polygonum sp.* (daun kesum) was the lowest (< 0.5%). The higher essential oils content, the stronger the aromatic properties of the plant. *Citrus hystrix* (limau purut) exhibit the strongest aromatic properties compared to other plant, whilst *Polygonum sp.* (daun kesum) was the least aromatic.

Analysis of Essential Oil Contents

The chemical contents in the essential oils were analyzed by gas chromatography and the profiles were determined by comparing with the gas chromatography standard obtained from library search.

The main chemical component of *Cymbopogon nardus* (serai wangi) was geraniol (61.4%). *Cymbopogon citratus* (serai makan) contains very high citral (75.7%) as its main chemical components. For *Citrus hystrix* (limau purut) the main chemical component of essential oil was beta-pinene (17.9%). The major chemical component for *Citrus limon* (limau nipis) was alpha-terpinol (25.4%). Both lime (*Citrus hystrix* (limau purut) and *Citrus limon* (limau nipis) contained other chemical component which was limonene, however at different percentage; *Citrus hystrix* (limau purut) contained 20.4% of limonene, and *Citrus limon* (limau nipis) contained 33.1%. *Polygonum sp.* (daun kesum) contained dodecanal (17.7%) and beta-caryophyllene (17.8%). The structures of the mentioned chemical components are presented in Figure 2. Chromatogram for *Cymbopogon nardus* (serai wangi), *Cymbopogon citratus* (serai makan), *Citrus limon* (limau nipis), *Citrus hystrix* (limau purut) and *Polygonum sp.* (daun kesum) are presented in Figure 3, 4, 5, 6 and 7 accordingly.

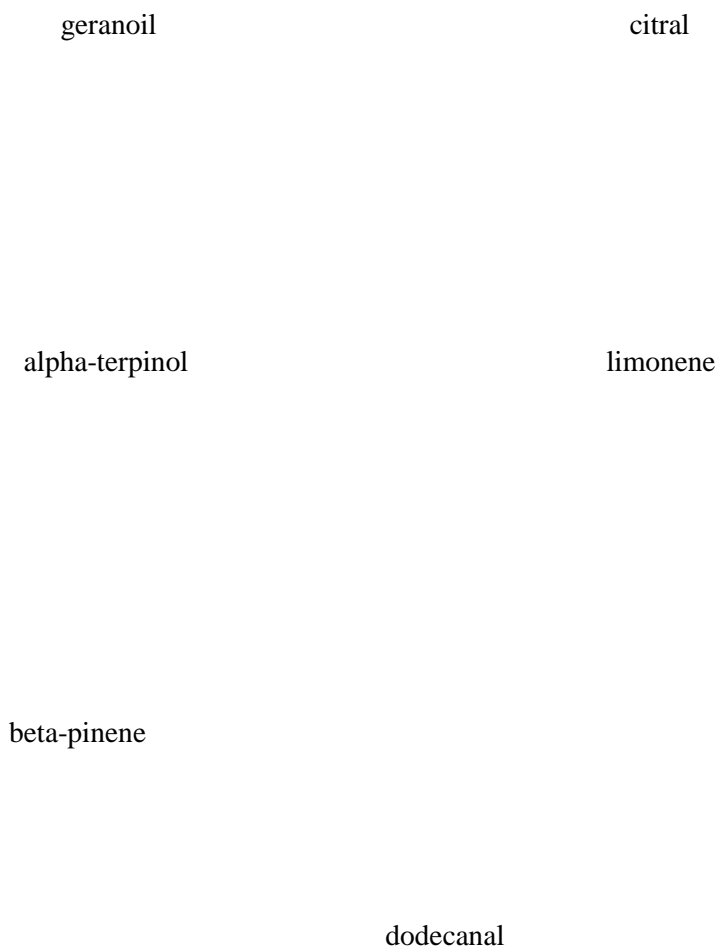


Figure 2 The Structure of Chemical Components Detected from Different Plants Studied

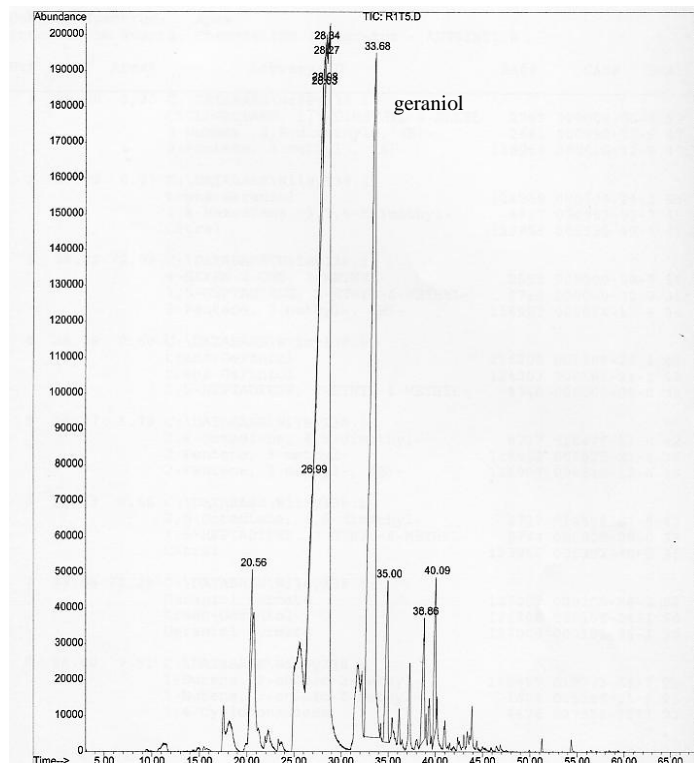


Figure 3 Chromatogram of *Cymbopogon nardus* (serai wangi) essential oil

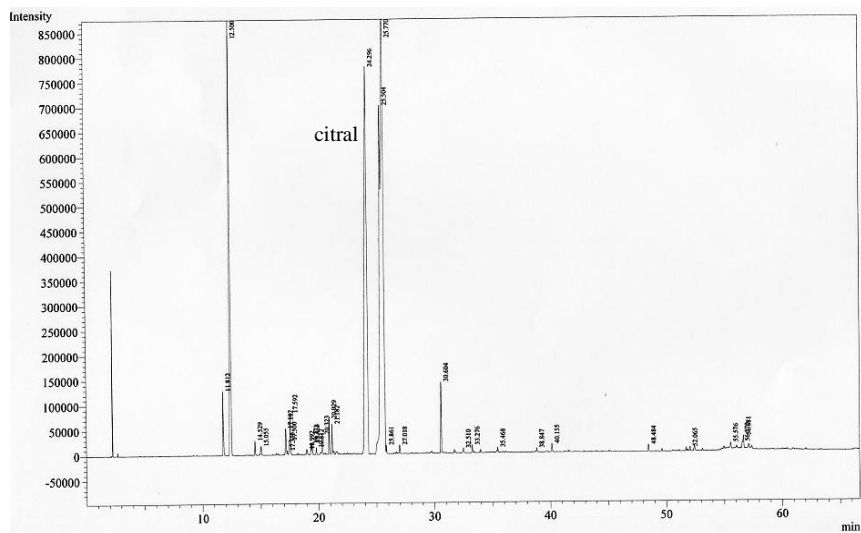


Figure 4 Chromatogram of *Cymbopogon citrates* (serai makan) essential oil

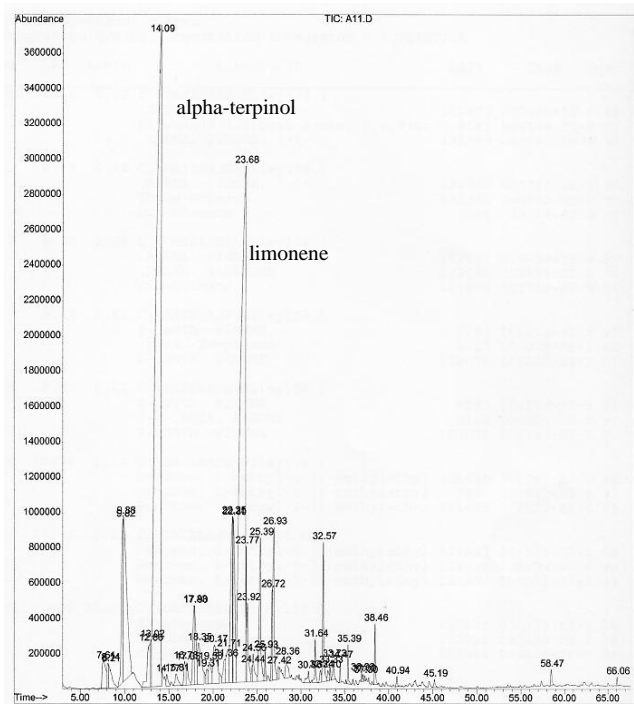


Figure 5 Chromatogram of *Citrus limon* (limau nipis) essential oil

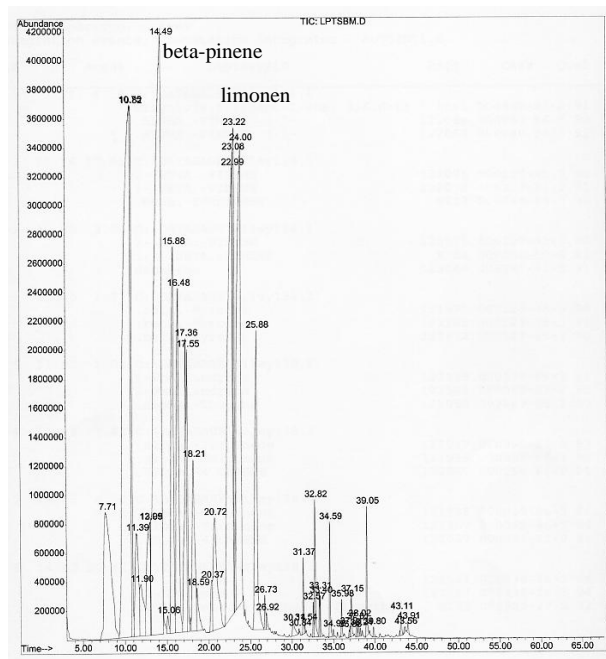


Figure 6 Chromatogram of *Citrus hystrix* (Limau Purut) essential oil

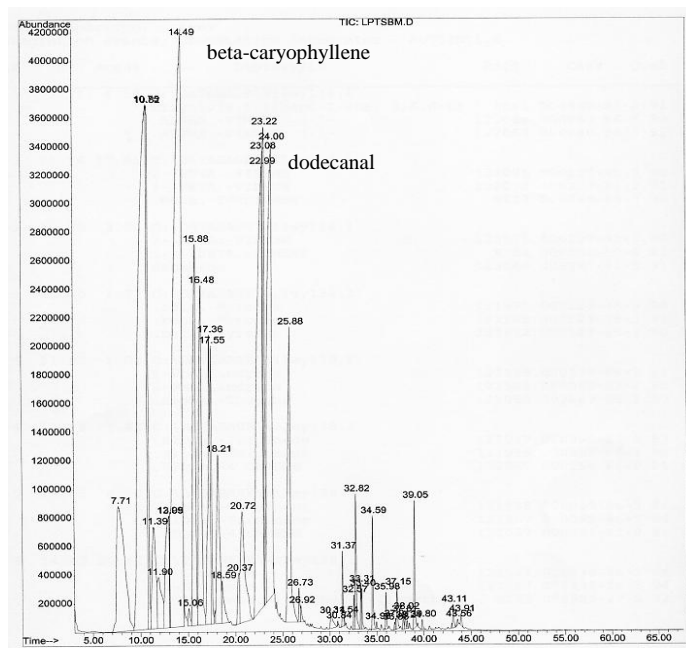


Figure 7 Chromatogram of *Polygonum sp.* (daun kesum) essential oil

Bioassay of Essential Oils on Tested Animal

Essential oil extracted from selected plants was tested on animals (white mice) for repellent or attractant properties. Plant extracts were applied to the guinea-pig skin and they were then exposed to the mosquitoes. Most animal's skin that have been applied with plant extracts and were exposed to mosquitoes showed repellent properties; i.e. mosquitoes did not bite the area applied with the extracts (Table 2).

Geraniol also found in other plants such as geranium, lemon, citronella, and many other essential oils and extensive testing by Dr. Jerry Butler at the University of Florida (Hunt, 1999) has shown geraniol to be one of nature's most effective insect repellents. Citral which is the major constituent of the oil of lemon grass and the oil of other plants in the genus *Cymbopogon*, also present in the oils of verbena, lemon myrtle, lemon and orange (Onawunmi, 1989). There was no report that citral has insecticides properties, however, citral has a natural anti-viral action (Schnaubelt, 1989). Out of 49 Australian essential oils including eucalyptus and tea tree species, citral exhibits the best killing power against *Staphylococcus aureus*, *Salmonella typhi* and *Mycobacteria phlei* (Atkinson & Brice, 1955). Beta-Pinene (β -pinene) which is a major constituent of *Citrus hystrix* (limau purut) also occurs naturally in rosemary, parsley, dill, basil and rose (Mann et al., 1994). Apart from *Citrus limon* (limau nipis), terpineol also has been isolated from a variety of sources such as cajuput oil and pine oil (Yuasa and Yuasa, 2006).

Table 2 Effect of Essential Oils to Mosquitoes

Sample	Chemical components	Repellent properties	Attractants properties
<i>Cymbopogon nardus</i>	Geraniol	+	-
<i>Cymbopogon citratus</i>	Citral	+	-
<i>Citrus limon</i>	Alpha-Terpineol	+	-
	Limonene	+	-
<i>Citrus hystrix</i>	β -pinene	+	-
	Limonene	+	-
<i>Polygonum sp.</i>	β -caryophyllene	+	-

Note: + = positive effect - = negative effect

Limonene as the main odour constituent of citrus plant (family *Rutaceae*) such as *Citrus limon* (limau nipis) and *Citrus hystrix* (limau purut). D-limonene has been reported used as botanical insecticide (EPA R.E.D., 1994). Caryophyllene was first totally synthesized in 1964 by E.J. Corey and was considered one of the classic demonstrations of the possibilities of synthetic organic chemistry at the time (Corey et al., 1964). Caryophyllene oxide extracted from *Valeriana jatamansi* (*Valerianaceae*) was reported to have insecticidal activity on mosquitoes (Dua et al., 2008).

Conclusion

As a conclusion all plants studied contain essential oils that have insect repellent properties. However, none of the essential oils tested have insect attractant properties.

Acknowledgement

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