Engineering Chemistry
ISSN: 2813-6535, Vol. 6, pp 27-33
doi:10.4028/p-30zZjJ

Submitted: 2023-02-08
Revised: 2023-09-27
Accepted: 2023-10-19
Online: 2024-03-05

Chemical Composition of Hydrodistillation and Solvent Free Microwave Extraction of Essential Oils from *Plectranthus amboinicus* and *Melaleuca cajupati* Leaves and their Repellent Activity

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Keywords: Hydrodistillation, Plectranthus amboinicus, Melaleuca cajupati, Essential Oil

Abstract. Extraction of the native plants Plectranthus amboinicus and Melaleuca cajupati leaves have been carried out using traditional hydrodistillation (steam distillation) and solvent free microwave-assisted extraction methods at different temperatures and microwave irradiation powers. The chemical compositions of the essential oils extracted were identified using gas chromatography analysis. The ideal extraction time for Pletranthus amboinicus and Melaleuca cajupati were 120 min with microwave power 200 W and 400 W, respectively with the calculated extraction yield were 0.034 % and 0.276 %, respectively. In this paper, the results of the selected microwave powers, first oil drops, and extraction time are presented as well as the findings of the chemical compositions present in both plants are also discussed.

Introduction

The heavy use of long-established synthetic repellents which commonly consist of diethyl m-toluamide (DEET), permethrin and malathion have raised several concerns in terms of human health risks and the environment. There are also shreds of evidence that point to a rapidly developing biological resistance of the insect vector to existing insecticidal chemical formulations [1]. Mosquitoes can become habituated to the smell of DEET over time, reducing its effectiveness as a mosquito repellent. Therefore, there is an increasing interest in the use of natural repellents that can substitute the existing synthetic compounds that are safe to use, non-toxic and environmental-friendly in protecting humans from insect vectors to prevent the transmission of serious and debilitating diseases [2].

The use of plant extracts essential oils with known effects on mosquitoes and other vectors is well known; essential oils from many plants such as Cymbopogon spp., Pelargonium citrosum and Eucalyptus maculata citriodon have been demonstrated to exhibit good repellent properties against mosquitoes [3]. In our bio-prospecting initiative for useful mosquito repellent plants, our overall objective is to identify candidates with potential repellent properties for the use as sources of essential oils or specific components that can be incorporated in personal protection products. Therefore, we report here the extraction data, analysis of constituents and mosquito repellent properties of two plants that are abundantly available and easy to grow in Malaysia i.e., Plectranthus amboinicus (P. amboinicus) and Melaleuca cajupati (M. cajupati) extracted using microwave-assisted extraction (MAE) technique and compare the data using conventional hydrodistillation (HD) method.

P. amboinicus is reported to have antimicrobial, antiepileptic and antioxidant effects properties that contain several chemicals such as monoterpenoids, diterpenoids, triterpenoids, phenolics, flavonoids, and aldehydes which are commonly used to treat coughs, sore throats, asthma, and skin problems [4 – 6]. It is also reported to possess many functions, including antibacterial, antiviral, antifungal, anti-inflammatory and antioxidative properties. As for M. cajupati, literatures reported that the oil is effective as an insect repellent and a remedy for headaches, rheumatism, and convulsions. It is known as a multi-purpose tree that is used for fuelwood, piles, and frame poles in buildings, whilst the leaves are used for essential oil distillation. and the flowers attract a honeybee [7]. The Melaleuca genus plants are reported to have known and used in traditional medicine for centuries, primarily due to their broad-spectrum antibacterial properties [9].

Materials and Methods

Plant materials preparation

Figure 1 shows the healthy plants species of P. amboinicus and M. cajupati were collected, specified and authenticated at Herbarium, Forest Research Institute Malaysia, (FRIM).

(a)

(b)

Fig. 1. Plants specified at FRIM, (a) M. cajupati and (b) P. amboinicus

Extraction and Characterization

In this work, essential oils of P. amboinicus and M. cajupati were isolated using two methods: i) classical hydrodistillation (HD) method carried out at FRIM for 24 h of extraction where fresh samples were used as soon as possible after their arrival from the field so that the leaves were still fresh and ii) solvent-free MAE technique using Milestone NEOS Microwave Extraction System at Food Science and Technology Research Centre, MARDI. The fresh leaves were cut into small pieces and ready for extraction. For MAE method, the time, temperature, and power of the first oil drop produced were recorded during the extraction process [11 – 13]. Mixture of essential oil, hexane and water were collected and dried over anhydrous sodium sulphate for a few hours leaving only a mixture of oil and hexane. The hexane was then separated from the oil using an evaporation method inside the fume hood leaving only the essential oils. The oils obtained were stored in amber-coloured vials at 0 °C until required for further work. The following equation found the yield of the extracted essential oils:

$$\text{Yield}\left(\%,\frac{\text{w}}{\text{w}}\right)=\frac{\text{Mass of extracted essential oil}}{\text{Mass of fresh leaves }\times(1-\text{water content})}\times 100$$

Characterisation and identification of the components of essential oils were performed through gas chromatography (GC). GC analysis was performed on Perkin Elmer Gas Chromatography Clarus 690 coupled with Perkin Elmer Mass Spectrometer Clarus SQ 8 T equipped with silica capillary column COL-Elite 5-MS (30 m × 0.25 mm id; film thickness 0. 25µm; Perkin Elmer). The temperature of the injector and detector were 250 ° and 280 °C, respectively. Nitrogen gas acts as a carrier, was applied at 1 mL/min flow rate, and in the split mode, 0.1 µl of essential oil samples diluted in acetone were injected. The oven temperature was programmed from 60 °C to 210 °C with a four °C/min gradient and isothermally kept for 8.5 min. A split 1:20 projection was maintained [14]. Mass spectra from the Wiley spectral library and the National Institute of Standards and Technology (NIST) were used to identify the components.

Results and Discussion

Extraction time and yield

Table 1 shows the overall data of P. amboinicus and M. cajupati fresh leaves extractions using MAE method that were carried out at different powers application of 200, 400 and 600 W with the temperatures for each power are determined as 60°, 120° and 180° C, respectively. It can be observed that as the power increased from 200 W to 600 W, the time for the first oil drop was also decreased, as shown in Figure 2(a). The longest time to produce the first oil drop of P. amboinicus was at ~ 30 min whilst for M. cajupati, the fastest first oil drop recorded was at ~18 min. As power increased, the first oil drops produced were faster i.e. less than 10 min and the volume of yield was also increased. Extraction time taken for M. cajupati at 200 W was the longest ~135 min, whilst P. amboinicus took 120 min and gradually decreased as the power increased, Figure 2(b); the increased in power application leads to rapid extraction process to occur.

Figure 2(c) shows the effect of power towards the yield of extraction; as the power increased [17], the higher yield of essential oils was obtained. The effective microwave powers in producing the highest yield for P. amboinicus and M. cajupati specimens were recorded at 200 and 400 W, respectively even though the highest temperature rise found was at 600 W. Furthermore, the extraction of essential oil performed at 600 W produced lower yield compared at 200 and 400 W. There is a material-specific element influencing the extraction process that could contribute to the 600 W power's inefficiency, which may not always produce the best or optimum yield. In this work, 200 W of microwave power produced the optimum yield of P. amboinicus specimens with 0.0347 %, whilst for M. cajupati, 0.275 % of yield was produced at 400 W. Nevertheless, we must take into consideration that the chosen of power application is vital since higher power may lead to burnt sample before the process of essential oil release occur. The prevalence of plant materials and essential oil components degrading may also be a factor in the decline in production at 600 W.

As for HD method, the essential oils were obtained at 24 h which consumes greater extraction time and energy. Commonly, the first droplet of essential oil using HD method recorded after 35 min compared to 5 min using MAE method. The extraction temperature for HD method was at the boiling point, 100°C for both plants and the extraction time was set at 6 h. The essential oil yield for P. amboinicus and M. cajupati were 0.05 % and 0.15%, respectively. Table 1 shows the comparison data for MAE and HD extraction methods for P. amboinicus and M. cajupati samples. Despite the differences in the volume of essential oils, the repellent compounds in both plants were found similar, as depicted in Table 2. The capacity of extraction method to release the bioactive compounds from the oil glands determine how many chemicals may be detected.

One of the important parameter that may affect the yield and the extraction time of essential oil is the power applied during the MAE extraction. It controls the amount of energy received by the plants which will then transformed into heat energy [17] that helps to release of essential oils from the plants. Power is also a driving force behind the breakdown of plant cell membranes, allowing the essential oil to seep out and dissolve in the solvent. [19]. The rise of microwave power could increase the depth of heat penetration as well as strengthen the molecular interaction between the electromagnetic field and samples which therefore, improve the extraction efficiency. However, the increase in microwave power may also contribute to the degradation of the target therefore, selection of the power application is significant to avoid elimination of the desired compounds.

Table 1. Comparison of extraction data for P. amboinicus and M. cajupati using MAE and HD methods.

Microwave Extraction Method (MAE)							
Plants	**Power (W)**	**T (°C)**	**1st Oil Drop (Min)**	**Extraction Time (Min)**	**Volume of Yield (ml)**	**Mass of EO (g)**	**Essential Oil Yield (%)**
P. amboinicus	200	60	30	120	0.2	0.1391	0.034775
	400	120	18	75	0.2	0.0666	0.01665
	600	180	9	60	0.3	0.0643	0.016075
M. cajupati	200	60	18	135	0.5	0.1209	0.030225
	400	120	8	105	1.5	1.1032	0.2758
	600	180	5	60	1.5	0.9358	0.23395
Conventional Hydrodistillation Method (HD)							
P. amboinicus	-	100	35	360	0.5	-	0.05
M. cajupati	-	100	35	360	1.5	-	0.15

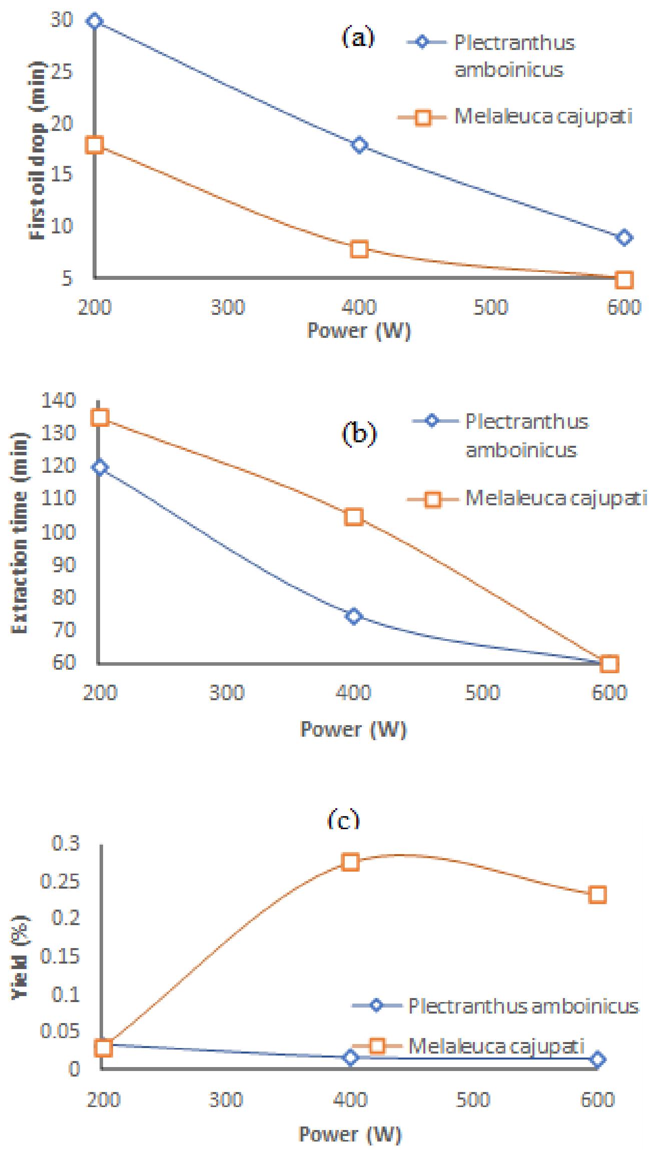

Fig. 2. Graphs of (a) first oil drops, (b) extraction time and (c) essential oils yield vs. different microwave power for P. amboinicus and M. cajupati extractions using MAE method.

Composition of essential oils

In this work, comparison of repellent compounds in both specimens extracted using HD and solvent-free MAE methods is presented. The chemical compositions of essential oils extracted from P. amboinicus and M. cajupati were characterised using GC-MS analysis to identify the bioactive compounds of the oil. As shown in Table 2, the preliminary results of chemical compositions in M. cajupati essential oils contains monoterpenes - linalool and limonene, with the main component is d-limonene whilst, essential oil in P. amboinicus contains sesquiterpenes – caryophyllene, with monoterpenes – thymol as the main component. Limonene [20], thymol and linalool [21], O-trifluoroacetyl-siopulegol [22], caryophyllene [5] and longiverbenone are reported among the several known compounds that possess mosquito repelling properties and can be found in other plants as well. The percentage of these repellent components varies depending on the type of extraction method and the power of microwave applied. HD method produced higher percentage for most of the components than the solvent-free MAE method, except for thymol in P. amboinicus where 60.73% is obtained at 600 W. Most of the components showed higher percentage at 600 W compared to 200 and 400 W, even though the effective microwave powers for P. amboinicus and M. cajupati were recorded at 200 and 400 W, respectively. Area percentage for all compounds using solvent-free MAE were lesser in all power applications, particularly for M. cajupati essential oil; the factor that may contribute to the decreasing percentage is the inappropriate specific parameters specified for M. cajupati specimen.

Table 2. List of repellent compounds and their percentage area for HD and MAE methods.

Types of plants	Repellent Compounds	Molecular formula	Area (%)			
			Hydro-Distillation (HD)	Microwave Assisted Extraction (MAE)		
				200W	400W	600W
M. cajupati	D-limonene	$C_{10}H_{16}$	9.89	1.11	1.55	1.1
	O-trifluoroacetyl-isopulegol	$C_{10}H_{17}O_2$	3.45	0.51	0.63	0.67
	Linalool	$C_{10}H_{18}O$	2.66	0.50	1.29	1.62
	Limonene	$C_{10}H_{16}$	3.45	0.51	0.63	0.67
P. amboinicus	Thymol	$C_{10}H_{14}O$	23.21	21.74	0.21	60.73
	Caryophyllene	$C_{15}H_{24}$	4.81	3.45	2.31	2.04

Conclusion

Extraction of P. amboinicus and M. cajupati essential oils have been carried out using HD and solvent-free MAE methods. In comparison to HD method, the presence of microwave power in MAE method contributes to the variation percentage of essential oil yield. The higher the microwave power application, the shorter the extraction time as well as the production of first oil drops. The effective microwave power that contributes to higher yield of essential oil from P. amboinicus and M. cajupati are at 200 and 400 W, respectively. The repellent compounds in P. amboinicus and M. cajupati have been identified which consists of monoterpenes and sisquiterpenes however, the percentage are found to be lower in MAE method compared to HD, particularly for M. cajupati which may indicate that M. cajupati essential oil may best extract using conventional HD method. Microwave technology is a viable alternative to conventional extraction techniques due to it rapidness, energy and time saving however, the process may also affect the structure of extracted molecules which may alter the characteristics of essential oils thus, affecting the quality and quantity of yield. Specific parameters such as temperature, time and irradiation power of the microwave need to be specified in every plant so that, the effective extraction can be performed.

Acknowledgements

We thank Universiti Pertahanan Nasional Malaysia for the facilities and financial support through the Short Term Research Grant (GPJP) no. UPNM/2021/GPJP/STG/3

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