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Comparison of Essential Oil of Lemongrass (Cymbopogon Citratus) Extracted with Microwave-Assisted Hydrodistillation (MAHD) and Conventional Hydrodistillation (HD) Method

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ABSTRACT

The increasing demand of essential oil has opened up wide opportunities for global marketing which leads to the requirement of its competitive product in market that comes with all the advantages in term of cost, quality and its production time. Microwave-assisted hydrodistillation (MAHD) method is an advance extraction technique that takes advantage of microwave heating with the conventional hydrodistillation (HD). This research was carried out to study the effect of different MAHD parameters which were water to plant material ratio (6:1, 8:1,10:1), microwave power (200 W,250W) and extraction time (30min,60min, 90min,120min) in extraction of essential oil from Lemongrass *(Cymbopogon Citratus)*. Its extraction yield and major constituents were analyzed and the results were compared with those of conventional HD. The optimum parameters were found at water to plant material ratio of 8:1, microwave power of 250W and 90 minutes of extraction and the yield obtained under this condition was 1.46%. The gas chromatography/mass spectrometric (GC-MS) analysis showed that the content of main constituents which were neral, geranial and myrcene were almost similar in the essential oil extracted using MAHD and conventional HD. This has proved that the use of microwave irradiation did not adversely influence the composition of essential oils. Overall, the results obtained indicate that MAHD method provided a good alternative for the extraction of essential oil from Lemongrass (*Cymbopogon Citratus*).

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INTRODUCTION

Essential oils are natural products obtained from plants. It is estimated that the global number of plants is of the order of 300,000 and about 10% of these contains essential oils and could be used as a source for their production (Husnu, K.C.B. and B. Gerhard, 2010). Their extracts are formed by combination of diverse and complex volatile mixtures of chemical compounds, with predominance of terpene associated to aldehydes, alcohols, and ketones that accumulated in various structure of the plant (Tajidin, N.E., 2012). In industry, the essential oils are typically extracted from fresh or partially dried leaves using numerous method of extraction. The most common extraction method will be hydrodistillation. Medicinal plants extracts are mainly applied in pharmaceuticals, food, cosmetics, and perfumery industry which is primarily to utilize their active substance benefits. The Asian continent with its diversity of climates appears to be the most vital producer of essential oils. China and India play a major role followed by Indonesia, Sri Lanka, and Vietnam with (Husnu, K.C.B. and B. Gerhard, 2010).

Lemongrass *(Cymbopogon Citratus)*, a perennial plant with long and thin leaves, is one of the largely cultivated medicinal plants for its essential oils in parts of tropical and subtropical areas of Asia, Africa and America (Chantal, S., 2012). It contains 1-2% of essential oil on dry basis (Carlson, L.H.C., 2001). The chemical composition of Lemongrass (*Cymbopogon Citratus*) essential oil is varying widely upon genetic diversity, habitat and agronomic treatment of the culture.The leaves of Lemongrass *(Cymbopogon Citratus)* present lemony characteristic flavor due to its main content, citral which present great importance to the industry. Citral, a combination of neral and geranial isomers, is used as a raw material for the production of

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ionone, vitamin A and beta-carotene (Carlson, L.H.C., 2001). There were a number of studies carried out to prove the anti-oxidant, anti-microbial and anti-fungal activities of Lemongrass *(Cymbopogon Citratus*) (Behboud, J., 2012; Nikos, G.T. and D.E. Costas, 2007).

The common methods to extract essential oil from medicinal plant, including for Lemongrass *(Cymbopogon Citratus)*, are hydrodistillation (HD), steam distillation, steam and water distillation, maceration, empyreumatic(or destructive) distillation and expression (Abderrahmane, D., 2013). It is proved through a number of studies (Abderrahmane, D., 2013; Ashgari, J., 2012) that the quality of essential oil mainly depends on its constituents which is primarily influenced by their extraction procedures. In contrast, these common methods can induce thermal degradation, hydrolysis and water solubililization of some fragrance constituents. . In addition, the oil obtained through solvent aided extraction contains residues that pollute the foods fragrances to which they are added. As a means to overcome this sort of drawbacks, an advance and improved method such as microwave-assisted extraction (Hong, W.W., 2010), ohmic-assisted hydrodistillation (Mohsen, G., 2012), subcritical water extraction and ultrasound-assisted extraction (Porto, C.D. and D. Decorti, 2009) have been applied to shorten extraction time, improve the extraction yield and reduce the operational costs.

Recently, microwave-assisted hydrodistillaton (MAHD) procedures for isolating essential oils have become attractive for use in laboratories and industry due to its effective heating, fast energy transfer and also an environmental friendly extraction technique. Its acceptance as potential and powerful alternative for conventional extraction techniques has been proved through several research (Abderrahmane, D., 2013; Ashgari, J., 2012; Mohammad, T.G. and R. Karamatollah, 2008). However, there are no reports of the simultaneous comparison of MAHD and HD extraction procedures on extraction of essential oil from Lemongrass (*Cymbopogon Citratus*) . Therefore, the aim of the present study was to investigate the applicability of microwave-assisted hydrodistillation(MAHD) technique as an alternative to conventional hydrodistillation (HD) in isolation of Lemongrass (*Cymbopogon Citratus*) extracts based on the extraction yield and constituents of oils obtained under optimized condition. The effect of operational parameter such as extraction time and water to raw material ratio were evaluated to identify its optimum condition for extraction and this applicability was appreciated by using the result of subsequent GC/MS analysis.

MATERIALS AND METHOD

Plant Samples:

Lemongrass (*Cymbopogon Citratus*) leaves were collected from home garden in northwest of Malaysia. The plant sample was freshly cut, 10cm from the root, in the morning of the day they were collected. According to Edwin et al., 2012, for Lemongrass (*Cymbopogon Citratus*) , the percentage essential oil yield for the partially dried leaves was found to be higher than that of the fresh leaves. Thus, once collected, the plant material were dried at room temperature for a week then kept in a sealed plastic bag at ambient temperature and protected from the light. The samples were ground using a kitchen grinder (Super Blender,Panasonic,Tokyo, Japan) at room temperature prior to extraction. Whereas, extraction yield increase by decreasing the particle size due to the higher amount of oil released as the leave cells are destroyed by milling (Guan et al., 2006). In order to improve the collection efficiency, the plant material was soaked in its distilled water for 30min before the extraction performed.

Reagents:

Anhydrous sodium sulfate and n-hexane used was analytical grade reagent purchased from Sigma Aldrich (US) and Fisher Scientific (US), respectively. Deionised water used was purified by Milli-Q purification system (Millipore) (Massachusetts,USA).

Microwave-Assisted Hydrodistillation

A modified domestic microwave oven model Samsung MW71E connected to the Clevenger apparatus was modified for MAHD operation. The Samsung MW71E has 1150 Watt power consumption, 800 Watt output power with 250v-50Hz power soure; 2450MHz. The cavity dimensions of the microwave oven were 306 x 211 x 320mm. The microwave-assisted hydrodistillation were conducted at water to raw material ratio of 6:1, 8:1 and 10:1 at 200 and 250W microwave power for durations of 30, 60, 90 and 120 min. The flask containing 50 g of Lemongrass (*Cymbopogon Citratus*) with its distilled water was placed within the microwave oven cavity. A condenser which has been set on the top, outside the oven, was used to collect the extracted essential oils. The essential was decanted from the condenser with 30min interval for a period of 2H since this period was sufficient enough to extract essential oil from the sample through MAHD (Mohammad, H.E., 2007).

Hydrodistillation:

50g of fresh Lemongrass (*Cymbopogon Citratus*) leaves were placed in a 1L flask containing 400ml of distilled water and hydrodistilled for 3h using a Clevenger-type apparatus. The essential were decanted from the condensate in 30 min interval. The system was operated at a fixed power of 500W and under atmospheric pressure (Liu, Y., 2012).

Analysis of Sample:

The collected extracts were dried over anhydrous sodium sulfate to remove the water. Then, the oil was being weighed and stored in vial at +4^{o}C prior to analysis. The yield obtained from the extraction was analyzed to evaluate the performance of MAHD in Lemongrass (*Cymbopogon Citratus*) oil extraction. Yield of oil that obtained for every run was calculated by using Equation (1):

$$Yield\ of\ essential\ oil = \frac{amount\ of\ essential\ oil\ (g)\ obtained}{amount\ of\ raw\ materials\ (g) used} \quad (1)$$

A GC-MS instrument (5973N, Agilent Technologies, Wilmington, DE, UAS) equipped with a mass selective detector operating in the electron impact mode (70eV) was used to study the composition of the essential oil at extracted various group of parameter condition to analyze its quality. The GC part (6890N, Agilent Technologies, Palo Alto, CA, USA) was equipped with an HP-5MS (Agilent BTechnologies) capillary column (30 m long, 0.25 mm id and 0.25 lm film thickness). Temperature-programming of the oven included an initial hold at 50 °C for 5 min and a rise to 240 °C at 3 °C min-1 followed by additional rise to 300 °C at 5 °C min-1. A final hold for 3 min was allowed for a complete column clean-up. The injector was set at 280 °C. The samples were diluted with *n*-hexane (1/10, v/v) and a volume of 1.0 µl was injected to the GC with the injector in the split mode (split ratio: 1/10). Carrier gas, He, was adjusted to a linear velocity of 1 ml min-1. The compounds of the extracted essential oils were identified by comparing their mass spectral fragmentation patterns with those of similar compounds from a database (Wiley/NBS library) or with published mass spectra. The components were quantified based on the comparison of compound's retention period, which were similar in both techniques. The normalization method was used; the value of total peak areas is considered 100% and the percentage of each component was calculated using the area of each pea

RESULT AND DISCUSSION

Optimization of MAHD method:

Quality and quantity of oil essential mainly depends on the extraction procedures. Therefore, optimizing extraction procedure is considered as a vital process. In current research, the following parameters were studied to identify the optimum operating condition of MAHD in extraction of essential oil from Lemongrass (*Cymbopogon Citratus*) : water to raw material ratio, 6:1, 8:1 and 10:1; microwave power, 200 W and 250 W; extraction time, 30 min, 60 min, 90 min, and 120 min.

Effect of water to plant material ratio – Fig.1A shows that the extracted yield at three different water to plant material ratio at 90 min of extraction and under 250 W microwave power. By decreasing the water to plant material ratio from 10:1 to 8:1, the extracted oil yield increased from 0.89% to 1.46%. However, the extracted oil yield decreased to 1.27% when the ratio is reduced to 6:1. Thus, ratio 8:1 is the selected as the optimum ratio for the extraction as it's gave the maximum yield. The effect of water to plant material ratio needs crucial consideration to achieve maximum extraction of yield during scaling up or down the sample preparation method. The small volume of water in the system failed to withstand at high microwave intensity for a longer extraction time and target extraction incomplete. While, presence of excess amount of water can cause excess thermal stress due to rapid heating of the solution on account of effective absorption of microwaves by water.

Effect of Microwave Power – Microwave power is one of the factors that influence the efficiency of MAHD extraction because the microwave energy significantly affects molecular interactions between the target compounds and others. Two microwave power levels were used in the extraction and their results were shown in Fig. 1B (at water to raw material ratio 8:1 and 90 min of extraction time). The extraction was improved by raising the microwave power from 200W to 250W with 1.21% and 1.46%, respectively. This is doubtlessly due to the rapid generation of heat inside the immersed Lemongrass (*Cymbopogon Citratus*) with the absorption of microwave energy and the subsequent formation of a higher pressure gradient inside the plant material when subjected to higher microwave power levels. Based on these results, 250W was chosen as the optimum microwave power. The obtained result is in accordance with Ashgari finding on effect of microwave power on extraction of *Echinophora platyloba DC* using MAHD.

Effect of Extraction Time – Fig. 1C shows the influence of microwave power on the extraction yields of Lemongrass (*Cymbopogon Citratus*) over the range 30-120 min under a fix microwave power of 250W and water to raw material ratio of 8:1. From the graph, the amount of yield does not change significantly after 90 minutes where the yield obtained up to this period was 1.46%. The rate of extraction was high at the beginning of the extraction but get slow gradually by time. The effect of this parameter in MAHD has been studied by number researches on various plant materials (Muhammad, H.H., 2012; Ashgari, J., 2012), and almost all of them obtained the same configuration as illustrated in Fig. 2C. These results confirmed the Fick's second law of diffusion which stated about the final equilibrium achieved by the solute concentrations in plant matrix and in the solvent after a certain time. This cause into no significant improvement in oil yield when prolonging the extraction time.

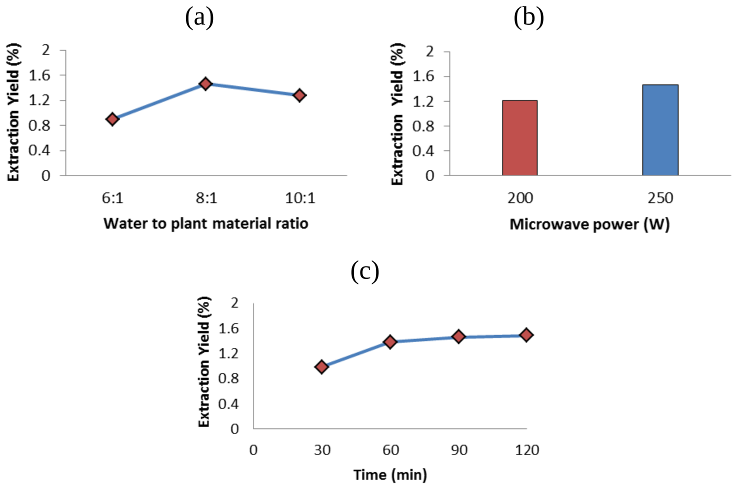

Fig. 1: Effect of (a) water to raw material ratio, (b) microwave power and (c) extraction time using MAHD on the extraction yield of essential oil from Lemongrass (Cymbopogon Citratus) .

Comparison of Lemongrass (Cymbopogon Citratus) oil obtained by Conventional Hydrodistillation method:

To evaluate the effect of microwave in the extraction of essential oil, the results of MAHD, at optimal conditions, were compared with those of conventional hydrodistillation (HD) in term of the oil yield and its constituents. HD is an accepted method that is used as reference for the quantification of essential oils.

The induction time for MAHD was only 18 minutes whereas for HD it was 30min. This show, 30% of the total oil can be extracted using MAHD by the time the extraction of essential oil by HD started. It is interesting to note that, the amount of yield extracted by MAHD after 60 min will similar as the oil resulted after 180 min by HD. At 90 min of extraction, the oil yield by MAHD and HD method were 1.46% and 0.98%, respectively. This momentous heating rate is due to the microwaves which deliver bulk heating within the system. These create an instant and high rate heating platform. All this results indicated a substantial saving in time, energy and as well as its cost.

GC-MS analyses were performed with the aim to compare the compositions of the Lemongrass (*Cymbopogon Citratus*) oil extracted by MAHD and HD. The comparison of major compounds, neral, geranial and myrcene, are shown in Table 1. Citral ,the combination of the neral and geranial isomers, is the key compound to evaluate the quality of Lemongrass (*Cymbopogon Citratus*) oil. From the results, it was observed that concentration of key compound found in the Lemongrass (*Cymbopogon Citratus*) oil were almost similar for both methods. Taking into account the comparison studies of extraction method between MAHD and HD by other researchers, it seems the constituents and their concentrations obtained by both of method were almost simila (Liu, Y., 2012; Djouahri, 2013). All these results have proved that microwave greatly accelerated the extraction process, but without causing significant affect in the volatile oil composition.

Table 1: Composition of Lemongrass (*Cymbopogon Citratus*) oil obtained by MAHD and HD.

Compound	Relative peak area (%)	
	MAHD	HD
Myrcene	3.96	9.91
Neral [a]	35.67	35.69
Geranial[b]	50.81	49.46
Citral[a+b]	86.48	85.15

Conclusion:

In this paper, microwave-assisted hydrodistillation method was optimized based on the three tested parameters namely water to plant material ratio, microwave power and extraction time. Then, the results were compared with essential oil obtained by conventional hydrodistillationy. MAHD offer great advantages over conventional HD. MAHD require shorter extraction time (90 min vs 180 min, respectively). The extraction of the essential oil from Lemongrass (*Cymbopogon Citratus*) by MAHD under this optimized conditions provided

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additional benefits of microwave irradiation in isolation of essential oils. In addition, GC-MS results proved that there were no significant difference between the constituents of essential obtained by MAHD and those obtained by conventional HD. Due to the substantial saving of time, cost and energy with no significant changes in its constituents, MAHD process is a good alternative in the extraction processes of essential oil from Lemongrass (*Cymbopogon Citratus*). The result obtained from this study encourage applying the MAHD for the extraction of the essential oil of some other plant materials.

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