

## **Comparison of Yield and Chemical Composition of Essential Oils from Lemongrass (*Cymbopogon Citratus*) Extracted with Microwave-Assisted Hydrodistillation (MAHD) and Conventional Hydrodistillation (HD) Methods**

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### **ABSTRACT**

The demand of essential oil in current industry has increased due to its bioactive compound that shows various therapeutic effects. Therefore, the qualities of essential oil mainly depend on its constituents. This study was carried out to determine the effects of Microwave-assisted hydrodistillation (MAHD) and hydrodistillation (HD) methods on yield, chemical composition and toxicity of essential oil from Lemongrass (*Cymbopogon citratus*). A pale yellow oil yield of 1.46 % (90 min) and 1.38% (180 min) were obtained from MAHD and HD methods, respectively. GC-MS results shows a total of twenty compounds in MAHD oil and seven compound in HD oil. MAHD oil contained slightly higher amount of citral, which is the key component of Lemongrass (*Cymbopogon citratus*), compared to HD oil, 86.48% and 83.15%, respectively. However, hydrodistilled oil showed the presence of higher  $\beta$ -Myrcene, 9.91%, and only 3.96% were found in the MAHD oil. Other identified constituents, include Linalool, 1,6-Octadiene, 3,5-dimethyl-, trans, Pinane, Geranic acid and 1,5-Octadiene. A minor variation in the total chemical composition of essential oil may contribute for different level of biological activity expression.

**Key Words:** essential oil, hydrodistillation, Lemongrass, Microwave-assisted hydrodistillation,

## INTRODUCTION

Essential oils are botanical extracts of various plant materials, and do not only originate from flowers, but from herbs, trees and various other plant material. 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 [1]. Their application in pharmaceutical and food industry gain growing attention due its biological activity, antioxidant, antibacterial and antifungal properties. Physically, essential oils are made up primarily of the elements; carbon, hydrogen and oxygen, and these primary elements form a wide variety of compounds that fall basically into two family groups which are hydrocarbons and oxygenated compounds. However, within essential oil chemistry, each functional family of compounds is known to have specific therapeutic effects.

Lemongrass (*Cymbopogon citratus*), a perennial plant with long, 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 [2]. It contains 1-2% of essential oil on dry basis [3] and the chemical composition of Lemongrass (*Cymbopogon citratus*) essential oil is varying widely upon genetic diversity, habitat and agronomic treatment of the culture [4]. 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 ionone, vitamin A and beta-carotene [3]. It possesses a high cytotoxic activity [5]. There were a number of studies carried out to prove the anti-oxidant, anti-microbial and anti-fungal activities of Lemongrass (*Cymbopogon citratus*) [6], [7], [8]. In industry lemongrass (*Cymbopogon citratus*) oil has many other uses, such as perfume in soaps, flavoring agent for tea and medicine to treat various health ailments, including acne, athlete's foot, flatulence, muscle aches and scabies [9].

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 distillation and expression [10]. Previous researches, [10], [11], [12], had proved the quality of essential oil mainly depends on their constituent which is primarily influenced by their extraction procedures. In contrast, these common methods can induce thermal degradation, hydrolysis and water solubilization of some fragrance constituents. According to [13], steam distillation caused susceptible chemical changes on monoterpenes compound. In addition, the oil obtained through solvent aided extraction contains residues that pollute the foods fragrances to which they are added and also leads to losses of more volatile compound during the removal of its solvent.

Recently, microwave-assisted hydrodistillation (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 [1], [4], [14]. Although, several studies has reported the benefits of MAHD over HD on several plant, but there are no reports of the simultaneous comparison of MAHD and HD extraction procedures on extraction of essential oil from Lemongrass (*Cymbopogon citratus*). Besides, earlier studies were mostly focused on the comparison of the yield through advance and conventional extraction method. For gain the clear knowledge of a method's efficiency, it is important to carry out the constituents comparison of the extracts so that their real clinical benefits can be studied. Therefore, the present investigation was designed to evaluate the yield and chemical composition of the essential extracted from MAHD and HD method. GC-MS was used for the analysis of chemical composition.

## **MATERIALS AND METHOD**

### **A. Plant Materials**

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 [7], 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. This is because, extraction yield increase by decreasing the particle size due to the higher amount of oil released as the leave cells are destroyed by milling. In order to improve the collection efficiency, the plant material was soaked in its distilled water for 30min before the extraction performed.

### **B. Extraction of Essential Oil**

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 source; 2450MHz. The cavity dimensions of the microwave oven were 306 x 211 x 320mm. 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 its condenser after 90 minutes since this period was sufficient enough to achieve exhaustive extraction of its essential oil through MAHD [14].

For hydrodistillation (HD), 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 [11].

### C. Analysis of Sample

To remove the water, the decanted essential oil were dried over anhydrous sodium sulfate, weighed and stored in vial at 40°C prior to analysis. The amount of 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):

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

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 μm 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<sup>-1</sup> followed by additional rise to 300 °C at 5 °C min<sup>-1</sup>. 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<sup>-1</sup> [12]. 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 (Massada 1976; Adams, 2001). 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 peak.

## RESULTS AND DISCUSSION

Current research is the first report on the simultaneous comparison of MAHD and HD effect on yield and chemical composition of Lemongrass (*Cymbopogon citratus*) oil. This comparison has been supported by the result of GC-MS analysis

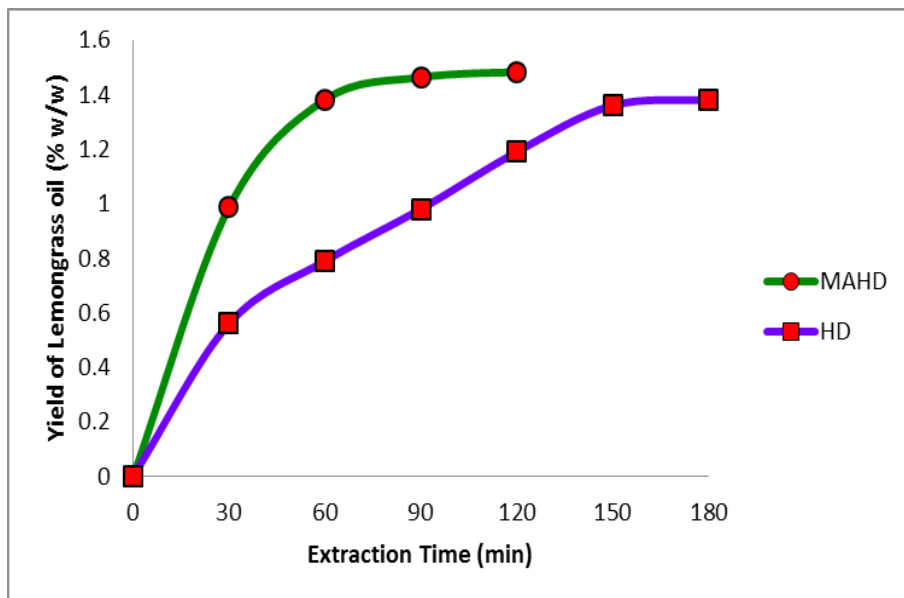
which provides the quantitative and qualitative information of Lemongrass (*Cymbopogon citratus*) oil content. HD is an accepted method that is used as reference for the quantification of essential oils [12].

The MAHD and HD of Lemongrass (*Cymbopogon citratus*) gave a pale yellow essential oil with yield of 1.46% and 1.38%, respectively. 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 was similar to the resulted oil after 180 min by HD. At 90 min of extraction, which was the optimum extraction time in MAHD extraction, the oil yield by MAHD and HD method were 1.46% and 0.98% respectively. The ultimate yield of essential oil by MAHD after 2 hr of extraction was higher than that obtained by HD after 3 hr of extraction (1.48% vs. 1.38%, w/w). These outcomes prove a substantial savings in time. The same configuration of results was also obtained by previous researchers in the activity of comparing effect of MAHD and HD on different oil bearing plants [15], [16], [17].

Table 1 lists the composition of Lemongrass (*Cymbopogon citratus*) oil obtained by MAHD and HD methods, at optimized condition, based on the results of GC-MS. It shows that twenty compounds were found for MAHD and seven compounds for HD. Both of the oils were characterized by monoterpenoids with oxygenated monoterpene which is isomers of citral dominating their compositions [18], [19], [8]. Citral is the key compound to evaluate the quality of Lemongrass (*Cymbopogon citratus*) oil [8]. In the comparison between MAHD and HD, the citral (combination of neral and geranial) percentage in the measured compounds was higher in extraction obtained by MAHD (86.48%) than HD (83.15%). The concentration of geranic acid and  $\beta$ -myrcene was the next highest to citral (combination of neral and geranial) of the measured compounds in MAHD and HD respectively. The percentage of flavour compounds recovery depends on the method [20]. The essential oil obtained by MAHD method contained more oxygenated compound including the 5 marker compounds. This can be explained when one considers that MAHD reduces the extraction time, allowing faster extraction with smaller amount of water. These lead to the minimisation of degradation in compound by hydrolysis, transesterification or oxidation. Another explanation may be found in dielectric properties of essential oil compounds and their interactions, as a material, with electromagnetic waves. Essential oil is composed of a variety of compound divided into two groups which are hydrocarbon and oxygenated compounds. It would be reasonable to believe that for the more polar compounds, the more readily the microwave irradiation is absorbed, the better the interaction between the electromagnetic wave and matters is established, and the more polar aromatic components are obtained. This would appear to correspond well to what has been observed in the case of Geranial (polar compound) and  $\beta$ -myrcene (non-polar compound) which presented opposite concentrations in MAHD

and HD respectively. It is worth noting that oxygenated compounds are highly odoriferous hence the most valuable components in the essential oils. Instead, monoterpene hydrocarbons, which are present in lower amounts in MAHD extract, are less valuable owing to their limited contribution to the fragrance of the essential oil. Taking into account the comparison studies of extraction method between MAHD and HD by other researchers on different plants [11], [12], [13], it seems the constituents and their concentrations obtained by both methods were almost similar. All these results have proven that microwave greatly accelerated the extraction process, but without causing significant effect in the volatile oil composition, and even produce essential oil with more preferable quality. [18], province on the same oil by Clevenger found the similar major components but in different percentage as well as dissimilarity in its minor components. This variability may due to the different climatic and soil growing conditions [25].

**Fig.1 : Effect of extraction method and extraction time on Lemongrass (*Cymbopogon citratus*) oil yield**



**Table 1: The Chemical Compositions of Lemongrass (*Cymbopogon citratus*) Oil Extracted by MAHD and HD Methods**

No.	Compound	Retention Time (min)	Percentage Composition (%)	
			MAHD	HD
1	<u><math>\beta</math>-Myrcene</u>	3.82	3.96	9.91
2	<u>Linalool</u>	6.04	0.48	-
3	<u>3,5-Dimethyl-2-cyclohexen-1-one</u>	6.43	0.17	-
4	<u>1,6-Octadiene, 3,5-dimethyl-, trans</u>	7.08	0.95	1.01
5	<u>Pinane</u>	7.65	1.77	-
6	<u>Cyclohexane</u>	8.18	0.14	1.48
7	<u>Neral</u>	10.13	35.67	33.69
8	<u>Geranial</u>	11.22	50.81	49.46
9	<u>Furane</u>	13.10	0.17	-
10	<u>1,5-Octadiene</u>	13.57	0.40	-
11	<u>Geranic acid</u>	15.05	1.33	-
12	<u>Nerolidol</u>	17.16	0.48	-
13	<u>2-Norpinene</u>	17.94	0.51	-
14	<u>2-Ethylcyclohexanone</u>	19.73	0.22	-
15	<u>Caryophyllene oxide</u>	22.71	0.24	-
16	<u>Selina-6-en-4-ol</u>	24.06	0.35	-
17	<u>1,19-Eicosadiene</u>	34.23	0.13	-
18	<u>Palmitic acid</u>	35.09	0.24	-
19	<u>Elaidic acid</u>	40.34	0.42	-
20	<u>R-citronellol</u>	42.98	0.50	-
21	<u>4-Tetra decyne</u>	49.87	-	0.18
22	<u>Olealdehyde</u>	50.10	-	0.30

## CONCLUSIONS

In current study, the advantage of MAHD over HD in extraction of essential from Lemongrass (*Cymbopogon citratus*) has been studied where by using MAHD shorter extraction time was achieved. In addition, GC-MS results proved that MAHD yield oil with higher main abundant compounds. 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 with the combination of various biological activity studies.

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