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Effect of Pretreatment on Extractions of Essential Oil from Kaffir Lime (*Citrus Hysteric DC.*) Leaves

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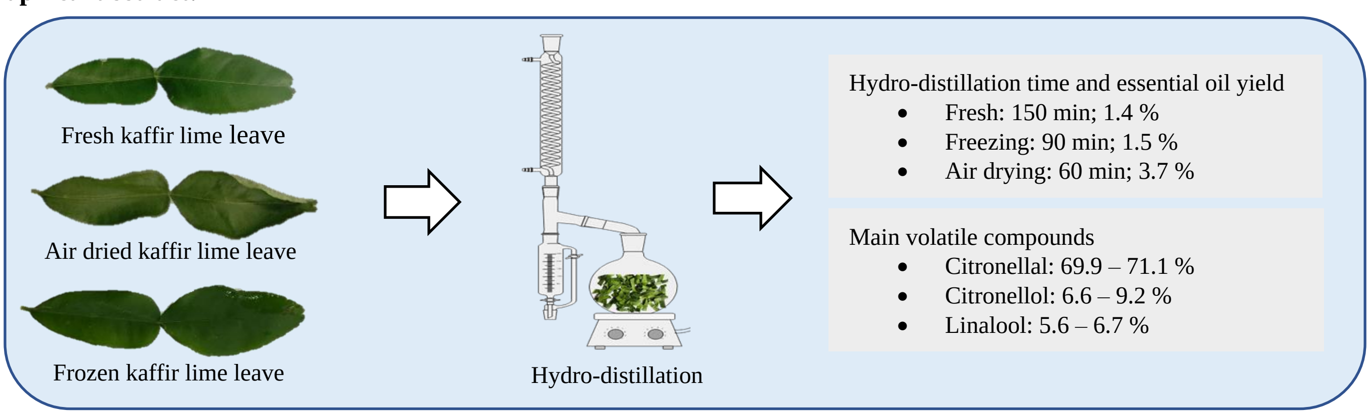
Abstract: *Pretreatment is an essential technique for enhancing the efficiency of extraction. Among many types of pretreatment processes, freezing and drying have been recognized as the thermophysical pretreatment techniques that can be applied to many solid samples before being subjected to extractions. However, the effectiveness of the pretreatment conditions highly dependent on the type of raw materials. Therefore, this study aimed to investigate the effect of pretreatments on the extraction of essential oil through hydro-distillation. Kaffir lime leaves was selected as the studied raw material because it is cheap and popularly used as an ingredient in various products. Pretreatment conditions such as air drying and freezing of the kaffiir lime leave before being subjected to hydro-distillation of essential oil was conducted in this study. Compared to fresh samples, it is found that freezing and air drying of kaffir lime leaves resulted in different essential oil accumulation rates, extraction yields of essential oil, and extracted volatile compounds. The dried sample provided the highest essential oil yield (3.7 %) with a shorter extraction time of about 60 min compared to the frozen sample (1.5 % of essential oil yield and about 90 min of extraction time). The fresh sample resulted in the lowest essential oils yield (1.4 %) with a longer extraction time of about 150 min. Furthermore, (GC-MS) results revealed that the main volatile compounds in essential oils extracted from kaffir lime leave are citronellal (69.9 – 71.1 %), citronellol (6.6 – 9.2 %), and linalool (5.6 – 6.7 %). The contents of volatile compounds in essential oils are affected by pretreatment techniques.. This finding can be very useful data for the extraction of essential oil from Kaffir lime leaves with better quality and yield by hydro-distillation.*

Keywords: Pretreatment, Extraction, Essential oil, Kaffir lime leave, Volatile compounds, Accumulation rate

Graphical abstract:



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1. INTRODUCTION

Citrus hystrix which is known as Kaffir lime, contains essential oils mainly in its leaves, fruits, and seeds, compared to other parts [1]. The kaffir lime leaves are normally used as a preservative in pharmaceutical products, aromatherapy, cosmetic products, and herbal drink [2]. In general, essential oils (EOs) are complex blends of aromatic molecules covering monoterpene hydrocarbons, sesquiterpenes, and their derivatives such as esters, alcohols, and aldehydes [3]. In the case of bioactive compounds, they are the main class of secondary metabolites of plants, including phenolic acids, flavonoids (flavonols, anthocyanins), tannins, lignans, and stilbenes. Both essential oils and bioactive compounds exhibit biological properties which can be functioned as anti-virus, antioxidant, antimicrobial, anti-inflammatory, anti-aging, and so on. Therefore, extraction of either essential oils or bioactive compounds from natural products is continuously studied at either laboratory or industry scale.

Extraction of essential oils and bioactive compounds from biomass products can be performed through various methods including hydro-distillation, steam distillation, cold-press, and other modern methods assisted by microwave, ultrasound [4], and supercritical carbon dioxide extraction methods [5]. Nevertheless, modern methods often required expensive costs for the installation [6], and were not easy to implement [7]. Among the mentioned methods, hydro-distillation is a common and traditional method that is widely used in terms of essential oil extraction from plant material. Hydro-distillation prevents the plant sample from clumping and compacting during the extraction process, but the extraction time takes a long time [8]. In this case, it causes chemical alternations of the molecules as they direct contact with boiling water, and some of the polar molecules present with relatively higher solubility in water will be lost in the distillate water [6]. However, in general, to complete the extraction, the accumulation of essential oils should have reached equilibrium. Therefore, it is necessary to study the effect of hydro-distillation time on the accumulation of essential oil during the extraction to determine the optimal duration for conducting the extraction.

In addition, the traditional extraction method is applied at high temperatures with a long extraction time. Therefore, it leads to a decrease in the quantity and quality of obtained extract [9]. Many pretreatment methods have been gradually developed in the last decade to maximize the yield of the extract by decreasing extraction time. The freezing and drying methods are common methods used to preserve herbs and aromatic plants. Drying is used to preserve food by reducing its moisture content [10] and increasing the mass transition coefficient owing to the porous structure plant created during drying, and speeding up essential oil free outside plant membranes [11]. Whereas, freezing is a method used to increase the solid content in forming mobilizing water into ice [12]. The formed ice can damage the structure of organs leading to enhance leaching rate of oils or other compounds from solid materials [13].

Therefore, the purposes of this current study aimed to determine the effect of pretreatment by freezing and drying on essential oil accumulation rate, essential oil yield, and the volatile composition of kaffir lime leaves.

2. METHODOLOGY

2.1. Chemicals

All chemicals used in this study were analytical grades. All needed reagents were gallic acid standard (Himedia, India), Quercetin standard and Folin-ciocalteau (Sigma Aldrich, Switzerland), ethanol, sodium hydroxide (Merck, Germany), sodium nitrite (Merck, Denmark), and aluminum chloride (Acros, Germany).

2.2. Sample collection and preparation

Kaffir lime leaves were collected from the local farm located in Svay Rieng province, Cambodia, in October 2021. The sample was cleaned and subjected to various pretreatment conditions as given in Table 1. The prepared samples obtained from each condition used for the extraction of essential oil are given in Fig 1. In this work, the moisture content of the sample after each pretreatment was also checked.

Table 1 Pretreatment conditions of kaffir lime leave for essential oil extraction

Pretreatment method	Pretreatment condition
Fresh	No pretreatment
Air drying	Dry at room temperature for 4 days
Freezing	Freeze at – 20 ºC for 24 h

2.3. Extraction of essential oil

In this study, hydro-distillation was applied for essential oil extraction from the kaffir lime leaves. Fig. 2 gives the image of an actual hydro-distillation system used in this study for the extraction of essential oil from kaffir lime leaves. The hydro-distillation tank contained 150 g of each raw material and 150 ml of distilled water. The mixture of sample and water was connected to the heater, then the oil receiver was connected to the extractor and cooling system (condenser). The extraction of essential oils was conducted at a temperature of water lower than 100 ºC. The essential oil accumulation has been performed by recording the volume of oil every 0.1 ml accumulated in the receiver with time. The collected essential oil was weighed and used for the calculation of essential oil (E.O) yield following Eq. 1. The essential oil yield is expressed as a percentage of dry basis.

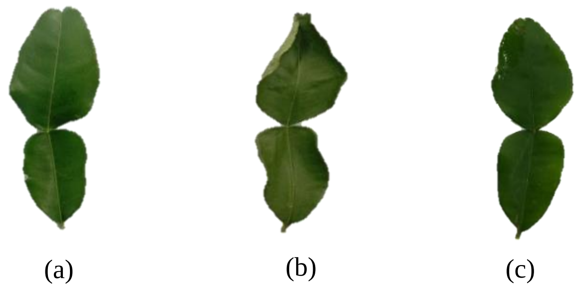

Fig. 1. Kaffir lime leaves obtained from different pretreatment condition, (a) Fresh kaffir lime leave, (b) Air dried kaffir lime leave, (c) Frozen kaffir lime leave

$$\text{E.O yield (\%)} = \frac{\text{Mass E.O (g)} \times 100}{\text{Mass sample (g)} \times \left(1 - \frac{\text{Moisture (\%)}}{100}\right)} \quad \text{(Eq. 1)}$$

Fig. 2. Hydro-distillation system used in this study

2.4. Gas chromatography mass spectrophotometry

Gas Chromatography-Mass Spectrophotometry *(*GC-MS) was applied to identify the specific volatile molecules of kaffir limes leave obtained from the hydro distillation. The GC-MS condition was followed by the study of Mohamed et al. [14]. Briefly, a mass of 10 mg essential oil was mixed with 1.0 ml of n-hexane. In addition, GC-MS analysis was performed using the HP5-MS column with 9.3 psi of head column pressure. Helium was used as a gas carrier with a flow rate of 1 ml/min, and a split ratio of 1:20. The oven temperature was held at 50 °C for 2 min and then raised to 240 °C at a rate of 8 °C /min. Flame ionization detector (FID) temperature was 280 °C and injector temperature was 240 °C; Helium was used as carrier gas with a linear velocity of 30 ml/min. The percentages of compounds were calculated by the area normalization method [15].

3. RESULTS AND DISCUSSION

3.1. Accumulation of essential oil during hydro-distillation

Fig. 3 depicted the result of kaffir lime leaves of essential oils accumulation rate obtained from hydro-distillation under different pretreatment conditions. The result expressed that the pretreatment techniques largely affected the accumulation of essential oil during the hydro-distillation.

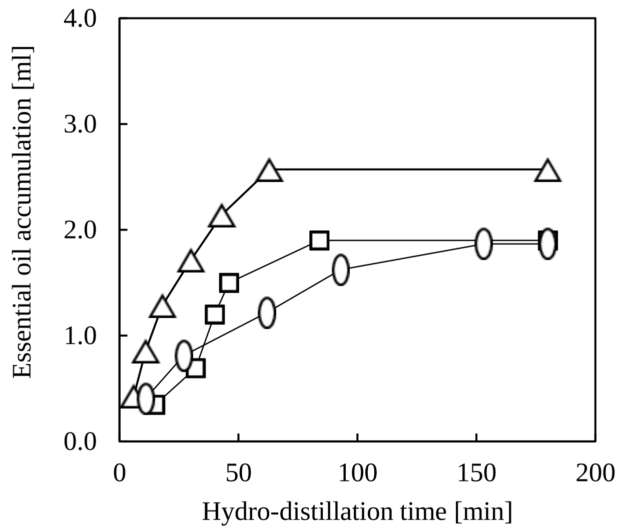

Fig. 3. Accumulation rate of essential oil during hydro-distillation of kaffir lime leave, (Δ) Air dried sample, (□) Frozen dried sample, and (0) Fresh sample

As seen in Fig. 3, using air-dried kaffir lime leave resulted in the extraction time of about 60 min to reach the equilibrium stage. Interestingly, the extraction time of essential oil to reach equilibrium for the dried sample is shorter than that resulting from fresh and frozen dried samples. This result is probably because of the different moisture contents of each sample. In this work, the moisture contents of each sample were found to be 56.8, 56.8, and 43.7 % for fresh, frozen, and air-dried samples, respectively. It seems that the accumulation rate of air-dried sample was faster and higher due to the lower moisture content of the sample. In addition to moisture content, pretreatment techniques generally can affect the microstructure of the sample [16]. Freezing of sample can soften the microstructure of solid sample which is commonly possible to enhance the accumulation rate of extraction compared to the fresh sample [17]. For kaffir lime leave, air drying probably can also modify the microstructure of the sample, however, checking the microstructure should be needed to confirm the change of microstructure of kaffir lime leave after the air drying.

3.2. Yield of essential oil

In this study, the yield of essential oil obtained from different pretreatment conditions was calculated based on a dried sample. As given in Fig. 4, the essential oil yield obtained from air-dried kaffir lime leave (3.7 ± 0.12%) is higher than that obtained from fresh (1.4 ± 0.05%) and frozen (1.5 ± 0.05%) samples. This result indicated that pretreatment by air drying of kaffir lime leave can be considered as a highly efficient method to enhance the extraction yield of essential oil through hydro-distillation. Air-dried kaffir lime leave provided a high yield of essential oil within a short equilibrium time because a porous structure of the surface plant was created while drying, leading to an increase of mass transition coefficient and speeding up the solutes like essential oil to be released from the plant matrix [11]. In the hydro-distillation process, a shorter extraction time is necessary since the sample is directly contacted with hot water which leads to losing some soluble volatile compounds into boiling water or evaporating to the atmosphere [7]. In addition, the presence of esters in an essential oil would be transformed into acid and alcohols while it exposed to a higher temperature for a long time, thereby resulting in low quality of the essential oils [3].

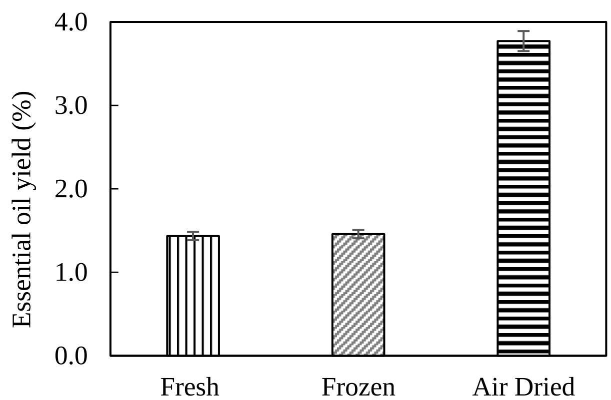

Fig. 4. Effect of pretreatment conditions on yield of essential oils extracted from kaffir lime leave

3.3. Volatile compounds of essential oils

In this study, volatile compounds of essential oils obtained from each sample were analyzed by GC-MS, to observe the effect of pretreatment conditions on the extraction of volatile compounds. Table 2 illustrates the volatile compounds found in kaffir lime leaves obtained from different samples using the hydro-distillation. The result from GC-MS expressed that 11 volatile compounds were presented in essential oils obtained fromfresh and air-dried kaffir lime leaves, whereas 12 volatile compounds existed in a frozen sample. However, the specific compounds present in the essential oils are not all the same. For example, citronellal, citronellol, and linalool compounds of essential oil decreased their percentage amount in the pretreatment samples (frozen and air dried) compared to the fresh sample. Another two main compounds such as citronellyl acetate (2.9 – 5.5 %) and nerolidol (0.5 – 1.7 %) increased after pretreatment. However, two other compounds such as β-phellandrene (4.1 – 4.8 %), and β-myrcene (2.1 – 2.6 %) were not affected by the pretreatment method. This result means pretreatment can affect the extraction of specific volatile molecules from the kaffir lime leaves.

Table 2 Volatile compounds in kaffir lime leaves obtained from different pretreatment condition

Volatile compounds	Percentages of compounds (%)		
	Fresh	Frozen	Air dried
β-Phellandrene	4.1	4.6	4.8
β-pinene	0	0.4	0
β-Myrcene	2.1	2.4	2.6
β -ocimene	0	1.4	0
Linalool	6.7	5.7	5.6
Citronellal	71.2	69.9	68.5
Citronellol	9.2	6.6	7.0
Geraniol	0	0.8	0.7
Citronellyl Acetate	2.9	5.5	4.1
Neryl Acetate	1.8	0	3.0
α-Farnesene	0.9	1.2	0
Nerolidol	0.6	1.3	1.7
Supraene	0.4	0	0
Caryophyllene	0	0	1.8

The highest citronellal was presented in essential oils in fresh, followed by frozen and air-dried samples. The study by Hien et al. [18] claimed that β-citronellal compounds were the main volatile compounds in kaffir lime leave presented from 61.7 to 80.8 %. In this study, it is found that the dominant volatile compound of kaffir lime leaves was citronellal (69.9 – 71.1 %) followed by citronellol (6.6 – 9.2 %), and linalool (5.6 – 6.7 %).

In summary, according to the result found in this study, pretreatment by freezing and air drying slightly affect the changes in quantitative and qualitative major volatiles compounds in kaffir lime leaves. The change of volatile constituents of essential oil is probably because of the chemical structure and the volatility of the compounds, and the formation of new constituents by oxidation, glycoside hydrolysis, and esterification during the pretreatments [19].

4. CONCLUSIONS

In this study, the effect of pretreatments through freezing and air drying on essential oils extracted from kaffir lime leaves was investigated. Hydro-distillation was applied to extract the essential oils and to study the effect of pretreatment. Results showed that pretreatment of samples affects the accumulation rate of essential oil, the yield of essential oils, and the volatile compounds extracted from the kaffir lime leave through the hydro-distillation. Air-dried samples resulted in higher yield of essential oil and a shorter equilibrium time. However, the essential oil obtained from fresh kaffir lime leave can retain a high amount of major volatile compounds (citronellal). The results of this study provided an useful information on the possibility of essential oil extraction from the kaffir lime leaves with better quality and yield by the hydro-distillation

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