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A REVIEW ON EXTRACTION METHODS OF ESSENTIAL OIL FROM KAFFIR LIME (*CITRUS HYSTRIX*) LEAVES

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ABSTRACT: Kaffir lime or "limaupurut," which are the common names for *Citrus hystrix* (*C. hystrix*), is a popular traditional herbal plant and an essential herb in Asian countries. *C. hystrix* was native to the Indonesian island known as Sumbawa; then, its cultivation was expanded to Malaysia, Thailand, Indonesia and other tropical countries in the Asian region. *C. hystrix* contains phenolic compounds and essential oil, and the rarity of its essential oil makes it a precious product in many industries. The essential oil is extracted from *C. hystrix* leaves using a few extraction methods; however, the best extraction methods up until now have yet to be ascertained. This narrative review paper highlights several extraction methods which determined the final yield of *C. hystrix* leaves 'essential oil and the comparison of advantages and disadvantages between each method. This review paper also covers the pre-treatment procedures as it is also an important element that affects the final yield. Essentially, each of the extraction and pre-treatment methods has its own pros and cons, and consequently, choosing a suitable technique depends on the demand and requirement of the producer.

INTRODUCTION: Kaffir lime, Makrut lime or "limaupurut,"also scientifically known as *Citrus hystrix* (*C. hystrix*), is an herbal plant from the Rutaceae family. The plant is widely cultivated in many Asian countries, such as Malaysia, Indonesia, and Thailand. The colour of *C. hystrix* leaves is dark green with a shiny sheen. Due to its aromatic, robust, spicy, and unique flavour, *C. hystrix* fruit and leaves become a popular ingredient in Asian culinary. Both fruit and leaves are regularly used as essential ingredients in soups and curries.

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The plant can also be used in the fermentation process fornon-alcoholic and alcoholic drinks. Its leaves, either fresh or dried, can be frozen for other purposes as well [1]. In Thailand, *C. hystrix* flavonoid-rich sachet is used to flavour the tea for drinking. Its leaves have also been used as a treatment for scurvy and to preserve healthy gums and teeth.

Furthermore, *C. hystrix* juice is commonly used to enhance appetite, dispel gas, as well as for blood detoxification, and maintaining healthy condition for hair and scalp [2]. In addition, the leaves' extracts have been reported to possess antioxidant, anti-inflammatory, and anti-cancer properties [3]. *C. hystrix* stands as a small tree with a width of 2.5 - 3m and a height of 3-6 m and is usually crooked, with spiny and glabrous branches **Fig. 1A**.

Its leaves are distinctive among the varieties of citrus species; it is unifoliate, alternate, roughly ovate-oblong to ovate shape, which is 7.5-10 cm long, with lighter bottom, dark green top, and immensely aromatic. The long petiole is prolonged into notable wings, which are about15 cm long and 5 cm wide. The leaves grow into two sections, apparently by a double leaf **Fig. 1B**. The prolonged petiole and leaves emerge as single "pinched" leaf. The leaf base is rounded or cuneate, moderately acuminate, apex obtuse, or notched [1]. The flower **Fig. 1C** is aromatic, small, white in colour, with a four-lobed calyx cuspidation, and has ovate-oblong shape white, a long by the violet fringe. The fruit has a large size, globose, verrucose, bumpy or warty, elliptic or ovoid, and colour may turn from green to yellowish-green when ripe. The diameter of the fruit is approximately 5-7 cm, and it has a thick rind, with yellowish pulp, which has bitter and acidic tastes **Fig. 1D**. The *C. hystrix* fruit produces numerous seeds, which are ridged and have an oval, oblong shape; the size of each seed is about 1-1.2 cm. Seed embryo is monoembryonic with white cotyledons **Fig. 1E** [1].

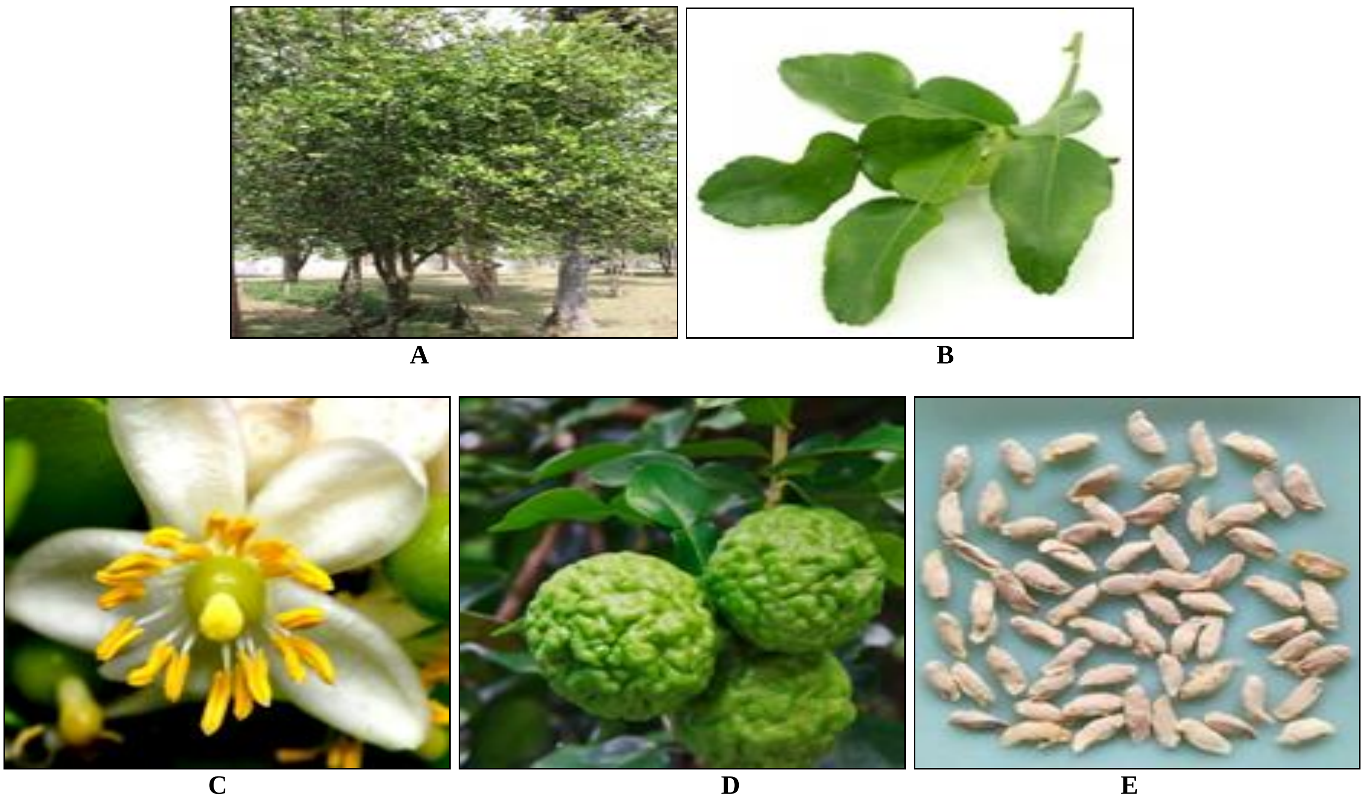

FIG. 1: (A) TREE (ADAPTED FROM NYE NOONA-WORDPRESS. COM); (B) LEAVES (ADAPTED FROM PURELYFRESH. COM); (C) FLOWERS (ADAPTED FROM FLICKR. COM); (D) FRUITS (ADAPTED FROM I NET-FARM. COM); (E) SEEDS (ADAPTED FROM EBAY. COM)

According to International Standard Organization (ISO 9235: 2013) and European Pharmacopoeia, "essential oil "is the term used for a product achieved from a plant or vegetable, through the process of distillation using steam or water, or from the *citrus* fruits flanked by dry distillation or mechanical process [1]. The merging of semi-volatile with volatile compounds makes essential oil, which commonly has a strong odour, infrequently colored, insoluble in water, and soluble in organic solvents. By using different biosynthetic paths and primary precursors to synthesize, the essential oil contains non-terpenoid and terpenoid origins of volatile compounds. The role of essential oil as an important component in various products, from aromatics and food to medicine and agriculture, has propelled essential oil as a product of high demand. Furthermore, the essential oil is frequently used in treating anxiety and depression due to its pleasant and soothing aromatic smell [4, 5, 6]. *C. hystrix* essential oil has been stated to exhibit sedative, analgesic, anesthetic, antimicrobial and anti-inflammatory properties [7, 8]. It is anticancerous, as evidence has suggested its ability in killing cancer cells and inhibiting cancer cell growth. Moreover, the presence of *β*-citronellal in *C. hystrix* essential oil contributes to the field of agriculture as it acts as an effective agent in eliminating rice weevil in rice production [9, 10].

The *C. hystrix* essential oil composition and content have been claimed to have diverse bioactivities. There are 38 identified constituents in *C. hystrix* leaves' essential oil, which represents 89% of the total composition of the oil. The essential oil is potent in monoterpenes (87%) and other minor components such as *β*-pinene (10%) and limonene (4.7%), and it is distinguished by a high content of *α*-terpineol (7.6%), terpinen-4-ol (13%), citronellol (6%) and 1.8-cineole (6.4%). On the other hand, other research has discovered that essential oilof *C. hystrix* leaves hads 29 compounds, with *β*-citronellal as the major component at 66.85% of the total oil [11]. In the study by Othman *et al*. (2016), it was identified that the main volatile compounds present in the *C. hystrix* leaves are citronellal (72.4%), *β*-citronellol (6.7%), *α*-pinene (1.9%) and citronellyl acetate (4.1%) while the minor component is limonene (0.1%) [10]. **I.**

However, dried leaves have different concentrations of components when compared to fresh leaves, as the major compounds are *β*-citronellal (69.96%), *β*-citronellol (6.67%), and linalool (3.86%) [12]. According to Wulandari *et al.* (2019), it was found that *β*-citronellal was a major compound in *C. hystrix* with a total of 66.85% of total oil yield [11]. By comparison to the outcomes of Loh *et al*. (2011), the *C. hystrix* essential oil was identified to contain*α*-farnesene and camphor. Rarely, the composition of *C. hystrix* essential oil differs for different plant parts, between the single leaves of oil glands through polymorphism of phytochemical [13]. However, the yield of oil is affected by the plant's growth phase, such as the fruiting stage, vegetative stage, and flowering stage [13, 14, 15]. The region and location where the plant was taken also affect the oil yields and its compositions [16].

Consequently, slight inconsistencies may occur between different studies, even though the same methodology is utilized. The composition and yield of oil are affected by using different extraction methods. Standard methods to extract bioactive compounds from plants and herb, such as hydro-distillation and Soxhlet extraction, have been used a long time ago. With advanced technology, more extraction methods have since been discovered. This review paper aims to summarize a few profound, impactful studies which reported various performances of the new techniques as well as the respective chemical compositions of the essential oils.

MATERIALS AND METHODS: In this review paper, Google Scholar was used as the search engine. References used for this paper were based on Springer Nature, Science Direct database, and other databases using a combination of different keywords such as "Kaffir lime," "*Citrus hystrix*," "Antimicrobial of *Citrus hystrix*," "*Citrus hystrix* essential oil," "Kaffir lime active compounds and "Kaffir lime extraction method". Relevant articles were also reviewed for additional background and support.

RESULTS AND DISCUSSION:

Extraction Methods:

1. Hydro-distillation: The characteristics of hydro-distillation, which are simple, eco-friendly, relatively cheap, and produces excellent oil quality, made it the most regularly used method to extract essential oils from medicinal plants and herbs [17, 18, 19, 20]. It is also advantageous to use the hydro-distillation method as it prevents damage to the leaves. The leaves are protected from degradation and charring because the technique does not expose direct heat to the leaves. However, cautious steps must be taken, such as avoiding excessive additional amounts of water that will cause high energy consumption in maintaining optimum temperature, increasing the extraction temperature, and avoiding hydrolytic effect to preserve the quality of oils and its yield [19]. The disadvantage of the hydro-distillation extraction process is the lengthy time required. It commonly takes up to 4 hours to complete the extraction process and averagely takes 3 h to achieve the maximum peak of oil yield [20, 21, 22], as exhibited in **Table 1.**

TABLE 1: THE EFFECT OF HYDRO-DISTILLATION PERIOD ON YIELD [20, 23]

Hydro-distillation Period (h)	Yield (%)
2.59	1.14
3.00	1.75
4.00	1.5
5.00	1.3
5.41	1.23

2. Steam Distillation: The extraction procedure of essential oil scan also is conducted using a Clevenger-type apparatus of steam distillation. The leaves for the steam distillation procedure are prepared by placing them in a packed bed just above the water level or plunged in the water. The water is heated, and steam passes over the leaves, causing cell structure to break down, thus releasing volatile components in the process. The flask is used to collect vapour or steam; both water and oil are contained in the condensate, whereby lower degree condensate will dissolve the water-soluble compounds 24. In this process, the maximum production yield will take almost 2 h 25. As the steam temperature is increased, more oil is extracted. This assumption was confirmed by Yusoff *et al.* (2013), whereby it was found that steam temperature increase would spontaneously increase the yield of essential oil. The maximum yield was obtained at 95 °C, at 2.94%.

3. Soxhlet Extraction: Volatile compounds can be obtained from raw materials by using Soxhlet extraction. The process starts by boiling the limited solubility solute and solution together in a percolator; the condensate of concentrated solute is collected from the reservoir. The final yield for essential oils using Soxhlet extraction and ethanol as a solvent for 22.5 h at 81-96 °C was 13.39%, while using n-hexane for 16 h produced 22.80% 26,27. Even though the extraction process consumes a longer time, it can extract a high yield value of oils [28, 29].

4. Solvent Extraction: Common solvents used in the solvent extraction process to extract bioactive compounds are ether, methanol, and hexane. This process is used for heat-sensitive and fragile material, *i.e.*, flowers that cannot withstand high temperature during the heating process of extraction and steam distillation. This process involves the use of many solvents, but it is also the most natural method to isolate volatile compounds. Tinjan & Jirapakkul (2007) reported that the separation of free bound compounds and volatile glycosidic compounds from leaves of Kaffir lime could be obtained using this extraction method. The characteristic scent of leaf from Kaffir lime was also present in the free bound and volatile glycosidic compounds. However, using solvent extraction as a methodology for extraction has some weaknesses. The oils were unsuited for pharmaceutical and food products due to the high-volume usage of synthetic chemical solvents, thereby causing unstable ecological equilibrium [8]. Moreover, the downstream process to concentrate and purify the process becomes complicated when solvent extraction is used. Zaibunnisa *et al.* (2009) revealed that removing complexes and forming emulsion were difficult and might lead to an unsuccessful process. According to Anuchapreeda *et al.* (2020), the highest yield could be obtained using ethanol extract as the compounds present in the leaves of Kaffir lime could be dissolved using ethanol [2].

5. Pressurized Liquid Extraction (PLE): PLE mechanism works by increasing the pressure and temperature of the liquid solvents. This method expands the ability of extraction when separating to room temperature and atmospheric pressure. The target compound partitions with solvent and water and the analyte solubility influence the efficacy of extraction. The advantages of this technique include a high yield of extraction, a shorter period of the process, production of clean extracts, and low consumption of solvents [30]. Since the target product is organic, the use of isopropanol, hexane, ethanol, and methylene chloride as organic solvents works well as extraction solvents. The unwanted result may be achieved when using water for non-polar compounds as water works well to extract polar compounds [29, 31].

A high amount of oil yields can be achieved using PLE with 47.27% on a dry weight base. It can be achieved at 1000 psi and 100 °C for 30 min using n-hexane as a solvent [27]. However, PLE's restriction is that only 10 g can be used as the maximum loaded quantity fixed on laboratory scale thimble [32]. Ghafar *et al*., (2013) reported that 56.16% was the final oil yield obtained using the optimized PLE method [33]. A custom-made PLEhad been found to lead to successful results, and this could be used to replace the other equipment, which could be very costly, as reported by Sanagi *et al.* (2005). This was evident as approximately 100% effectiveness for extraction recovery was achieved compared to Soxhlet extraction method [34]. The comparison of several extraction processes in terms of final oil yield as well as their advantages and disadvantages are summarized in **Table 2.**

TABLE 2: OVERVIEW OF THE YIELD, ADVANTAGES AND DISADVANTAGES OF VARIOUS EXTRACTION METHODS

Methods	Essential Oil Yield (%)	Advantages	Disadvantages
Hydro-distillation	0.78 [12] 0.82 [20,35] 0.83 [25] 1.1 [22] 1.26 [11]	Prevents degradation and charring. Simple to configure.	Extended time is taken to process and requires a high amount of water and heat energy.
Steam Distillation	3.11 [37] 4.26 [36]	The handling of heat-sensitive materials can be manipulated by pressure. The higher the steam temperature, the more abundant the final oil yields.	May singe the leaves and requires a significant amount of heat energy to produce steam continuously
Soxhlet Extraction	13.39[26] 22.80 [27]	Conventional and standard method which can produce high oil yield.	Very long process and can be costly due to the usage of a large amount of expensive solvents.
Solvent Extraction	10.36, 3.51, 1.12, 2.78 and 10.31 [20]	Suitable for heat-sensitive materials. Simple to setup. Effective at separating glycosidically and free bound volatile compounds.	Consumesa large amount of solvents. It can negatively affect the ecological equilibrium. Complicates the downstream process.
Pressurized Liquid Extraction	47.27 [27] 56.16 [33]	Extraction yieldis higher, production of clean extracts, short processing time and low solvent consumption.	Expensive initial cost. Using water may yield less-satisfactory results.

Pre-treatment Methods: Pre-treatment is a technique for enhancing the extraction process before it begins. In this case, pre-treatment methods uninvolved with the oil extraction but combined to refine the ingredients for extraction. The different results of this step depend on the variety of raw materials used and the treatment method. The pre-treatment method may affect the yield of the final product and its purity, reduce other procedure workloads, elevate the method's efficiency and intercept equipment harm.

1. Ultrasound: Ultrasonic pre-treatment can obtain target heat-sensitive bioactive compounds without altering the material's structure, and this is commonly known as the non-thermal methods. This pre-treatment method can significantly affect dehydrating kinetics.

Investigation made by Śledź *et al*. (2014) found that an ultrasound pre-treatment causeda minor decrease in the total content of phenolic and decreased the frying period by 56%. Elevated mass and heat transfer and modification of microstructure tissue could be caused by ultrasound treatment. The study also found that a significant decrease in drying time was contributed by a more extended period of exposure to ultrasound treatment. The sonification parameters have to be adjusted as microstructure is involved in determining sonification effectiveness. As the implosion of cavitation bubbles affects the cell wall separation, ultrasound helps in enhancing the quality and yield of oil, consequently assisting in the mechanism of rapid exudation and solvent penetration [38, 39, 40]. Degradation and damages of thermolabile and volatile compounds can be prevented when using ultrasound to find the target compounds. Moreover, ultrasound is more efficient and faster than other conventional extraction processes [40]. Ultrasound can also reduce colour changes, lower water activity, and decrease loss of nutrient elements [41].

As the duration of pre-treatment increases, the average oil yield increases [35]. The increase in temperature depends on the released energy made by popping bubbles, thus controlling the time exposed to the ultrasound treatment. The volatile components may be vaporized before the extraction start, causes by the extended time treatment of ultrasound, consequently decreasing oil yield, as indicated by Anjazi & Sauid (2015) in **Table 3** [20]. The optimal frequency and time for optimal ultrasound pre-treatment are 53 kHz and 120 min, respectively [23].

TABLE 3: EFFECTS OF DURATION OF ULTRASONIC PRE-TREATMENT ON ESSENTIAL OIL YIELD [20]

Ultrasonic pre-treatment (min)	Yield (%)
0	1.3
60	1.5
120	1.75
145	0.82

2. Drying: Drying is a pre-treatment procedure that is essential to remove excess water from the samples before the extraction process. As compared to fresh or dried leaves, the concentration of volatile compounds arises when the drying process is involved [43]. Exposure to a temperature above 70 °C will damage the oil glands, so precautionary steps must include using moderate temperature appropriate to dry medicinal herbs. Kumar *et al.* (2016) had proven that the amount of oil yields decreased when exposed to high temperatures [44]. Besides, moisture content must be present before the analysis because drying causes evaporation of water, which pulls the compounds from the surface of the leaves to keep away from active chemical constituent impairment. Consequently, a higher yield of oils can be obtained from dried leaves in comparison to fresh leaves [45, 46]. The comparison of the hydro-distillation process (with ultrasonic pre-treatment) using fresh and dried leaves of Kaffir lime is shown in **Table 4.**

TABLE 4: OIL YIELD OF FRESH AND DRIED KAFFIR LIME LEAVES FROM ULTRASONIC PRE-TREATMENT

Time (min)	Average of Yield from Fresh Leaves (%) [23]	Average of Yield (%) from Dried Leaves [35]
0	0.265	0.537
60	0.295	0.818

3. Physical size Alteration: Extraction can be enhanced by reducing the sample's physical size either by cutting or grinding. It can elevate the efficiency of extraction and reduce energy and time for distillation. Based on a study by Singh *et al.* (2014), as the particle size decreased, the final yield would increase irrespective of the extraction methods [46]. This is due to an increase of area at the surface as leaves are cut into small pieces. With increasing surface area, the resistance of mass transfer decreases which leads to excellent transfer of heat. This phenomenon can slow down transporting oil from cells when using the solvent extraction process [46]. Therefore, to achieve an excellent yield of essential oils, particle size reduction is crucial and this can be done by reducing the diameter of sample [47]. Moreover, controlling the extraction process can be balanced by reducing internal mass transfer resistance, thus decreasing distillation time [18]. The authors also reported that cut leaves produced higher oil yields than whole leaves, at 2.3% and 2.0%, respectively.

CONCLUSION: This review paper presents various methods of conventional extraction and pre-treatment appropriate to the production of Kaffir lime leaves' essential oil. The differences between pre-treatment and the process of extraction differ in the percentage of oil yields, and the specified methods possess their respective advantages and disadvantages. For example, extraction techniques such as PLE and Soxhlet technique produce high oil yields, but they require an extended period of extraction and low loadings of materials. Thus, the selection of the methods to be applied must meet the manufacturer's requirements. To effectively elevate the yield of oils in an alow-cost manner as well as and enhancing the process of extraction, a pre-treatment method may be employed. Effective pre-treatment methods such as grinding, cutting, and drying of the samples can be used in the line of progression to increase productivity. It is the purpose of this narrative review to compare and provide an overview of the available current methods in extracting essential oil from Kaffir lime leaves.

Nonetheless, the availability of appropriate extraction methods or techniques is not limited to the ones listed in this paper. The aspiration to enhance the current methodologies is progressively ongoing, which can drive and shape the future of numerous industries.

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