

## REVIEW ON EXTRACTION METHODS OF ESSENTIAL OIL FROM KAFFIR LIME (*Citrus hystrix*) LEAVES

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### Abstract

Kaffir lime or “limau purut” (*Citrus hystrix*) is a popular traditional herbal plant with aromatic leaves. It produces fruits which are commonly used in Asian cuisines as a flavouring agent. Essential oil has been a popular and valuable product applicable in various industries, but its availability is scarce. Essential oil from the leaves of *Citrus hystrix* is a complex mixture of volatile and semi-volatile compounds. It is rich in bioactive molecules that act synergistically to improve the well-being of an individual. Essential oil is extracted from *Citrus hystrix* leaves using a few extraction methods; however, the most excellent extraction methods have yet to be ascertained. This review paper highlights several extraction methods that determined the final yield of *Citrus hystrix* leaves' essential oil and the comparison of advantages and disadvantages between each method. Two types of methods are discussed, which are conventional and modern methods. Conventional methods include hydro-distillation, steam distillation, Soxhlet distillation and solvent extraction, while the modern method includes pressurized liquid extraction (PLE). In addition, pre-treatment processes are also included as they can significantly affect the performance of other important processes and production yield. This paper also found that the highest yield of 56.16% was obtained using PLE method. Essentially, each of the extraction and pre-treatment methods has its own pros and cons; hence choosing a suitable method depends heavily on the producer's demand and requirement.

**Keywords:** *Citrus hystrix*, Kaffir lime, Essential oil, Leaves, Extraction method.

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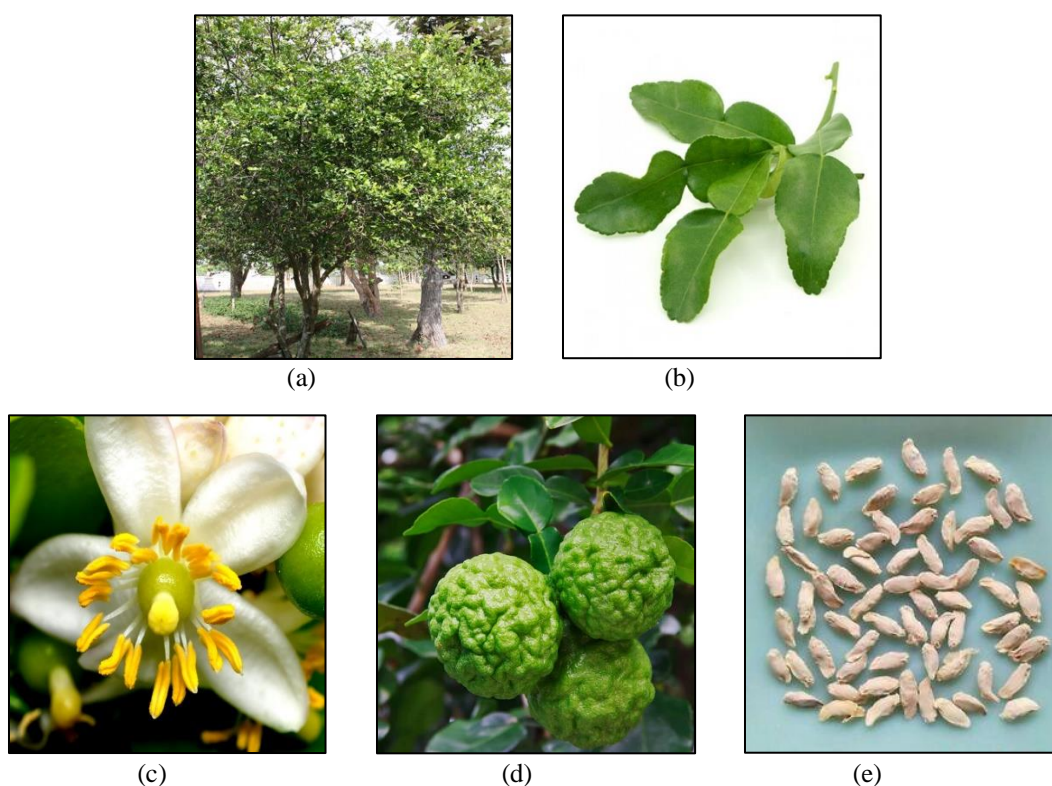
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### Introduction

Kaffir lime or “limau purut,” also scientifically known as *Citrus hystrix* (*C. hystrix*), is an herbal plant from the Rutaceae family. The plant is widely cultivated in several Asian countries, such as Malaysia, Thailand and Indonesia. The colour of *C. hystrix* leaves is dark green with a shiny shine. Due to its aromatic, robust, spicy and unique flavour, *C. hystrix* leaves and fruits are regularly used as essential ingredients in soups and curries, especially in Asian culinary dishes. The plant can also be used in the fermentation process for non-alcoholic and alcoholic drinks. Its leaves, either fresh or dried, can be frozen for other purposes as well (Lim, 2012; Budiarto *et al.*, 2019). In Thailand, *C. hystrix* flavonoid-rich sachet is used to flavour 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 (Anuchapreeda *et al.*, 2020). In addition, the leaves' extracts have been reported to possess anti-inflammatory, antioxidant and anticancer properties (Abirami *et al.*, 2014; Nararak *et al.*, 2016).

*C. hystrix* stands as a small tree with a width of 2.5 - 3 m and a height of 3 - 6 m, and is usually crooked, with spiny and glabrous branches (Figure 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, has a dark green top with lighter bottom, and immensely aromatic. The long petiole is prolonged into notable wings, about

15 cm long and 5 cm wide. The leaves grow into two sections, apparently by a double leaf (Figure 1b). The prolonged petiole and leaves emerge as single “pinched” leaf. The leaf base is rounded or cuneate, moderately acuminate, notched or apex obtuse (Lim, 2012; Kusuma and Mahfud, 2017). The flower (Figure 1c) is aromatic, small, white in colour, with a four-lobed calyx cuspidation, and has an ovate-oblong shape with a long 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 fruit's diameter is approximately 5 - 7 cm. It has a thick rind with yellowish pulp, which has bitter and acidic tastes (Figure 1d). The *C. hystrix* fruit produces numerous seeds that are ridged and have an oval, oblong shape; each seed's size is about 1 - 1.2 cm. Seed embryo is monoembryonic with white cotyledons (Figure 1e) (Lim, 2012; Kusuma and Mahfud, 2017).



**Figure 1:** (a) Tree (adapted from Nye Noona-WordsPress.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 European Pharmacopoeia and International Standard Organization (ISO 9235: 2013), “essential oil” is the term used for a product achieved from a plant or a vegetable, through the process of distillation using steam or water, or from the *citrus* fruits flavedo by dry distillation or mechanical process (Lim, 2012). The merging of semi-volatile compounds with volatile compounds makes essential oil, which commonly has a strong odour, infrequently coloured, insoluble in water and soluble in organic solvents. 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 agriculture and medicine, has propelled essential oil as a high demand product. Furthermore, essential oil is frequently used in treating anxiety and depression due to its pleasant and soothing aromatic smell (Ades, 2009; Russo, 2011; Guzmán-Gutiérrez *et al.*, 2015). *C. hystrix* essential oil has been stated to exhibit analgesic, sedative, spasmolytic, anaesthetic, anti-inflammatory and antimicrobial properties (Luangnarumitchai *et al.*, 2007; Bakkali *et al.*, 2008). It is

anticancerous, as evidence has suggested its ability in killing cancer cells and inhibiting cancer cell growth (Bakkali *et al.*, 2008). Moreover, the presence of  $\beta$ -citronellal in *C. hystrix* essential oil contributes to agriculture, whereby the oil acts as an effective agent in eliminating rice weevil in rice production (Loh *et al.*, 2011; Othman *et al.*, 2016; Agouillal *et al.*, 2017).

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 oils, representing 89% of the oil's total composition. The essential oil is potent in monoterpenes (87%) and other minor components such as limonene (4.7%) and  $\beta$ -pinene (10%) and it is distinguished by a high content of  $\alpha$ -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 oil of *C. hystrix* leaves have 29 compounds, with  $\beta$ -citronellal as the major component at 66.85% of the total oils (Wulandari *et al.*, 2019). It was also identified that the main volatile compounds present in the *C. hystrix* leaves were citronellal (72.4%),  $\beta$ -citronellol (6.7%),  $\alpha$ -pinene (1.9%) and citronellyl acetate (4.1%) (Othman *et al.*, 2016). In comparison, the minor component was limonene (0.1%) (Othman *et al.*, 2016; Agouillal *et al.*, 2017). However, dried leaves have different concentrations of components when compared to fresh leaves, as the major compounds were  $\beta$ -citronellal (69.96%),  $\beta$ -citronellol (6.67%), and linalool (3.86%) (Ismail and Sauid, 2016). Another study found that  $\beta$ -citronellal was a major compound in *C. hystrix*, with 66.85% of total oil yield (Wulandari *et al.*, 2019). By comparison to the outcomes of a separate study, the *C. hystrix* essential oils were identified to contain  $\alpha$ -farnesene and camphor (Loh *et al.*, 2011). Rarely, *C. hystrix* essential oils' composition differs for different parts of the plant, between the single leaf of oil glands through polymorphism of phytochemical (Johnson *et al.*, 2004). However, the yield of oils is mostly affected by the plant's growth phases, such as vegetative, fruiting, and flowering (Johnson *et al.*, 2004; Novak *et al.*, 2006; Slavkovska *et al.*, 2013). The region and location where the plant was taken also affects oil yield and its compositions (Dardioti *et al.*, 2012). Consequently, slight inconsistencies may occur between different studies, even though the same methodology is utilized.

In addition to discrepancies between plants, the composition and yield of oils are also affected by the different extraction methods. Each method has its effects on the sample. Conventional methods to extract bioactive compounds from plants and herbs, 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 that reported various performances of the new techniques and the essential oils' respective chemical compositions. Hydro-distillation, steam distillation, solvent extraction, Soxhlet extraction and pressurized liquid extraction methods were investigated in terms of their yield, process conditions for extraction and major compounds.

### Material and methods

In this review paper, Google Scholar was used as the search engine. References used for this paper were based on Springer Nature, ScienceDirect 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

#### A. Kaffir lime extraction method

Extraction methods majorly affect the production yield and composition of essential oil. Conventional methods like hydro-distillation, steam distillation, Soxhlet extraction and solvent extraction are cost-efficient and easy to implement. On the other hand, modern method like pressurized liquid extraction (PLE) requires high installation cost and technical knowledge. However, modern method can provide high-quality essential oil (El Asbahani *et al.*, 2015). Therefore, the method and solvent used should be selected carefully as both affect the quality and quantity of the product to be obtained. In extracting essential oil from

*C. hystrix*, the leave's peels in fresh and dry forms were utilized. Similarly, hydro-distillation, steam distillation, solvent extraction, Soxhlet extraction and PLE methods are commonly used to extract essential oil from Kaffir lime as they are simple and easy to handle.

### **Hydro-distillation**

Hydro-distillation, also known as water distillation, is a conventional method for extracting essential oil from aromatic plant materials. The characteristics of hydro-distillation (Figure 2a) are simple, eco-friendly, relatively cheap, and able to produce excellent oil quality, making it the most regularly used method to extract essential oils from medicinal plants and herbs (Chinese Pharmacopoeia Committee, 2010; Saudid and Md Anjazi, 2015; Kusuma and Mahfud, 2016; Kusuma and Mahfud, 2017). 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 the leaves to direct heat. However, cautious steps must be taken, such as avoiding excessive 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 yield (Kusuma and Mahfud, 2017). 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 hours to achieve the maximum peak of oil yield (Chanthaphon *et al.*, 2008; Rosli and Saudid, 2016; Bousbia *et al.*, 2019), as exhibited in Table 1.

**Table 1:** The effect of hydro-distillation period on yield ((Chanthaphon *et al.* 2008; Rosli and Saudid, 2016)

| 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      |

### **Steam distillation**

Steam distillation is one of the preferred distillation methods for extracting essential oil from sensitive to high temperature from plant materials. It is one of the most widely used methods for extracting essential oil from plant materials on a large scale (Tongnuanchan and Benjakul, 2014; Reyes-Jurado *et al.*, 2015). The extraction procedure of essential oils can also be conducted using a Clevenger-type apparatus (Figure 2b) of steam distillation. The steam distillation procedure leaves 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 (Tongnuanchan and Benjakul, 2014; Saudid and Aswandi, 2018). In this process, the maximum production yield will take almost 2 hours (Yusuff and Saudid, 2016). As the steam temperature is increased, more oil is extracted. This assumption was confirmed, whereby it was found that steam temperature increase would spontaneously increase the yield of essential oil (Yusuff and Saudid, 2016). The maximum result was attained at 2.94%, 95 °C.

### **Soxhlet extraction**

Soxhlet extraction is a continuous solid/liquid extraction process used commonly when the compound of interest has limited solubility in the solvent. Volatile compounds can be obtained from raw materials by using Soxhlet extraction (Figure 2c). The process starts by boiling the limited solubility solute and solution together in a percolator; the concentrated solute condensate is collected from the reservoir. The final yield for essential oils using Soxhlet extraction and ethanol as a solvent for 22.5 hours at 81 - 96 °C was 13.39%

while using *n*-hexane as a solvent for 16 hours produced 22.80% (Munawaroh and Astuti, 2010; Haiyee and Winitkitcharoen, 2012). Even though the extraction process consumes a longer time, it can extract a high yield value of oils (Ong, 2004; Pourmortazavi and Hajimirsadeghi, 2007).

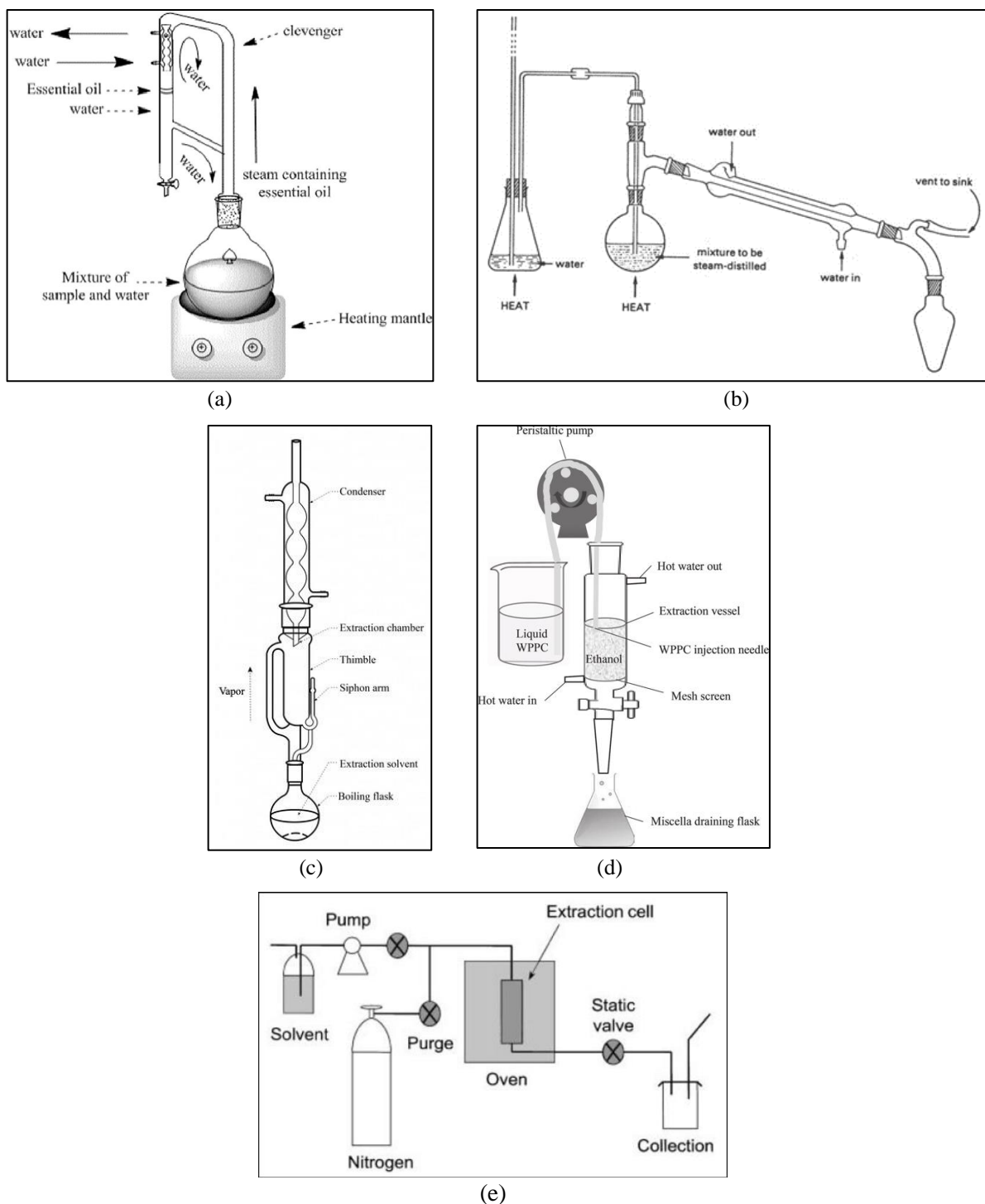
### ***Solvent extraction***

Common solvents used in the solvent extraction (Figure 2d) process to extract bioactive mixtures are methanol, hexane and ether. This process is used for fragile and heat-sensitive materials, i.e., plants that cannot stand at high temperature during the heating process of extraction and steam distillation. This process involves many solvents, but it is also the most natural method to isolate volatile compounds. It was reported that the separation of free bound compounds and volatile glycosidic compounds from leaves of *C. hystrix* could be obtained using this extraction method (Tinjan and Jirapakkul, 2007). The characteristic scent of leaf from *C. hystrix* was also present in the free bound and volatile glycosidic compounds. Based on a study, the highest yield could be obtained using ethanol extract as the compounds existing in the leaves of *C. hystrix* could be dissolved using ethanol (Budiarto *et al.*, 2019). 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 (Luangnarumitchai *et al.*, 2007). Moreover, the downstream process to concentrate and purify the process becomes complicated when solvent extraction was used. It was revealed that removing complexes and forming emulsion were difficult and might lead to an unsuccessful process (Zaibunnisa *et al.*, 2009).

### ***Pressurized liquid extraction***

Pressurized liquid extraction (PLE) is an advanced extraction technique that uses liquid solvents for extraction at high temperature and pressure, below its critical point, which maintains the solvent at liquid state (Figure 2e). This method expands the ability of extraction when differentiating to room temperature and atmospheric pressure. The target compound partitions with solvent and water, and the analyte solubility influences the efficacy of extraction (Sanagi *et al.*, 2005). 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 (Delgado-Zamarreño *et al.*, 2004; Sanagi *et al.*, 2005; Raut *et al.*, 2015; Otero *et al.*, 2018). 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 (Ong, 2004; Marriott *et al.*, 2011). A high amount of oil yields can be achieved using PLE with 47.27% on a dry weight base. It can be achieved at 100 °C and 1000 psi for 30 min using *n*-hexane as a solvent (Haiyee and Winitkitcharoen, 2012). However, PLE's restriction is that only 10 g can be used as the maximum loaded quantity fixed on laboratory scale thimble (Suchan *et al.*, 2004). One study reported that 56.16% was the final oil yield obtained using the optimized PLE method (Ghafar, 2013). A custom made PLE had been found to lead to successful results and this could be used to replace other equipment, which could be very costly (Sanagi *et al.*, 2005). This was evident as approximately 100% effectiveness for extraction recovery was achieved compared to Soxhlet extraction method (Sanagi *et al.*, 2005).

The comparison of several extraction processes in terms of final oil yield as well as their advantages and disadvantages are summarized in Table 2.



**Figure 2:** (a) Hydro-distillation (adapted from <https://www.researchgate.net/>); (b) Steam distillation (adapted from <https://www.researchgate.net/>); (c) Soxhlet extraction (adapted from <https://www.researchgate.net/>); (d) Solvent extraction (adapted from <https://www.researchgate.net/>); (e) Pressurized liquid extraction (adapted from <https://www.researchgate.net/>)

**Table 2:** Outline of the yield, disadvantages and advantages of various extraction methods

| Methods                              | Essential oil yield (%)  | Advantages   | Disadvantages   |
|--------------------------------------|--|--|---|
| <b>Hydro-distillation</b>            | 0.78 (Othman <i>et al.</i> , 2016)<br>0.82 (Chanthaphon <i>et al.</i> , 2008; Zakaria and Saud, 2016)<br>0.83 (Yusuff and Saud, 2016)<br>1.1 (Delgado-Zamarreño <i>et al.</i> , 2004)<br>1.26 (Agouillal <i>et al.</i> , 2017) | Prevents degradation and charring. Simple to configure.  | Extended time is taken to process and requires a high amount of water and heat energy.  |
| <b>Steam Distillation</b>            | 3.11 (Yusoff <i>et al.</i> , 2013)<br>4.26 (Kasuan <i>et al.</i> , 2013)   | 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                           |
| <b>Soxhlet Extraction</b>            | 13.39 (Munawaroh and Astuti, 2010)<br>22.80 (Haiyee and Winitkitcharoen, 2012)   | 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.                                 |
| <b>Solvent Extraction</b>            | 10.36, 3.51, 1.12, 2.78 and 10.31 (Pourmortazavi and Hajimirsadeghi, 2007)   | Suitable for heat-sensitive materials. Simple to set up. Effective at separating glycosidically and free bound volatile compounds.                 | Consumes a large amount of solvents. It can negatively affect the ecological equilibrium. Complicates the downstream process. |
| <b>Pressurized Liquid Extraction</b> | 47.27 (Haiyee and Winitkitcharoen, 2012)<br>56.16 (Ghafar, 2013)   | Extraction yield is higher, production of clean extracts, short processing time, and low solvent consumption.                                      | Expensive initial cost. Use of water may yield less satisfactory results.   |

## B. Pre-treatment methods

Pre-treatment methods are techniques used to complement the extraction process. In this case, it is not directly involved with extracting the oil, but it improves the materials for the extraction process. The effects of pre-treatment vary depending on the method of treatment and types of raw materials. It may increase final product purity, decrease another process workload, increase the efficiency of other processes, and prevent equipment damage.

### *Ultrasound*

Ultrasonic pre-treatment can obtain target heat-sensitive bioactive compounds without altering the material's structure, and this is commonly known as non-thermal methods. This pre-treatment method can significantly affect dehydrating kinetics. An investigation found that an ultrasound pre-treatment caused a minor decrease in phenolic content and decreased the frying period by 56% (Śledź *et al.*, 2017). Elevated mass and heat transfer and modification of microstructure tissue could be caused by ultrasound treatment. The study also found a significant decrease in drying time 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 to enhance the quality and yield of oil, consequently assisting in the mechanism of rapid exudation and solvent penetration (Zhang *et al.*, 2008; Santos *et al.*, 2014; Chemat *et al.*, 2017). 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 (Chemat *et al.*, 2017). Ultrasound can also decrease water activity, reduce colour changes, and decrease the loss of nutrient elements (Roldan-Gutierrez *et al.*, 2008; Fan *et al.*, 2017). As the duration of pre-treatment increases, the average oil yield increases (Zakaria and Sauid, 2016). The increase in temperature depends on the released energy made by popping bubbles, thus controlling the ultrasound treatment time. The volatile components may be vaporized before the extraction starts, caused by the extended time treatment of ultrasound, consequently decreasing oil yield, as indicated in Table 3 (Sauid and Md Anjazi, 2015). The optimum frequency and time for optimal ultrasound pre-treatment are 53 kHz and 120 minutes, respectively (Rosli and Sauid, 2016).

**Table 3:** Effects of duration of ultrasonic pre-treatment on essential oil yield (Rosli and Sauid, 2016)

| Ultrasonic pre-treatment (min) | Yield (%) |
|--------------------------------|-----------|
| 145                            | 0.82      |
| 120                            | 1.75      |
| 60                             | 1.5       |
| 0                              | 21.3      |

### **Drying**

Drying is a pre-treatment procedure that is essential to remove excess water from the samples before the extraction process. Compared to fresh or dried leaves, the concentration of volatile compounds arises when the drying process is involved (Raksakantong *et al.*, 2011). 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. A study had proven that the amount of oil yields decreased when exposed to high temperatures (Kumar *et al.*, 2016). 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 compared to fresh leaves (Kamal *et al.*, 2011; Singh *et al.*, 2014). Comparison of the hydro-distillation process (with ultrasonic pre-treatment) using fresh and dried leaves of *C. hystrix* is shown in Table 4.

**Table 4:** Oil yield of dried and fresh *C. hystrix* leaves from ultrasonic pre-treatment

| Time (min) | Yield from fresh leaves (%) (Rosli and Sauid, 2016) | Yield (%) from dried leaves (Tinjan and Jirapakkul, 2017) |
|------------|---|---|
| 0          | 0.265   | 0.537   |
| 60         | 0.295   | 0.818   |

### **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. As the particle size decreased, the final yield would increase irrespective of the extraction methods (Singh *et al.*, 2014). 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 (Singh *et al.*, 2014). Therefore, to achieve an excellent yield of essential oils, particle size reduction is crucial. This can be done by reducing the diameter of the sample (Ouzzar *et al.*, 2015).



Moreover, controlling the extraction process can be balanced by reducing internal mass transfer resistance, thus decreasing distillation time (Slavkovska *et al.*, 2013). 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 presented current methods used to extract the essential oil of *C. hystrix*. Conventional methods such as hydro-distillation, steam distillation, Soxhlet distillation and solvent extraction, and modern methods such as pressurized liquid extraction (PLE) are commonly used methods to extract *C. hystrix* leaves' essential oil. The method of extraction is important as it affects the quality and quantity of essential oil significantly. The differences between the extraction process and pre-treatment differ in the percentage of oil yield, and the specified methods possess their respective advantages and disadvantages. For example, extraction techniques such as PLE and Soxhlet technique produce high oil yields. However, 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 a low-cost manner and enhance the process of extraction, a pre-treatment may be employed. Effective pre-treatment of the sample (cutting, grinding, and drying) would increase the mass transfer efficiency, resulting in improved quality and quantity. 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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