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Essential oil of *Citrus hystrix* DC.: A mini-review on chemical composition, extraction method, bioactivities, and potential applications in food and pharmaceuticals

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Abstract

Citrus hystrix DC. is a common herb in tropical regions. Its essential oils are now widely researched and applied because of their high economic value and safety for humans and are interesting materials for future trends. This review provides an extensive overview of the biological activities of *C. hystrix* essential oil, characterized predominantly by citronellal, β-pinene, sabinene, limonene, and terpinene-4-ol, which are deciding factors in antimicrobial, antioxidant, insect repellent, anti-tumor, and anti-inflammatory properties. Therefore, it is applied in the fields of food preservation and pharmaceuticals. However, these applications should consider the ratio of these components in the essential oil, which is variable when using materials from different parts of the plant and depending on the original location of the plant, growth stages, traditional or modern extraction methods, and pre-treatment methods.

Keywords

bioactivities; *Citrus hystrix*; essential oil; food; medicine

Introduction

The scientific name of this plant is *Citrus hystrix* DC., and it is part of the genus *Citrus* belonging to the Rutaceae family. It grows well in the typical warm and humid climate of tropical and subtropical regions and is native to Southeast Asia and South China. Being known and commonly used in daily life by indigenous peoples, it is called by different names, including Kaffir lime, Thai Lime, and Chúc. *C. hystrix* is a small tree that is about 3-6 m high and 2.5-3 m wide with crooked spiny branches. Its leaves are unifoliolate, alternate, 7.5-10 cm long, 5 cm wide. It has small and white flowers. Fruits are large, warty, or bumpy, verrucose, globose to ovoid, green turning yellowish-green when ripe (1). In addition to its trunk and branches, its fruit, which is unpopular and generally harvested with its leaves in June and July, is a by-product of the exploitation process and is used to make essential oil (EO) through distillation (2).

In traditional medicine, *C. hystrix* is also used to treat colds, fever, hypertension, colic, and diarrhea in infants. In addition, the EO from its fruit and leaves is commercially used as a flavouring, and in cooking, perfume, insecticides, food technology, and medical treatments (1). In Malaysia, fruit and leaves are used to wash hair, and fruit juice is applied to the skin to soften and control body odors. Besides, the fruits are also used to separate leeches from the skin, while fresh leaves are frequently used to brush teeth and gum to improve oral health (3). Moreover, Thai people use fresh leaves to make *Tom Yum Gai* and *Tom Yum Kung*, as well as *Kroeung* in Cambodia and Laos, and *Sayur asem* in Indonesia (1). Those are the special dishes and spices of these countries.

Chemical composition of essential oil from the leaves and fruit peels of C. hystrix

According to the study of Sato *et al*., 58 chemical compounds were found in *C. hystrix* EO. Citronellal, a major component (81%) of leaf oil, has the highest antioxidant activity, making it a feature of *C. hystrix* leaf oil. In contrast, the oil from the fruit peel is rich in β-pinene (25.93%) and sabinene (20.36%) (4). There is also regional variation in the composition of leaf oil. For example, citronellal concentration in leaf oil from Long Xuyen province (Vietnam) is significantly high at 86.89% (5).

Moreover, the chemical composition of EO varies between different stages of plant development. According to Ravi *et al*. (6), numerous compounds were exclusively detected in the oil from recently matured leaves, namely 5-Propyldihydro-2 (3H) –Furanone (32.9%), β-Pinene (7.6%), Terpinen-4-ol (2.4%), 1-Hexanol (1.3%) and (Z) -3-Hexen-1-ol-Acetate (1.0%), while 1-Nonanol (30.8%), 3-Axit Methyl Butanoic (4.6%), p-Methyl Acetophenone (1.5%), Trans-Hex-2-Enyl Acetate (1.4%) and Methyl Eugenol (1.1%), which were only found in the old leaf oil.

Biological characteristics of C. hystrix EO

Antimicrobial activity

The chemical composition of *C. hystrix* EO was found to have antimicrobial activity. In the study of Chanthaphon *et al*., peel oil showed higher antimicrobial activity than other citrus fruit oils. It acted against *Saccharomyces cerevisiae*, *Bacillus cereus*, *Staphylococcus aureus*, and *Escherichia coli* due to antibacterial components, such as β-pinene (30.6%), limonene (29.2%) and sapinene (22.6%) (7). Additionally, Sreepian *et al*. (8) indicated that gram-positive bacteria were more sensitive to *C. hystrix* oil than gram-negative bacteria. Overall, the peel oil of *C. hystrix* could be considered a potent ingredient with high antimicrobial properties and natural origin, suitable for use as an alternative to synthetic substances in the pharmaceutical and food industries.

Antioxidant activity

Similarly, *C. hystrix* EO has antioxidant properties, and oils extracted from different plant parts show differing antioxidant activity. According to Warsito et al. (9), leaf oil had an antioxidant activity that was twice as high as peel oil and oil from branches. Citronellal, which is abundant in leaf oil at 85.07%, typically demonstrates the antioxidant characteristics of leaf oil (9). Note that, the extraction method also influences antioxidant activity and this property can be attributed to bioactive compounds obtained (10). In addition, Paramitha and Nabila evaluated the leaf oil produced by steam distillation and the extract collected by macerating *C. hystrix* leaves in ethanol. The results showed that the extract had higher antioxidant activity than the leaf oil because the oil was impaired by the high temperature in the steam distillation process (11). These studies also indicate that the extraction method affects antioxidant properties because antioxidant constituents are lost during extraction. In addition, the *C. hystrix* EO from different parts also showed differing antioxidant activity, and the leaf oil was considered to have higher antioxidant activity, which explains its more common use.

Insect repellent activity

Another exciting feature that local people have long used daily is repelling insects with fresh leaves and fruit, which are toxic to insects but safe for humans. Experimental findings showed that all fly larvae were killed at a 50 percent lethal concentration (LC_{50}) of 71 g/L, and mature flies were killed at a 50% lethal dose (LD_{50}) of 408.63 μg/fly (12). The study has also indicated that compounds, such as citronellol, limonene, pinene, and terpinene-4-ol, in EO, positively impact mosquito repellent, and an addition of 5% vanillin prolongs the protection time to three hours because pure oil is volatile at room temperature (13). This shows that this material has a very high potential for controlling insects that cause infectious diseases.

Anti-inflammatory and anti-tumor activity

C. hystrix is also used as an herbal medicine to treat common diseases, such as cold, fever, cough, and abdominal pain. However, recent studies on the action of this material at the molecular level have shown that it directly or indirectly inhibits protein and enzyme molecules, which in turn induces anti-inflammatory and anti-tumor effects. For example, Lertsatitthanakorn *et al.* reported the anti-inflammatory property of *C. hystrix* EO when it could act against *Propionibacterium acnes* and suppress 5-lipoxygenase, which is an inflammatory enzyme, with the contribution of D-Limonene (14). In addition, Othman *et al*. also revealed that citrus EO had effective anti-proliferative activity against the HeLa cervical cancer cell line with a half maximum inhibitory concentration (IC_{50}) of 25.91 μg/mL (15).

Other reports also showed that *C. hystrix* peel oil with a 220–300 μg/mL concentration had a potential anti-melanoma effect on three human cell lines, WM793, A375, and HTB140, by inhibiting hyaluronidase and tyrosinase. Furthermore, it is safe and non-toxic to human skin cells (16).

Extraction of C. hystrix EO

Pre-treatment methods

Pre-treatment aims to improve the efficiency of the oil extraction process by using physical or biological agents. One of the most traditional, simple, and economical methods is reducing material size with physical processes such as cutting, grinding, and crushing, which increase the surface area of the material, increase heat transfer, and weaken the cell wall. In addition, a high percentage of broken cells leads to better mass transfer, effectively transporting constituents to the outside. A size reduction of *C. hystrix* leaves from 600 to 400 μm increased the yield efficiency from 15.97 to 23.52% (17).

Most materials must be dehydrated to maintain quality and minimize the growth of fungi and other microbiomes during storage and transport. Dehydration helps reduce free water, and the compounds are pulled out, which makes the solvent penetration process easier before extraction. This also explains why volatile substances are lost or altered during dehydration (18). Specifically, prolonged dehydration time decreases oil yield efficiency. In one study, *C. hystrix* leaves were dehydrated at ambient temperature. The results showed that the yield efficiency peaked at 1.26 percent on the 4^{th} day and then decreased gradually (19). Therefore, choosing the appropriate method, temperature, and time during the dehydration process is crucial, which consequently influences EO quality. Dehydration temperatures between 40 and 60°C, and material size reduction, microwave, ultrasound, or shortening processing time are reasonable options.

Another pre-treatment method related to biology is using microbiome enzymes. Enzyme activity breaks down cellulose linkages, thus weakening the cell wall. The compounds are subsequently pulled out, which assures extraction efficiency and the best EO quality. The effects of enzyme pre-treatment are presented in Table 1.

material is completely immersed in water, which protects it from heat. Moreover, steam distillation is also a preferred and popular method on an industrial scale. It is used to extract plant materials that have a high oil content and are heat-sensitive. In the same way as hydro-distillation, however, the material does not directly contact water in this method; therefore, extraction efficiency and oil quality are higher (24).

In addition, soxhlet extraction is a standard method used in oil extraction. The material continuously contacts the new solvent, which prevents solvent saturation and optimizes the distillation procedure. Nowadays, at a higher technical level, people use pressurized liquid extraction (PLE) with liquid solvents at high temperatures and pressures to extract EOs. The extraction process takes place within a set period once the desired pressure and temperature are reached. Haiyee and Winitkitcharoen also applied this method to extract the EO from *C. hystrix* leaves, obtaining an excellent yield efficiency of 42.27% (dry weight basis), respectively (25). Based on the points mentioned above, depending on the technical and economic conditions, we can choose an appropriate extraction method (Table 2).

Table 1. Pretreatment time and microbiome species in correlation with yield efficiency of *Citrus hystrix* essential oil

Ref.		**(20)**	**(21)**	**(22)**
Species		Tempeh yeast	Microbes from pineapple peels	Microbes from pineapple peels
Material		Fresh leaves	Dried leaves	Fresh leaves
Yield of oil from *C. hystrix* leaves at different processing times (%)	0 h	0.55	0.196	0.09
	12 h	-	0.833	0.78
	18 h	-	0.750	0.52
	24 h	0.56	0.596	0.47
	30 h	-	0.459	0.46
	36 h	-	-	-
	48 h	0.64	-	-
	72 h	0.67	-	-
	96 h	0.34	-	-

Modern pre-treatment techniques, such as ultrasound, are applied to highly bioactive and thermally unstable compounds, which helps reduce the alteration and loss of essential chemical compounds. Ultrasound involves the formation and implosive rupture of cavitation bubbles, which disrupt the cell wall and facilitate solvent penetration. The extracted compounds then easily flow out without altering their nature, thus enhancing oil yield and quality, shortening extraction time by up to 70%, and improving the oil's antimicrobial and antioxidant activity. However, a prolonged ultrasound pre-treatment time results in the escape of volatile substances and diminishes oil quality and output (23).

Essential oil extraction methods

Hydro-distillation is a method for distilling EOs in which water vaporizes to form a heterogeneous mixture and then liquefies by indirect cooling in a condenser that flows to a separator. One feature of hydro-distillation is that the

Applications in food technology and pharmaceuticals

People utilize the antibacterial and antioxidant properties of *C. hystrix* EO in food preservation. However, the direct use of EOs is quite limited because they are volatile compounds. Therefore, many methods are used to slow down the loss of EOs by combining them with other materials, such as proteins, lipids, and polysaccharides, which prolong the action time of EOs (31). For example, *C. hystrix* leaf oil can be combined with cassava starch to form an edible coating, which prolongs the shelf life of fresh beef, according to Utami *et al*. (32). This coating prevents the accumulation of by-products related to lipid oxidation and inhibits microbial growth. As a result, this method extends the shelf life of fresh beef (Table 3).

In addition, Pumival *et al*. also evaluated the antifungal ability of *C. hystrix* leaf EO. They applied a microemulsion of *C. hystrix* leaf oil to treat foot fungus caused by *Trichophyton mentagrophytes* var. *interdigitale*. The results

Table 2. Comparison of the efficiency, advantages, and disadvantages of popular *Citrus hystrix* essential oil extraction methods

Method	Yield (%)	Advantages	Disadvantages
Water distillation	Leaves:1.75 (26) Leaves: 0.91 Peels: 1.74 (27)	Simple, eco-friendly, economical, industrial scale applicable	Prolonged extraction time Water and heat energy wasting
Steam distillation	Peels: 4.26 (28) Peels: 3.11 (29)	Short time Heat-sensitive materials can be manipulated by pressure, industrial scale applicable	Materials can be burnt Wasting heat energy to produce steam continuously
Soxhlet extraction	Leaves: 22.8 (25) Leaves: 13.39 (30)	High oil yield Combining with pretreatment methods helps save time	Expensive due to high solvent consumption Prolonged time, component alteration
Pressurized liquid extraction (PLE)	Leaves: 42.27 (25)	High oil yield, short time, clean extracts Low solvent consumption, high quality	High demand for technique, high cost, small sample

Table 3. Application of *Citrus hystrix* essential oil combined with other compounds in food

Technique	Combination material	Application	Result	Ref.
Edible film	Fish skin gelatin	Antioxidant	ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity and ferric reducing antioxidant power	(34)
Edible coatings	Cassava starch-based	Refrigerated fresh beef	Microbiologically, there is a significant reduction in total plate count (TPC) in beef after 14 days Beef quality does not exceed value standards pH stable at 5.34-5.56	(32)
Microencapsulation	Gelatin and gum Arabic	Antibacterial activity	Size about 30-40 µm Antibacterial activity against *Staphylococcus aureus*	(35)
Microemulsion	Tween 80, propylene glycol, water	Medicine	Microemulsion has dimensions of 12.82 ± 0.40 nm The strong antibacterial ability of *T. mentagrophytes* var. *interdigitale*.	(33)

showed an excellent ability to inhibit this fungus and hence offered worthy pharmaceutical applications (33).

So, using *C. hystrix* EO is a solution for future trends because citrus EOs that come from natural plants are considered to be safe, eco-friendly, antimicrobial, and antioxidant, and they can replace synthetic preservatives.

Conclusion

Concern for public health worldwide is increasing; therefore, research to discover a new direction from natural ingredients to replace synthetic substances in food and pharmaceutical products is an inevitable trend. *C. hystrix* EO is a potential research field because of its antimicrobial, antioxidant, anti-inflammatory, and anti-tumour properties and its abundant material resources.

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Authors contributions

Le Pham Tan Quoc and Do Minh Long designed this study. Two authors searched and handled the data, drafted the manuscript, and resolved all the queries of editors and reviewers.

Compliance with ethical standards

Conflict of interest: No conflict of interest was declared by the authors.

Ethical issues: None.

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