Extraction of Essential oils and volatile compounds of Kaffir lime (Citrus hystrix D.C) by hydrodistillation method

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Abstract: Kaffir lime (Citrus hystrix D.C.), a member of the Citrus family, is a lemon tree naturally grown in tropical regions of Asia. Kaffir lime contains a specific aroma currently used worldwide as spices and cosmetics. Kaffir lime leaves are a famous spice for many dishes of Thai cuisine. The peel of Kaffir lime collected in the southwest of Vietnam was used for essential oil extraction using the process of hydro-distillation. During extraction, 100 g Kaffir lime peel was input at 1:3 ratio, at 120 °C for 90 min. The phytochemical profile of Kaffir Lime essential oil was analyzed by using gas chromatography/mass spectrometry (GC/MS) assay. The optimal essential oil yield reached 4.6%. A total of twenty-six components have been identified to account for 99.998% of essential oils. Β-pinene (47.926%) was characterized as the main ingredient of peel oil. Other major components of Kaffir lime essential oil included D-limonene (24.121%), citronelal (11.84%), and α-pinene (2.834%). The present study evaluated the chemical compositions of Kaffir lime essential oil and compared with other previous studies. Future research on Kaffir lime EO can be expanded by using different extraction methods to improve its bioactive and applicability in many regions.

1. Introduction

In many developing countries, traditional medicinal herbs have been widely used as a source of health treatment [1-4] due to easy accessibility, cost-effectiveness and minimal side effects [5-8]. Essential oils (EOs) from these herbs have become popular in the fields of food, cosmetics and pharmaceuticals [4, 9-13]. Vietnam with tropical weather conditions is very favorable for the growth and development of plants, especially those containing EOs are affirmed to be abundant and unique.

Although the Citrus family (Rutaceae) is well-known for its great potentials, the EOs from fruit peels have not been exploited and utilized. Kaffir lime (Citrus hystrix D.C.), from Citrus family, is a very aromatic fruit originating from tropical Asian countries such as Vietnam, Laos, Indonesia, Malaysia and Thailand.
The native plants are now grown worldwide for spices, foods, and cosmetics. The leaves of this plant are a typical spice in Thai cuisine [14].

In Vietnam, Kaffir lime are commonly grown as an endemic species in Bay Nui, An Giang province. Kaffir lime is a woody tree that grows naturally under direct sunlight and has strong vitality. When the tree reaches about 2-3 m of height, fruits and leaves are harvested for food processing. The volatile components in Kaffir lime EOs are a mixture of hydrocarbons, monoterpenes, sesquiterpenes, ketones, acids, aldehydes, alcohols, and esters.

Many methods and techniques have been used to extract the EOs and volatile compounds from Kaffir Lime such as steam distillation, hydro-distillation, microwave-assisted hydro-distillation, supercritical extract (CO2), cold pressing and Soxhlet extraction methods [15-16]. Among the listed methods, hydro-distillation is simple, safe, reproducible, inexpensive and suitable for industrial applications. The quality of an EO is determined by volatile compounds which can be analyzed by gas chromatography-mass spectrum (GC-MS) analysis. Several volatiles in Kaffir lime peel are known as α-pinene, limonene, camphene, myrcene, γ-terpinene, terpinolene, trans-sabinene hydrate, sabine, β-pinene, copaene, linalool, citronellal, terpinen-4-ol, geraniol, citronellol and δ-cadinene [17-19]. These constituents are highly versatile and mainly used for perfumes, soaps, cosmetics, flavorings, fruit drinks and household products [20, 21]. They are also known for exhibiting antimicrobial properties, such as antibacterial, antifungal and antiviral properties [22, 23]. Composition of the peel EO is influenced by ripening stages of the fruits, conditions and methods of preservation of extraction such as temperature, time and grain size) [14].

Therefore, the purpose of the present study is to analyze the chemical constituents of Kaffir lime peel EO obtained by hydro-distillation and compared with species grown in different areas reported in previous studies. This finding would provide the foundation for promoting research on the application of volatile ingredients in Kaffir lime and other Citrus oils.

2. Materials and methods

2.1. Plant material

Kaffir lime fruits used for EO extraction were purchased from the local market in Bay Nui area, An Giang province (Vietnam). Peel samples were prepared using a sharp knife and the intestine in order to removing pith and control the size and thickness of the sample at approximately 2 mm.

2.2. Extraction of Kaffir lime EO

Kaffir lime peel EO is extracted by hydro-distillation method with Clevenger type device. 100 g of fresh Kaffir lime peels were placed into a flask of distillation system with a ratio of 1:3 peels/solvent. The distillation process took place continuously at a temperature of 120 ºC for 90 minutes. The EO was separated from the aqueous phase, dried on anhydrous sodium sulfate and calculated for resulting EO yield. EOs were stored in dark, glass bottles under low-temperature conditions (4 ºC) until GC-MS analysis.

2.3. GC-MS analysis

The chemical ingredient of Kaffir lime peel EO were determined by GC-MS analysis using GC Agilent 6890 N instrument coupled with the HP5-MS column and MS 5973 inert. The head column pressure was set as 9.3 psi. 25 μL of EO was added with n-hexane and dehydrated with Na2SO4. The flow rate was constant at 1 mL/min. Injector temperature is 250 ºC and the rate of division was 30. Thermal program for samples: 50 ºC kept for 2 min increased by 2 ºC/min to 80 ºC, continued to increase by 5 ºC/min to 150 ºC, continued to increase by 10 ºC/min to 200 ºC, increase 20 ºC/min to 300 ºC hold for 5 min. The compounds were determined by comparing retention indices with Wiley library and published mass spectra.
3. Results and discussion
In this study, the hydro-distillation of Kaffir lime peel collected in Vietnam resulted in a pale yellow almost transparent liquid, with a yield of 4.6% based on fresh weight. The amount of EO yield was varied from region to region. For example, EO extraction of the materials collected from Caringin Central Market, Bandung, West Java, Indonesia was conducted for eight hours by Aripin et al. (2015) to extract the EO with an efficiency of 2.26% [15]. On the other hand, Kaffir lime grown in Songkhla (Thailand) recovered 2.56% of content through hydro-distillation by Chanthaphon et al [14]. Sreepian et al. (2016) conducted an analytical study on Kaffir lime peels from Chiang Rai province (Thailand), which had been extracted by using steam distillation. The yield of EO extracted from Kaffir lime was 2.5% (w/v). As compared to these previous studies, the amount of EO yield obtained in the present study was relatively higher.

Table 1 listed the retention time indices, names and the percentage content of the 26 identified volatile compounds present in Kaffir lime EO peel, accounting to 99.998% of the EO obtained by hydro-distillation.

| Peak | R.T. | Compounds               | Pct Total |
|------|------|-------------------------|-----------|
| 1    | 7.01 | α-thujene               | 0.189     |
| 2    | 7.24 | α-pinene                | 2.834     |
| 3    | 7.826| Camphene                | 0.165     |
| 4    | 9.123| β-pinene                | 47.926    |
| 5    | 9.907| β-myrcene               | 1.159     |
| 6    | 11.099| Unknown                | 0.528     |
| 7    | 11.789| D-limonene            | 24.121    |
| 8    | 13.473| γ-terpinene            | 0.86      |
| 9    | 14.309| cis-Linalool oxide     | 1.015     |
| 10   | 15.219| Cyclohexene            | 0.123     |
| 11   | 15.271| trans-Linalool oxide   | 0.655     |
| 12   | 16.108| β-linalool             | 0.575     |
| 13   | 19.224| Citronellal            | 11.84     |
| 14   | 20.207| 4-terpineol            | 2.514     |
| 15   | 20.897| α-terpineol            | 1.015     |
| 16   | 22.696|(R)-(+)β-citronellol   | 0.72      |
| 17   | 27.109| Citronellyl acetate    | 0.273     |
| 18   | 27.673| Copaene                | 0.597     |
| 19   | 28.133| β-cubebene             | 0.372     |
| 20   | 28.196| β-elemen               | 0.168     |
| 21   | 28.991| Caryophyllene          | 0.503     |
| 22   | 29.995| α-caryophyllene        | 0.163     |
| 23   | 30.779| Germacrene D           | 0.388     |
| 24   | 31.208| Cyclohexane            | 0.123     |
| 25   | 31.877| β-cadinene             | 0.866     |
| 26   | 32.483| Elemol                 | 0.306     |
|      |      | Total                  | 99.998    |
As indicated by five peaks in the GC-MS result, five main constituents included d-limonene (24.121%), β-pinene (47.926%) and α-pinene (2.834%) of monoterpane hydrocarbons, while citronellal (11.84%) and 4-terpineol (2.514%) were the constituents of oxygen monoterpenes (Figure 1). The composition of the remaining 21 compounds ranges from 0.123 to 1.159%. However, in another study, the main compounds of Kaffir lime peels EO in the Pallepola region, Sri Lanka were reported as 3-carene (18.310%), d-limonene (11.538%), citronellal (12.267%), α-pinene (9.244%), copaene (4.290%) and α-cadinene (4.290%), caryophyllene (3.988%), linalool (4.020%) and γ-cadiene (3.544%) accounting for about 71% of the total amount of detected compounds [23]. While Kaffir lime EO in Banda Aceh in northwestern Indonesia showed 30 identified compounds, including pin-pinene (23.03%), sabine (13.37%), terpinene-4-ol (11.43%), limonene (10.59%) and citronella (10.41%) [24].

On the other hand, d-limonene (31.24%), β-pinene (13.81%), citronella (13.41%), terpinene-4-ol (8.28%), citronellol (5.62%), bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl) (7.18%) and γ-terpineol (3.63%) were extracted from Kaffir lime peel oil collected from Caringin Central Market, Bandung, West Java, Indonesia [15]. Chanthaphon et al has employed methods of solvent extraction (i.e. ethyl acetate) and hydro-distillation to extract Kaffir lime EO. The chemical composition resolution results were completely different, in which ethyl acetate extract majorly consisted of limonene (31.64%), β-pinene (6.83%) and citronellal (25.99%), while hydro-distillated contained β-pinene (30.48%), citronellal (15.66%) and sabinene (22.75%) as the main components [14]. The diversity of volatile compounds in Kaffir lime may be due to the different techniques used for extraction. In addition, it is also dependent on climatic conditions, growth stages of trees and harvesting time [25]. The presence of different bioactive components determines a variety of biological activities, typically the antimicrobial activity which can affect different groups of microorganisms, thereby contributing to quality of the EO.
4. Conclusion

Extraction process of Kaffir lime EO employed hydro-distillation method and achieved high efficiency of 4.6%. Results of GC-MS analysis have identified 26 main ingredients, accounting for 99.998% of the total content of EO. β-pinene (47.926%) and d-limonene (24.121%) are the two compounds that had the highest content and determined the quality of Kaffir lime EO. The results of the study also provided some information about the volatile compounds present in Kaffir lime peel EO in Vietnam as compared to other previously reported studies. At the same time, the research has opened the potential of Kaffir lime EO, contributing to economic development in the region. However, Kaffir lime EO needs to expand its research, using different extraction methods to improve its bioactive and applicability in many regions.

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