Korean J Food Preserv, 30(3), 395-404 (2023)
https://doi.org/10.11002/kjfp.2023.30.3.395
pISSN: 1738-7248, eISSN: 2287-7428
Korean Journal of Food Preservation

Research Article

Chemical profiles and biological activities of essential oil of *Citrus hystrix* DC. peels

Do Minh Long[1], Le Pham Tan Quoc[1]*, Tran Thi Phuong Nhung[1], Vuong Bao Thy[2], Nguyen Le Quynh Nhu[3]

[1]*Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City, Ho Chi Minh City 700000, Vietnam*
[2]*Faculty of Health Sciences, University of Cuu Long, Vinh Long 85000, Vietnam*
[3]*Pham Ngoc Thach University of Medicine, Ho Chi Minh City 700000, Vietnam*

Abstract Essential oil (EO) was extracted from the peel of *Citrus hystrix* DC. originating from Tinh Bien, An Giang province (Vietnam), using steam distillation. The study aimed to determine some physicochemical properties of *Citrus hystrix* peel EO (ChpEO), including the acid value (AV), saponification value (SV), ester value (EV), density, specific gravity, and freezing point. The chemical composition was also analyzed by gas chromatography-mass spectrometry (GC-MS). Compounds like β-pinene (30.19%), D-limonene (22.15%), and sabinene (21.37%), with antioxidant and antibacterial properties, had a relatively high content. The EO was also capable of inhibiting the growth of both Gram-positive and Gram-negative bacteria, including *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 25923), *Salmonella typhimurium* (ATCC 13311), and *Bacillus cereus* (ATCC 11778) specifically.

Keywords biological activities, chemical profiles, *Citrus hystrix* DC. peels, essential oil

OPEN ACCESS

Citation: Long DM, Quoc LPT, Nhung TTP, Thy VB, Nhu NLQ. Chemical profiles and biological activities of essential oil of *Citrus hystrix* DC. peels. Korean J Food Preserv, 30(3), 395-404 (2023)

Received: April 21, 2023
Revised: May 30, 2023
Accepted: June 09, 2023

***Corresponding author**
Le Pham Tan Quoc
Tel: +084 028-38940 390, 666/555/891
E-mail: lephamtanquoc@iuh.edu.vn

1. Introduction

Kaffir lime, also known as Thai lime, is a tropical fruit of the genus Citrus in the Rutaceae family (Kaffir lime is the more common name). Its scientific name is *Citrus hystrix* DC. (Srifuengfung et al., 2020). The kaffir lime tree is about 3-5 m high and its fruit is round to egg-shaped and often has a distinct nipple-like structure near the apex. They have thick, wrinkled green skin that turns yellow after ripening (Abirami et al., 2014). Essential oils (EO) exist in the leaves, flowers, and fruit; the oils exist in the highest concentrations in the green skin of the fruit. The EO content of the kaffir lime fruit is 3 to 5 times higher than other citrus fruits in the same family (Giao et al., 2022).

People use almost every part of the plant, such as the leaf, fruit, seed, and root, to treat many diseases. Phytochemicals isolated from different parts of *C. hystrix* have been found to have various pharmacological activities, such as antibacterial, antifungal, antioxidant, anti-inflammatory, and anticancer activities (Abirami et al.,

2014). The EO of *C. hystrix* was reported to have antibacterial and anti-inflammatory activity against some typical bacteria, such as *Propionibacterium acnes*. D-limonene in EO could also inhibit *P. acnes* and reduce inflammation, which in turn reduced post-acne scar formation and faded acne dark spots (Lertsatitthanakorn et al., 2006). On the other hand, *C. hystrix* EO extracted from fruit peel showed anti-proliferative effects on human skin fibroblasts and melanoma cells A375 and WM793 (Borusiewicz et al., 2017). It also inhibited the growth of dandruff-causing *Candida albicans* on the scalp (Foo-trakul and Watchiradatsatiean, 2005).

The bioactive compounds in *C. hystrix* peel EO (ChpEO) are pretty diverse such as D-limonene, β-pinene, sabinene, citronellal, terpinen-4-ol, etc. (Sreepian et al., 2019). Their chemical composition and content differ when collected in different regions, harvest times, and extraction methods. The obtained EOs have usually been isolated using steam or hydro distillation. In addition to its medical and pharmaceutical technology application, ChpEO is also noticed in the agricultural field. It can be considered an antifungal agent, for instance, Chitaree et al. (2021) proved that *C. hystrix* EO could be applied to protect against mango fungal contamination (*Colletotrichum gloeosporioides*). For the food industry, the EO of *C. hystrix* peel is of little interest and is mainly used for culinary purposes.

In fact, there have been many studies on *C. hystrix* EO; however, the EO from different locations has different chemical compositions and properties. Meanwhile, *C. hystrix* is quite commonly cultivated in Tinh Bien, An Giang province (Vietnam), mainly for its fruit. The EO in the fruit peel of *C. hystrix* is considered a by-product, so its value has not been fully exploited yet. This study aims to determine the chemical composition and some physicochemical and biological properties of *C. hystrix* EO to further understand the value of this EO and its potential application in food technology and pharmacology.

2. Materials and methods

2.1. Plant extraction

The EO was extracted from the peel of *C. hystrix* harvested in the Tinh Bien district, An Giang province (Vietnam) (Coordinates: 10°37'55.6"N 104°59'34.8"E). The fruits had reached the desired maturity (3-4 months old) and the weight of each fruit was 46.84±5 g. The peel was used to extract the EO by steam distillation. The strong point of steam distillation is that it produces clean and pure oils. The obtained EOs can contain many temperature-sensitive compounds with specific biological properties that may be damaged or changed by other extraction methods. The fruits were washed and dried naturally at room temperature, then peeled and cut into small pieces. On average, about 2.5 kg of whole fruit produced 1 kg of peel; the per-batch yield was 50-60 kg peel/batch. The distillation time was 3 h at 100℃. The yield of the distillation process was about 2.8%. The obtained EO was stored in dark glass jars at room temperature.

2.2. Bacterial strains

The bacteria used in this study included 4 strains: 2 gram-positive bacteria, *Staphylococcus aureus* (ATCC 25923) and *Bacillus cereus* (ATCC 11778), and 2 gram-negative bacteria, *Pseudomonas aeruginosa* (ATCC 27853) and *Salmonella typhimurium* (ATCC 13311). They were provided by the Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City.

https://doi.org/10.11002/kjfp.2023.30.3.395

2.3. Chemicals

The chemicals used in this study included 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma, St. Louis, Missouri, USA) and dimethyl sulphoxide (DMSO, Nanjing, China). The culture and antibacterial media included Mueller Hinton Agar (HiMedia, Thane, India), Nutrient Broth (HiMedia, Thane, India), and other chemicals meeting analytical standards.

2.4. Determination of physicochemical properties of the EO

The physicochemical properties of the EO, such as the freezing point, acid value (AV), saponification value (SV), ester value (EV), specific gravity, and density, were determined respectively according to following standards: ISO 1041 (1973), ISO 1242 (2023), ISO 3657 (2013), and ISO 279 (1998).

2.5. Analysis of chemical composition of the EO

The chemical composition of the EO was analysed by gas chromatography-mass spectrometry analysis method (GC-MS) according to the method described by Van et al. (2022) with some minor changes. First of all, 20 μL of sample was dissolved in methanol and made up 1 mL with methanol. Then, 1 mL of EO was injected into an Agilent DB-5MS gas chromatograph with a capillary column (30 m×0.25 mm, 0.25 μm) at an injection temperature of approximately 220℃. The temperature was set up to 70℃ in 1 min, then increased by 12℃/min to reach 280℃ and then held for 10 min. The mass range was 29-650 amu. Helium was used as the carrier gas at a constant flow rate of 1.2 mL/min.

2.6. Determination of antioxidant capacity (AC) of the EO

The antioxidant capacity (AC) of ChpEO was assessed in terms of the radical-scavenging activity (RSA) against DPPH according to the procedure described by Quoc (2020) with some slight modifications. The EO was dissolved in ethanol (96%) to obtain different concentrations (1,040, 520, 260, 130, 65, and 32.5 mg/mL). Then, 0.3 mL of the obtained EO was mixed with 2.7 mL of 0.1 mM DPPH. The mixture was kept in the dark at room temperature for 30 min. The decolourisation of DPPH was determined by measuring the absorbance at a wavelength of 517 nm using a spectrophotometer (Genesys 20, Thermo Scientific, Waltham, Massachusetts, USA). The AC of the EO was compared with ascorbic acid, as a control. The AC was calculated using the formula below:

$$DPPH_{RSC}\ (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

$A_{control}$: Absorbance of a solution containing only DPPH solution.

A_{sample}: Absorbance of the EO sample in the presence of DPPH.

The AC of the EO was estimated by the IC_{50} value (concentration of the sample required to inhibit 50% of DPPH free radical scavenging).

2.7. Determination of antibacterial activity (AA) of the EO

The antibacterial activity (AA) of the EO was determined by the paper disc diffusion method according to the procedure of Quoc (2021). First, 100 μL of bacterial suspension (0.5 McFarland, approximately 1.5×10^8 CFU/mL) was spread on a Petri dish containing Mueller Hinton Agar medium. Then, sterile paper plates (6 mm diameter) impregnated with EO (5 μL/disc) were placed on the plate with gentamicin (10 μg/disc) and DMSO

solution (5%), which were used as the positive and negative controls, respectively. The plates were then incubated at 37℃ for 24 h. The AA was assessed by measuring the diameter of the bacterial inhibition zone in millimetres (mm).

2.8. Data analysis

Three independent experiments were performed, and the results were expressed as mean±standard deviation (SD). The Statgraphics Centurion XV software was used for the statistical analysis of the results, using one-way analysis of variance (ANOVA) and Fisher's least significant difference (LSD) procedure with a significance level of 5%.

3. Results and discussion

3.1. Physicochemical properties of C. hystrix peel EO

The obtained EO is an insoluble liquid in water, has a pale yellow colour, a characteristic aroma, and is very volatile. Its smell is similar to grapefruit EO and lemon EO.

The physical and physicochemical properties of the EO are expressed in Table 1 through parameters such as the freezing point, density, specific gravity, AV, SV, and EV. The freezing point of ChpEO was measured at -40℃, but there was no crystallisation phenomenon at this condition. This experiment has shown that the ChpEO has a very low freezing point when compared to *Ageratum conyzoides* L. EO, which had a freezing point of -10.33℃ in the study by Quoc (2020). Thus, the freezing point of ChpEO is much lower than that of *A. conyzoides* L. The freezing point of ChpEO has not been mentioned in previous studies, so it is difficult to compare our results.

Table 1. Physicochemical properties of essential oil from *Citrus hystrix* peel

No.	Items	Value
1	State	Liquid
2	Colour	Pale yellow colour
3	Odour	Has a characteristic smell
4	Freezing point	<-40℃
5	Absolute density	0.8478±0.018 g/mL
6	Specific gravity	0.8533±0.0058
7	Refractive index (RI)	1.4699±0.0002
8	Acid value (AV)	0.561±0 mg KOH/g
9	Ester value (EV)	10.66±1.405 mg KOH/g
10	Saponification value (SV)	11.22±1.405 mg KOH/g

The absolute density and specific gravity of the ChpEO was 0.8478 g/mL and 0.8533, respectively. In previous research, a study performed by Wulandari et al. (2019) showed that *C. hystrix* leaf oil had a specific gravity of 0.843, while Husni et al. (2021) indicated an absolute density of 0.87 g/mL for *C. hystrix* peel oil, the materials for both of these studies were collected in Indonesia. It can be clearly seen that there is a negligible difference between these results and the results of our study. In addition, the refractive index (RI) of ChpEO reached 1.4699, compared with a value of 1.5 for *Thymus zygis* EO and 1.33 for *Selaginella willdenowii* EO (Radi et al., 2022). This shows that the RI is completely discrepant with different materials. The RI of EO also indicates the quality of the EO.

The AV and EV are criteria used to evaluate the quality of EOs. In this study, the AV and EV of ChpEO were 0.561 mg KOH/g and 10.66 mg KOH/g, respectively. In comparison, patchouli EO harvested in Indonesia had an AV and EV of 23.9 mg KOH/g and 8.48 mg KOH/g, respectively (Lubis et al., 2022), indicating that the AV of ChpEO was 42.5 times lower than that of patchouli EO, while the EV was not significantly higher. The above results

https://doi.org/10.11002/kjfp.2023.30.3.395

demonstrate the special physicochemical properties of ChpEO and highlight its high potential EO in the food and pharmaceutical industry in the future, with a low acid index, and a higher ester index compared with other EOs.

The difference in physicochemical indexes with other studies is due to the difference in raw material sources, the different parts of the plant used to extract the EO, such as the leaves, roots, bark, fruits, etc., as well as the difference in harvest time, which leads to difference in the properties and chemical compositions of the EOs.

3.2. Volatile compounds of C. hystrix peel EO

Table 2 shows that ChpEO has 22 volatile compounds identified, which account for 99.98% of the total number of compounds identified. The number of compounds in this study was significantly

Table 2. Volatile compounds of essential oil from *Citrus hystrix* peel

No.	Rt. (min)	Compounds	Percentage (%)
1	3.16	2-Thujene	0.28
2	3.27	α-Pinene	3.74
3	3.46	Camphene	0.17
4	3.69	**Sabinene**	**21.37**
5	3.78	**β-Pinene**	**30.19**
6	3.82	β-Mycrene	1.72
7	4.19	α-Terpinene	0.86
8	4.35	**D-Limonene**	**22.15**
9	4.38	β-Phellandrene	1.04
10	4.68	γ-Terpinene	1.46
11	4.83	cis-Linalool oxide	1.20
12	5.03	trans-Linalool oxide (furanoid)	0.96
13	5.16	Linalool	0.62
14	5.81	Citronellal	6.17
15	6.24	Terpinen-4-ol	4.88
16	6.41	α-Terpineol	1.77
17	8.15	Citronellol acetate	0.18
18	8.57	Copaene	0.31
19	8.69	β-Cubebene	0.20
20	9.09	Caryophyllene	0.22
21	9.75	Germacrene D	0.15
22	10.10	Cadina-1(10), 4-dien	0.34
Total:			**99.98%**
Monoterpene hydrocarbon			83.52%
Monoterpenoids			15.78%
Sesquiterpene hydrocarbon			0.68%

different from *C. hystrix* EO obtained from other locations. For instance, leaf oil originating in Malaysia had 29 compounds accounting for 99.75% (Loh et al., 2011), while peel oil from Thailand had 27 compounds accounting for approximately 89.98% (Sreepian et al., 2019). In general, the chemical composition of *C. hystrix* EO fluctuates quite considerably, which might be due to climate, plant variety and soil characteristics in each area, as well as differences in conditions and extraction methods. The main compounds of ChpEO were also present in juniper EO, as in the study by Zheljazkov et al. (2017), including α-pinene, β-pinene, β-myrcene, sabinene, and limonene.

The major compounds of ChpEO identified in this study were β-pinene (30.19%), D-limonene (22.15%), and sabinene (21.37%), and these compounds were also present in ChpEO from Malaysia and Thailand. However, there was an insignificant variation in the content of the main chemical compositions; for instance, β-pinene, limonene, and sabinene accounted for 21.1%, 25.28% and 14.99% respectively in ChpEO from Thailand (Sreepian et al., 2019). Chanthaphon et al. (2008) also reported β-pinene as having the highest concentration in ChpEO in Thailand. In addition, sabinene might contribute to the anti-microbial activity (Chanthaphon et al., 2008) and D-limonene has been shown to have a powerful antioxidant activity against cancer (Miller et al., 2011). Thereby, it can be seen that ChpEO had many precious compounds with different biological activities. The combination of these substances with different ratios in the EO can also create special effects due to their ability to interact synergistically. Some precious compounds with high biological activity but low content were also identified in the oil, such as germacrene D (0.15%), camphene (0.17%), caryophyllene (0.22%), etc. This finding demonstrates that ChpEO is a potential material worthy of future research for application in the food and medical fields.

Based on the obtained results, the chemical composition of ChpEO includes: Monoterpene (monoterpene hydrocarbon and monoterpenoids) and Sesquiterpene. There were 19 of the most predominant substances belonging to the Monoterpene group (99.3%), of which Monoterpene hydrocarbons accounted for 83.52% and Monoterpenoids accounted for 15.78%. Three compounds belonged to the Sesquiterpene group, with 0.68% Sesquiterpene hydrocarbon including copaene, caryophyllene, and germacrene D. Therefore, it can be seen that the characteristic composition of ChpEO is Monoterpenes (99.3%), which is also the most common group present in most EOs.

3.3. Antioxidant capacity (AC) of C. hystrix peel EO

The AC is one of the most important properties for determining the quality of an EO or its potential for application in the preservation of food products. With an increasing concentration of EO from 32.5 to 1,040 mg/mL, Fig. 1 shows that the AC becomes more effective when the amount of ChpEO increases.

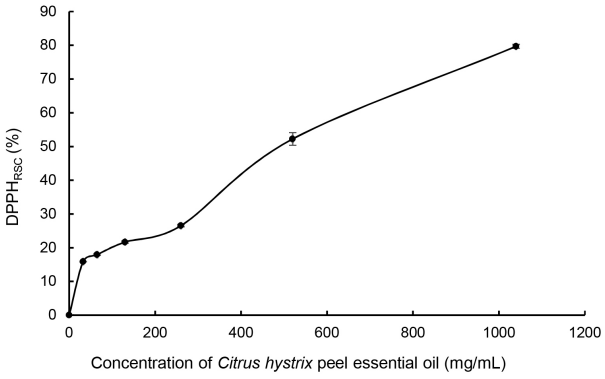

Fig. 1. The antioxidant capacity of essential oil from *Citrus hystrix* peel.

https://doi.org/10.11002/kjfp.2023.30.3.395

The AC increased rapidly from a concentration of 130 to 520 mg/mL and increased slowly from a concentration of 520 to 1,040 mg/mL. The IC_{50} of ChpEO was determined at approximately 500 mg/mL and the AC reached 79.6% at an EO concentration of 1,040 mg/mL. In particular, the AC of the pure EO only inhibited about 92%. ChpEO showed a much lower AC than the control sample ascorbic acid. This is acceptable because ascorbic acid is a potent antioxidant and its IC_{50} was determined at a concentration of 36 μg/mL (Fig. 2).

According to previous studies, most EOs have antioxidant properties. When compared with ChpEO from other regions, the IC_{50} of this study was approximately 500 mg/mL, while ChpEO from Indonesia had an IC_{50} of 72.78 mg/mL according to the study of Zuhra et al. (2014), and this value (IC_{50} = 500 mg/mL) was 〉250 μg/mL for material from Thailand (Wungsintaweekul et al., 2010). According to the study by Othma et al. (2023), the IC_{50} of EO from *C. hystrix* leaves was 279.03 mg/mL, which is 1.8 times lower than the peel oil in this study, indicating that the AC of leaf oil is stronger. The difference in AC of EOs can greatly depend on their origin, growing regions, and chemical composition. The AC of ChpEO is caused by the presence of several strong antioxidant compounds, especially β-pinene (30.19%), D-limonene (22.15%), and sabinene (21.37%). These substances have been shown to have good AC (Amorati et al., 2013).

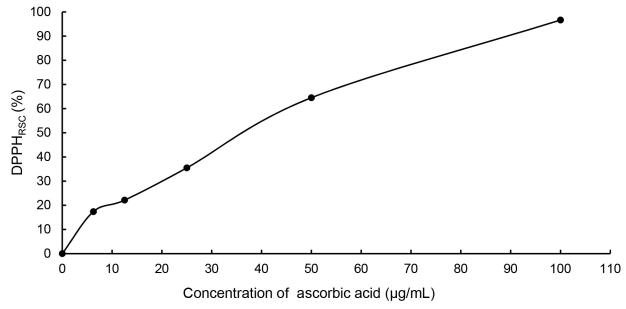

Fig. 2. The antioxidant capacity of ascorbic acid.

3.4. Antibacterial activity (AA) of C. hystrix peel EO

Table 3 shows that the inhibitory ability of gentamicin in ascending order is as follows: *S. typhimurium* / *S. aureus* / *P. aeruginosa* 〈 *B. cereus*. The diameters of the inhibition zone of gentamicin range from 12.67 to 15.33 mm. The obtained results indicate that the tested bacteria, including *S. aureus*, *S. typhimurium*, and *P. aeruginosa* (inhibition diameter between 8 and 14 mm), are sensitive to gentamicin, while *B. cereus* (inhibition diameter between 14 and 20 mm) is very sensitive to gentamicin (Sebei et al., 2015). In addition, for ChpEO, the ascending order of bacterial inhibition is as follows: *S. typhimurium* 〈 *P. aeruginosa* / *B. cereus* 〈 *S. aureus*. The AA is strongest against *S. aureus* (inhibition diameter of 22.33 mm) and weakest against *S. typhimurium* (inhibition diameter

Table 3. Antibacterial activity of essential oil from *Citrus hystrix* peel

No.	Bacteria	Diameter of the inhibition zone of ChpEO (mm)	Diameter of the inhibition zone of positive control (gentamicin, mm)
1	*B. cereus*	21 ± 0^{Bb}	15.33 ± 0.58^{Ab}
2	*S. aureus*	22.33 ± 0.58^{Bc}	13 ± 1^{Aa}
3	*S. typhimurium*	19 ± 1^{Ba}	12.67 ± 1.15^{Aa}
4	*P. aeruginosa*	20.67 ± 0.58^{Bb}	13.33 ± 0.58^{Aa}

$^{a-c}$Different lowercase in the same column denote significant differences ($p<0.05$) with respect to bacterial strains.
^{A,B}Different capital in the same row denote significant differences ($p<0.05$) with respect to the antibacterial agents.

of 19 mm). *B. cereus*, *S. aureus*, and *P. aeruginosa* are extremely sensitive to ChpEO (inhibition diameter ≥ 20 mm). Notably, all inhibitory zone diameters for the EO were superior to those of the positive control (gentamicin) with regard to the 4 strains of Gram-negative and Gram-positive bacteria used in the study.

The mechanism of action of EOs on microbial cells follows various pathways. For instance, EOs can penetrates into the cell membrane and increase cell membrane permeability, and denature proteins. It can also inhibit ATP synthesis through energy metabolism, or damage the cell membrane and inhibit peptidoglycan synthesis through the cell wall. Through a genetic material pathway, it can inhibit gene expression or impede DNA repair. The antibacterial mechanism of the compounds present in the EO may be different. Therefore, the antibacterial mechanism of an EO is often not a single mode of action but includes many different activities. Genetic material plays a key role in the process of growth, development, and reproduction; and can control the synthesis and metabolism of protein in the body, hence controlling life activities. Previous reports have shown that terpenoids and phenolic compounds in EOs inhibit bacterial DNA replication and repair (Ni et al., 2021).

Given the superior antibacterial properties of ChpEO above, the application of a combination of this oil with other agents is a possibility in the food industry, to increase the effectiveness of the preservation process in future.

4. Conclusions

In summary, the chemical composition of *C. hystrix* EO from fruit peel harvested in Tinh Bien, An Giang province (Vietnam) was analysed by GC-MS and found to contain 22 volatile compounds. The main compounds accounting for a high concentration of the oil include β-pinene (30.19%), sabinene (21.37%), and D-limonene (22.15%). The ChpEO could inhibit the growth of both specific Gram-positive and Gram-negative bacteria, including *P. aeruginosa*, *S. aureus*, *S. typhimurium,* and *B. cereus*. The oil also had specific physicochemical properties (RI, AV, SV, EV, etc.) compared to other raw materials and potentially has a wide application in food preservation technology in order to replace synthetic food preservatives.

Acknowledgements

The authors would like to express their gratitude to the Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City for supporting this research. The authors also thanks to Nguyen Thi Kieu Tham, Dang Thi Anh Thu and Nguyen Thi Kim Phung for their helpful advice on various technical issues examined in this paper.

Conflict of interests

The authors declare no potential conflicts of interest.

Author contributions

Conceptualization: Long DM, Quoc LPT. Methodology: Long DM, Quoc LPT. Formal analysis: Nhung TTP, Thy VB. Validation: Nhu NLQ. Writing - original draft: Long DM. Writing - review & editing: Quoc LPT.

Ethics approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

ORCID

Do Minh Long (First author)
https://orcid.org/0009-0004-3768-8272

https://doi.org/10.11002/kjfp.2023.30.3.395

Le Pham Tan Quoc (Corresponding author)
https://orcid.org/0000-0002-2309-5423
Tran Thi Phuong Nhung
https://orcid.org/0000-0001-5733-8545
Vuong Bao Thy
https://orcid.org/0009-0005-1753-4433
Nguyen Le Quynh Nhu
https://orcid.org/0009-0006-4492-3255

References

Abirami A, Nagarani G, Siddhuraju P. The medicinal and nutritional role of underutilized citrus fruit *Citrus hystrix* (kaffir lime): A review. Drug Invent Today, 6, 1-5 (2014)

Amorati R, Foti MC, Valgimigli L. Antioxidant activity of essential oils. J Agric Food Chem, 61, 10835-10847 (2013)

Borusiewicz M, Trojanowska D, Paluchowska P, Janeczko Z, Petitjean MW, Budak A. Cytostatic, cytotoxic, and antibacterial activities of essential oil isolated from *Citrus hystrix*. ScienceAsia, 43, 96-106 (2017)

Chanthaphon S, Chanthachum S, Hongpattarakere T. Antimicrobial activities of essential oils and crude extracts from tropical *Citrus* spp. against food-related microorganisms. Songklanakarin J Sci Technol, 30, 125-131 (2008)

Chit-aree L, Unpaprom Y, Ramaraj R, Thirabunyanon M. Valorization and biorefinery of kaffir lime peels waste for antifungal activity and sustainable control of mango fruit anthracnose. Biomass Convers Biorefin, 1-15 (2021)

Foo-trakul P, Watchiradatsatiean C. Development of anti-dandruff shampoo from kaffir lime which is the by-product of food industry. Kasetsart J (Nat Sci), 39, 725-729 (2005)

Giao DH, Thao NHT, Ni LTA, Nhi LTT, Thanh LHV, Hanh CLN, Mai NTN. Distillation of kaffir lime (*Citrus hystrix* DC.) essential oil and making of antibacterial liquid soap. Can Tho Univ J Sci, 58, 1-10 (2022)

Husni E, Putri US, Dachriyanus. Chemical content profile of essential oil from kaffir lime (*Citrus hystrix* DC.) in Tanah Datar regency and antibacterial activity. Proc Int Conf Contemp Sci Clin Pharm, 176-183 (2021)

ISO 1041. Essential Oils - Determination of Freezing Point. International Organization for Standardization, Geneva, Switzerland, p 1-4 (1973)

ISO 1242. Essential Oils - Determination of Acid Value by Two Titration Methods, Manual and Automatic. International Organization for Standardization, Geneva, Switzerland, p 1-10 (2023)

ISO 279. Essential Oils - Determination of Relative Density at 20℃. International Organization for Standardization, Geneva, Switzerland, p 1-4 (1998)

ISO 3657. Animal and Vegetable Fats and Oils - Determination of Saponification Value. International Organization for Standardization, Geneva, Switzerland, p 1-9 (2013)

Lertsatitthanakorn P, Taweechaisupapong S, Aromdee C, Khunkitti W. *In vitro* bioactivities of essential oils used for acne control. Int J Aromather, 16, 43-49 (2006)

Loh FS, Awang RM, Omar D, Rahmani M. Insecticidal properties of *Citrus hystrix* DC. leaves essential oil against *Spodoptera litura* Fabricius. J Med Plants Res, 5, 3739-3744 (2011)

Lubis A, Mandang T, Hermawan W, Sutrisno. Characterization of the yield and quality of patchouli oil based on the size of chopping and drying type. IOP Conf Ser: Earth Environ Sci, 1038, 012075 (2022)

Miller JA, Thompson PA, Hakim IA, Chow HHS, Thomson CA. d-Limonene: A bioactive food component from citrus and evidence for a potential role in breast cancer prevention and treatment. Oncol Rev, 5, 31-42 (2011)

Ni ZJ, Wang X, Shen Y, Thakur K, Han J, Zhang JG, Hu F, Wei ZJ. Recent updates on the chemistry,

bioactivities, mode of action, and industrial applications of plant essential oils. Trends Food Sci Technol, 110, 78-89 (2021)

Othman HIA, Alkatib HH, Zaid A, Sasidharan S, Rahiman SSF, Lee TP, Dimitrovski G, Althakafy JT, Wong YF. Phytochemical composition, antioxidant and antiproliferative activities of *Citrus hystrix*, *Citrus limon*, *Citrus pyriformis*, and *Citrus microcarpa* leaf essential oils against human cervical cancer cell line. Plants, 12, 134 (2023)

Quoc LPT. Physicochemical properties and antibacterial activity of essential oil of *Ageratum conyzoides* L. leaves. Agric Conspec Sci, 85, 139-144 (2020)

Quoc LPT. Physicochemical properties, chemical components, and antibacterial activity of *Melaleuca cajuputi* Powell essential oil leaves from Quang Tri province, Vietnam. Bull Chem Soc Ethiop, 35, 677-683 (2021)

Radi ZF, Bouhrim M, Mechchate H, Al-zahrani M, Qurtam AA, Aleissa AM, Drioiche A, Handaq N, Zair T. Phytochemical analysis, antimicrobial and antioxidant properties of *Thymus zygis* L. and *Thymus willdenowii* Boiss. essential oils. Plants, 11, 15 (2022)

Sebei K, Sakouhi F, Herchi W, Khouja ML, Boukhchina S. Chemical composition and antibacterial activities of seven *Eucalyptus* species essential oils leaves. Biol Res, 48, 7 (2015)

Sreepian A, Sreepian PM, Chanthong C, Mingkhwancheep T, Prathit P. Antibacterial activity of essential oil extracted from *Citrus hystrix* (kaffir lime) peels: An *in vitro* study. Trop Biomed, 36, 531-541 (2019)

Srifuengfung S, Bunyapraphatsara N, Satitpatipan V, Tribuddharat C, Junyaprasert VB, Tungrugsasut W, Srisukh V. Antibacterial oral sprays from kaffir lime (*Citrus hystrix* DC.) fruit peel oil and leaf oil and their activities against respiratory tract pathogens. J Tradit Complementary Med, 10, 594-598 (2020)

Van HT, Dam SM, Phan UTX, Nguyen TNA, Nguyen TBT, Tran TL, Luu TN, Le VS, Huynh NTA. Chemical diversity of essential oils from aerial parts of eight species of Zingiberaceae family from Vietnam. Acta Univ Agric Silvic Mendelianae Brun, 70, 273-281 (2022)

Warsito W, Noorhamdani N, Sukardi S, Suratmo S. Assessment of antioxidant activity of citronellal extract and fractions of essential oils of *Citrus hystrix* DC. Trop J Pharm Res, 17, 1119-1125 (2018)

Wulandari YW, Darmadji P, Anwar C, Supriyadi S. Comparison between hydrodistillation with steam explosion and conventional hydrodistillation in kaffir lime oil extraction. J Agritech, 39, 306-314 (2019)

Wungsintaweekul J, Sitthithaworn W, Putalun W, Pfeifhoffer HW, Brantner A. Antimicrobial, antioxidant activities and chemical composition of selected Thai spices. Songklanakarin J Sci Technol, 32, 589-598 (2010)

Zheljazkov VD, Semerdjieva IB, Dincheva I, Kacaniova M, Astatkie T, Radoukova T, Schlegel V. Antimicrobial and antioxidant activity of *Juniper galbuli* essential oil constituents eluted at different times. Ind Crops Prod, 109, 529-537 (2017)

Zuhra CF, Lenny S, Nurtjahya K. Comparison of antimicrobial and antioxidant activities of essential oils from the leaf and peel *Citrus hystrix*. Proc Int Conf Nat Environ Sci, 67-72 (2014)

https://doi.org/10.11002/kjfp.2023.30.3.395