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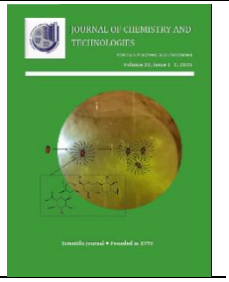

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EVALUATION OF THE CHEMICAL COMPOSITION, ANTIOXIDANT CAPACITY, AND ANTIBACTERIAL ACTIVITY OF ESSENTIAL OIL FROM MANDARIN PEELS (*CITRUS RETICULATA* L.) GROWN IN DONG THAP PROVINCE, VIETNAM

Pham My Hao, Le Pham Tan Quoc*, Do Thi Quynh Hoa, Dang Thi My Tram, Truong Quoc Thao
Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City, Ho Chi Minh City, 700000, Vietnam
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Abstract

***Citrus reticulata* L. essential oil (CrEO) is widely used in food technology, cosmetics, and pharmaceuticals due to its distinct aroma and bioactive properties. In this study, *Citrus reticulata* L. was collected in Dong Thap province, Vietnam and its peel essential oil was extracted through distillation. Several physicochemical properties of the oil were analyzed, including acid, saponification, and ester values, as well as relative and absolute density, freezing point, and fragrance retention. The results showed that CrEO exhibited an acid value of 0.748 mg KOH/g, a saponification value of 67.320 mg KOH/g, and an ester value of 66.572 mg KOH/g, with fragrance retention lasting over 33 h. The chemical composition of CrEO was determined by gas chromatography-mass spectrometry (GC-MS), and the results revealed six main components namely *α*-Pinene (0.93 %), *β*-Pinene (0.61 %), *β*-Myrcene (1.88 %), D-Limonene (96.09 %), Linalool (0.16 %), and *α*-Terpineol (0.33 %), which are monoterpenes. Furthermore, the study showed the weak antioxidant capacity of oil and strong antibacterial activities against pathogenic bacteria were determined using the paper disc diffusion method.**

Keywords: antibacterial activity; antioxidant capacity; oil; physicochemical properties.

ОЦІНКА ХІМІЧНОГО СКЛАДУ, АНТИОКСИДАНТНОЇ ЗДАТНОСТІ ТА АНТИБАКТЕРІАЛЬНОЇ АКТИВНОСТІ ЕФІРНОЇ ОЛІЇ ЗІ ШКІРКИ МАНДАРИНА (*CITRUS RETICULATA L.*), ВИРОЩЕНОГО В ПРОВІНЦІЇ ДОНГТХАП, В'ЄТНАМ

Фам Мі Хао, Ле Фам Тан Куок, До Тхі Куїн Хоа, Данг Тхі Мі Трам, Труонг Куок Тхао
Інститут біотехнології та харчових технологій, Індустріальний університет Хошиміну, Хошимін, 700000, В'єтнам

Анотація

Ефірна олія *Citrus reticulata L.* була зібрана в провінції Донгтхап, В'єтнам. Були визначені деякі фізико-хімічні властивості (кислотність/омилення/ефірне число, відносна/абсолютна густина, температура замерзання та збереження аромату) ефірної олії. Хімічний склад ефірної олії мандарина визначали методом газової хроматографії-мас-спектрометрії (ГХ-МС), і результати виявили шість основних компонентів, а саме: α-пінен (0.93 %), β-пінен (0.61 %), β-мірцен (1.88 %), D-лимонен (96.09 %), ліналоол (0.16 %) та α-терпінеол (0.33 %), які є монотерпенами. Крім того, дослідження показало слабку антиоксидантну здатність олії та сильну антибактеріальну активність проти патогенних бактерій, визначену за допомогою методу дифузії на паперовому диску.

Ключові слова: антибактеріальна активність; антиоксидантна здатність; олія; фізико-хімічні властивості.

**Corresponding author: e-mail: lephamtanquoc@iuh.edu.vn*

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Introduction

Citrus reticulata L. is a plant species that produces small-segmented fruits and is widely cultivated in tropical and subtropical regions. This plant species is quite common and can be found in almost all provinces and cities in Vietnam. However, the largest production of mandarins is concentrated in regions including the Mekong Delta in provinces including Dong Thap, Tien Giang, Vinh Long, An Giang, and Can Tho. They contain important chemical compounds, such as vitamins, phenolic compounds, and flavonoids, which have been widely used in basic scientific research and the pharmaceutical industry. In addition, it also contains a significant amount of ascorbic acid (vitamin C), which plays an important role in the food industry as a food additive and antioxidant [1]. Furthermore, mandarin peel essential oil is widely used as a food additive and antioxidant snd has been shown to exhibit antioxidant, anti-diabetic, insecticidal, antibacterial, and antifungal properties, including against *Staphylococcus aureus* and *Escherichia coli* [2]. Brah et al. found that mandarin essential oil is used in food production, perfume manufacturing, and skincare products [3]. Moreover, *C. reticulata* essential oil (CrEO) contains secondary metabolites that help protect plants against insects, herbivores, and microorganisms.

There are various methods for extracting volatile compounds such as essential oil (EO) from plant materials, ranging from traditional to modern techniques. Each method has its own advantages and disadvantages, which determine the biological and physicochemical properties of EOs. Traditional methods include water distillation, steam distillation, water distillation by diffusion, and solvent extraction, while modern methods include supercritical fluid extraction, subcritical fluid extraction, and solvent-free microwave extraction [4]. Although modern methods can reduce energy consumption and shorten the extraction time, the number of by-products from Dong Thap mandarin raw materials is relatively high. Therefore, a feasible method in terms of raw material input is needed. Water distillation is an optimal choices because of its higher yield and lower sensitivity compared to other traditional methods [5].

Although there have been many studies related to mandarin EO, most of research focused on the properties or applications of mandarin EOs in different regions. In Vietnam, Dong Thap mandarins are mainly grown for fruit production, but the amount of fruits that meets this standard is not high. Therefore, utilizing the by-products of mandarin fruits to produce EO is a potential solution. This not only solves the problem of reducing agricultural waste but also enhances the income of mandarin growers. This is the first time that mandarin EO has been extracted and produced in this region. It is also a prerequisite for clarifying the characteristics and chemical composition of mandarin EO, as it may have unique differences from that produced in other regions.

Materials and methods

Plant material

The mandarin fruits originated from Dong Thap, Vietnam, and during harvesting and sorting, fruits that did not meet the standard were collected for EO extraction. The EO was then extracted using the steam distillation method with an average yield of 0.9 % (w/w) [6].

Bacterial strains

In this study, four bacterial strains including two Gram-positive strains, *Staphylococcus aureus* (ATCC 29213) and *Bacillus cereus* (ATCC 11778), and two Gram-negative strains, *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853), were used to determine the antibacterial activity in this study.

Chemicals and reagents

The DPPH (1,1-diphenyl-2-picrylhydrazyl) reagent was purchased from Sigma-Aldrich (USA). The culture and antibacterial media included Nutrient Broth (NB, HiMedia, India) and Mueller Hinton Agar (MHA, HiMedia, India). The chemicals used in this study included dimethyl sulfoxide (DMSO, China), ethanol (Vietnam), and others meeting analytical standards.

Determination of physicochemical properties of CrEO

The following physicochemical properties of EOs were evaluated: acid value (AV), saponification value (SV), relative density (RD), absolute density (AD), freezing point (FP) and refractive index (RI). They were determined according to the following standards: ISO 1242, 3657, 279, 1041, and 280, respectively [7–11].

Determination of fragrance retention (FR) of CrEO

The fragrance retention (FR) of CrEO was determined following Mahajan [12]. FR was determined using the CrEO concentrations and FR time with some minor adjustments. The EO was dissolved in 96 % ethanol at different ratios (100, 80, 60, 40, and 20 %, v/v). FR test paper

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was used to determine the FR time of the EO at the above ratios under normal conditions.

Chemical component analysis of essential oils by GC-MS analysis

The chemical composition of ObEO was analyzed by GC–MS according to the procedure described by Hao and Quoc [13]. A volume of 1 µL of EO was injected into a gas chromatograph (Shimadzu, Nexis GC-2030, Japan) with a universal capillary column (Rtx-5sil-MS, 30 m×0.25 mm×0.25 µm, Restek Technologies, USA) equipped with a quadrupole mass analyzer (Shimadzu GC-MS-QP2020 NX, Japan). Helium was the carrier gas with a constant flow rate of 3 mL/min and a 10 : 1 split ratio. The injection temperature was 250 °C and the temperature program was set as follows: initial temperature at 50 °C, hold for 2 min, increase until 250 °C at a rate of 10 °C/min and hold for 5 min, increase to 280°C at a rate of 10°C /min and hold for 3 min. Mass spectra were recorded at 70 eV ionization energy in EI mode.

Determination of antioxidant capacity (AC) of CrEO

The antioxidant capacity of the EO was determined following Gouderm and Ligamallu [14] with some minor modifications. CrEO was dissolved in 96 % ethanol at different concentrations, and 0.3 mL of the solution was mixed with 3.7 mL of DPPH solution (0.1 mM) and incubated for 30 min in the dark. The absorbance was measured at 517 nm using a spectrophotometer (Genesys 20, Thermo Scientific, USA). Vitamin C was used as the control sample. The free radical scavenging rate of DPPH was determined based on the inhibitory IC_{50}. The percentage of inhibition was calculated using the following formula:

$$\% \text{ Inhibition } = \frac{A_0 - A_1}{A_0} \times 100\ \%$$

where A_0 is the absorbance of DPPH and A_1 is the absorbance of the mixture of EO and DPPH.

Determination of antibacterial capacity (AC) of CrEO

The antibacterial activity (AA) was determined using the paper disc diffusion method described by Hindler et al. [15] with minor modifications. First, 100 µL of bacterial suspension (0.5 McFarland standard, approximately 1.5×10^8 CFU mL^{-1}) was spread on Mueller-Hinton agar (MHA) medium using a swab. Then, sterile paper discs (6 mm diameter) were impregnated with 5 µL of EO. Gentamicin (10 µg/disc) and dimethylsulfoxide (DMSO) (5 %, v/v) were used as positive and negative controls, respectively. All discs were incubated at 37 °C for 24 h and AA was evaluated by the inhibition zone.

Data analysis

All the experimental results were analysed using Statgraphics Centurion software (Version 15.1.02). Every assay was done in triplicates, and the obtained results were expressed as mean±standard deviation, except for the GC–MS analysis, which was done once. Analysis of variance (ANOVA) with Fisher's least significant difference procedure was used to determine the significant differences (P<0.05) between means.

Results and their discussion

Determination of the physicochemical properties of CrEO

Table 1 shows the physicochemical properties evaluated for CrEO. The AV of CrEO was 0.748 mg KOH/g, which is lower than that of other types of EOs, including kaffir lime EO (AV=0.95 mg KOH/g) [16] and orange EO (AV=1.56 mg KOH/g) [17].

In general, the SV of CrEO (67.320 mg KOH/g) was lower than that of most other EOs, including local Madurai *C. reticulata* in India (SV=140.3±0.31 mg KOH/g) [18]. Similarly, it is lower than that of *C. limetta* EO (SV= 121.6± 0.90 KOH/g) and yellow lemon *C. limon* EO (112.2 KOH/g) [18]. However, this value is higher than that of *Fortunella japonica* (Thumb.) peel oil (33.66 mg KOH/g) [19].

The difference between SV and AV mainly depends on the distillation method, climate conditions, variety, harvesting region, and harvesting time. In addition, AV and SV are quality indicators for EOs. On the other hand, the acid value (AV) specifically indicates the free fatty acid content in the sample. EOs are mainly composed of volatile compounds, notably free fatty acids. Therefore, monitoring and controlling acidity and free fatty acid content is essential to ensure essential oil products' quality and longevity.

Table 1

Physicochemical properties of CrEO

No.	Physicochemical properties	Value
1	Acid value (AV, mg KOH/g EO)	0.748±0.324
2	Saponification value (SV, mg KOH/g EO)	67.320±14.605
3	Este value (EV, mg KOH/g EO)	66.572±12.189
4	Relative density (RD)	0.776

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Continuation of Table 1

5	Absolute density (AD, g/mL)	0.774
6	Freezing point (FP, °C)	−70
7	Refractive index (RI)	1.4734
8	Fragrance retention (FR, h)	
	20% EO	6.5
	40% EO	13
	60% EO	20
	80% EO	26.5
	100% EO	33.5

The AD and RD of CrEO were 0.774 g/mL and 0.776 respectively, indicating that CrEO is insoluble in water and floats on its surface. The AD anf RD and of *Mentha arvensis* L. oil, are 0.8959 g/mL and 0.8987, respectively, according to the study by Quoc [20], which is significantly higher than the values observed for CrEO. In addition, the chemical composition of the oil is a direct factor affecting the FP. According to this study, the FP of CrEO is −70 °C.

An important characteristic of this study was the RI, which was is 1.4734 at 20°C. This index is similar to that of *C. hystrix* EO with RI=1.4699 [21]. The RI reflects the purity of the EO [22].

When conducting the comparison experiment to determine of the longevity of CrEO at different concentrations, we observed that at a pure concentration of 100 %, the longevity was 5 times longer than that of the diluted 20 % concentration. Compared to rosemary leaf EO, CrEO has a higher FR. At a concentration of 40%, the FR time of rosemary leaf EO is about 12 h [23], while that of CrEO is 13 h. According to Mahajan [12], depending on the purpose of creating different products, dilution with different doses of water and alcohol is used to achieve the desired fragrance product.

Determination of the chemical compositions of C. reticulata essential oil (CrEO)

The chemical composition of CrEO was determined by gas chromatography-mass spectrometry (GC/MS) and 6 main components were identified: *α*-Pinene, *β*-Pinene, *β*-Myrcene, D-Limonene, Linalool, and *α*-Terpineol (Table 2).

In CrEO, the active ingredient was D-Limonene (96.09 %), comprising the highest proportion. Other compounds made up a relatively small proportion, although they belonged to the monoterpenes group. In addition to D-Limonene, which is dominant and plays an important role in antioxidant applications according to Bacanli et al. [24], another compound with a relatively small proportion was *α*-Pinene (0.93 %). It is not only widely used in the flavor and fragrance industry but also evaluated for its other properties, such as antioxidant, antibacterial, and anti-inflammatory activities, as reported by Sales et al. [25].

The difference in the number of chemical components discovered in each type of EO and their proportions is mostly due to differences in origin, genetic source, season, climate, age, ripening stage, and extraction method [26].

Table 2

Chemical composition of CrEO

No.	Compounds	RT. (min)	Content (%)
1	*α*-Pinene	7.433	0.93
2	*β*-Pinene	9.082	0.61
3	*β*-Myrcene	9.852	1.88
4	D -Limonene	11.423	96.09
5	Linalool	14.909	0.16
6	*α*-Terpineol	19.49	0.33
Total:			100%

Determination of the antioxidant capacity (AC) of C. reticulata essential oil (CrEO)

Fig. shows that the AC of CrEO depends on the concentration. When the concentration increased, the AC increased in a second-order trend with an R^2 value of 0.9936. At a concentration of 2000 mg/mL, the AC reached its highest value within the experimental range, and an IC_{50} of about 1600 mg/mL was determined, which was much higher than the control sample comprised of vitamin C (70±2 μg/mL). This indicates that the AC of CrEO is very weak. The AC may depend on the chemical composition, as observed when compared with other citrus EOs using different raw materials; the inhibitory capacity against DPPH varies. For example, in the EO of *Citrus*

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sinensis L., the IC_{50} value is 7.73±2 mg/mL [17], which is higher than that of CrEO. Similarly, the EO of *Citrus aurantifolia* is 2.36 mg/mL [27]. Based on these results, it can be concluded that the antioxidant capacity of CrEO is lower than that of other EOs.

Although D-Limonene accounts for a high percentage (96.06 %) and plays a major role in the antioxidant capacity of CrEO, the antioxidant capacity of orange EO is relatively poor. This is one of the weaknesses of CrEO and provides a basis for further research.

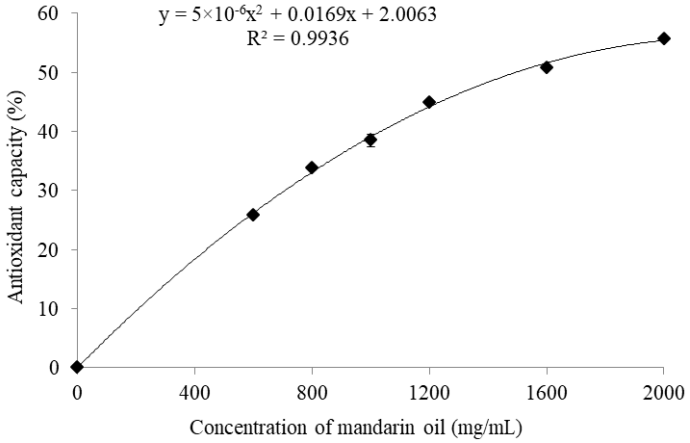

Figure. Antioxidant capacity (AC) of *Citrus reticulata* essential oil (CrEO)

Determination of the antibacterial activity (AA) of C. reticulata essential oil (CrEO)

As shown in Table 3, Gentamycin control showed strong resistance against 4 types of bacteria, with the size of inhibition zones being in the following order: *P. aeruginosa* > *S. aureus* > *B. cereus* > *E. coli*. CrEO exhibited antibacterial activity, with inhibition zone sizes being in the following order: : *E. coli* > *B. cerius* > *S. aureus* > *P. aeruginosa*. The ability of the EO to inhibit *E. coli* was superior to that of the positive control, but the ability to inhibit *P. aeruginosa* was three times weaker.

All 4 bacterial strains showed extremely high sensitivity to the positive control (D > 20 mm), while 2 Gram-negative bacterial strains showed very high sensitivity to CrEO (D > 15–20 mm). Although both were Gram-positive bacterial strains, *E. coli* was extremely sensitive to CrEO (D > 20 mm), while *P. aeruginosa* was almost insensitive (D < 8 mm) [28].

Comparing citrus EOs, the inhibitory zone diameter of CrEO is smaller for Gram-negative bacteria compared to grapefruit EO (*Citrus paradisi* L.). However, it shows a higher sensitivity to Gram-positive bacteria, particularly *E. coli*, compared to orange peel EO (*Citrus sinesis* L.) [29].

Compared to two types of mandarin EOs of the same origin in the southern part of Guangxi province, China, YKEO (*Citrus japonica* Thunb.) has a stronger inhibitory effect on *E. coli* than on CrEO. However, XMEO (*Citrus reticulata* Blanco) has a smaller inhibitory zone diameter for *E. coli* (only 7.44 ± 0.13 mm), which is three times smaller than that of CrEO [30].

To explain the AA of mandarin EO, according to Al-Harrasi et al. [31] found that the AA of EOs can be attributed to cell membrane disruption, changes in permeability, cell lysis, and a reduction in intracellular ATP synthesis. The chemical composition of CrEO included *α*-Terpineol, which accounted for only 0.33%; however, *α*-Terpineol had a strong AA against both Gram-positive and Gram-negative bacteria. Study of Gadhoumi et al. have shown that α-terpineol can disrupt bacterial cell membranes, causing leakage of cell components and affecting the synthesis of bacterial proteins and DNA. Additionally, α-terpineol has been found to have bactericidal activity, not just bacteriostatic activity, especially against antibiotic-resistant *Staphylococcus aureus* [32].

Table 3

Antibacterial zones of *C. reticulata* essential oil (CrEO)

No.	Microorganisms	Inhibition zone diameter of Gentamicin (mm)	Inhibition zone diameter of CrEO (mm)
1	*Escherichia coli*	$22.4±0.1^{Aa}$	$27.57±0.01^{Bd}$
2	*Bacillus cereus*	$23.24±0.03^{Ab}$	$21.33±0.13^{Bc}$
3	*Staphylococcus aureus*	$24.07±0.13^{Ac}$	$19.36±0.16^{Bb}$
4	*Pseudomonas aeruginosa*	$24.47±0.25^{Ad}$	$7.93±0.08^{Ba}$

Within a row (A–B) or a column (a–c), different letters denote significant differences (P<0.05) between samples or microorganisms, respectively.

Conclusions

In summary, the extraction of EO in Dong Thap, Vietnam, is quite feasible. Six chemical components, namely *α*-Pinene, *β*-Pinene, *β*-Myrcene, D-Limonene, Linalool, and *α*-Terpineol, were identified in CrEO. The antioxidant activity IC_{50} was 1600 mg/mL. Additionally, CrEO resisted certain types of pathogenic bacteria. These results demonstrate that CrEO is comparable to other types of EOs from different regions. The utilization of waste byproducts is one of the potential applications of citrus EOs in the fields of medicine, food, and cosmetics.

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