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PHYSICOCHEMICAL PROPERTIES, CHEMICAL COMPOSITION, AND BIOACTIVITY OF ORANGE (*Citrus sinensis)* PEEL ESSENSTIAL OIL

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Abstract: *Orange (Citrus sinensis) peel essential oil (CsEO) is used extensively in pharmaceuticals, cosmetics, and food preservation because of its bioactive qualities and aroma. Nevertheless, different extraction techniques and environmental factors affect its chemical composition and antioxidant capacity. To evaluate the potential uses of CsEO, this study considered the characteristics just mentioned above. The physicochemical properties of CsEO were determined, including acid value (3.561 mg KOH.g^{-1}), saponification value (8.392 mg KOH.g^{-1}), ester value (4.831 mg KOH.g^{-1}), etc. According to GC-MS analysis, the main component was D-limonene (86.96%), which was followed by α-pinene (5.15%) and β-myrcene (4.69%). The IC_{50} values for antioxidant assays were 78.47 mg.ml^{-1} (ABTS) and 96.69 mg.ml^{-1} (DPPH), indicating weaker radical scavenging activity than Vitamin C. Antibacterial tests revealed inhibition zones of 14.16–20.82 mm against Salmonella enteritidis, Escherichia coli, Salmonella enteritidis, and Bacillus cereus, demonstrating moderate antimicrobial properties. These findings confirm the consistency of CsEO's physicochemical properties and chemical profile across studies. While CsEO exhibits moderate antioxidant activity, further research is needed to enhance its bioactivity and optimize extraction methods for industrial applications.*

Key words: *essential oil, chemical composition, orange, physicochemical properties.*

1. Introduction

Citrus sinensis (L.), commonly known as the sweet orange, boasts a rich heritage rooted in Southeast Asia. This versatile fruit thrives in tropical and subtropical climates worldwide, with significant cultivation efforts across various countries [33]. In

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Vietnam, orange trees are a familiar sight in diverse regions, including the northern provinces of Ha Giang and Hoa Binh, the central province of Nghe An, and the fertile lands of the Mekong Delta [32]. A substantial byproduct of the burgeoning juice and food processing industries is orange peel. Often discarded as waste or relegated to livestock feed, these peels hold immense, yet often overlooked, potential [17]. This potential lies in their wealth of bioactive compounds, most notably orange-peel essential oil (*CsEO*).

With the main component being limonene used up to 85-96%, grapefruit peel essential oil (*CsEO*) is like a natural treasure containing countless precious molecules. Together with other important compounds such as myrcene, α-pinene and linalool [18], they not only create a fresh, especially pleasant aroma but also are the source of power for a series of strong biological activities. Many studies have confirmed the impressive antibacterial, ruby and antioxidant properties of *CsEO* [10]. On the other hand, this essential oil is also loved for its positive contributions to mental health, such as reducing stress, improving mood and supporting sleep [21].

The attractive aroma and rich active ingredients have made *CsEO* a sought-after material in many fields. In the cosmetics and personal care industry, it is an ideal ingredient that helps with fragrance properties and benefits the skin [23]. In food technology, *CsEO* opens up new prospects for flavour enhancement and its potential application as a natural preservative. Its insecticidal and antibacterial properties also give it a place in agriculture [12]. Although research on *CsEO* is abundant globally, surveys of this essential oil originating from Vinh Long - a province in the Mekong Delta - are still very limited. This gap is even more evident when it comes to its physical, chemical properties and potential applications in product preservation. Further research focusing on *CsEO* from Vinh Long promises to bring unique discoveries and expand its practical application.

2. Materials and Methods

2.1. Plant Extraction

CsEO was extracted from the peels of *Citrus sinensis* L., a citrus variety cultivated and harvested in the Vinh Long province, Vietnam (coordinates: 10°03'31.0"N 106°08'04.6"E). The essential oil was obtained by hydrodistillation using a Clevenger-type apparatus, with 250 g of fresh peels distilled in 1.25 l of distilled water for 3 h. The distillation process resulted in an essential oil yield of approximately 1.2% (v/w). To preserve its quality and properties, the extracted essential oil was stored in amber glass bottles at room temperature.

2.2. Bacterial Strains

Four bacterial strains were selected for this research: *Staphylococcus aureus* (ATCC 33591) and *Bacillus cereus* (ATCC 11778) as Gram-positive strains, and *Escherichia coli* (ATCC 25922) and *Salmonella enteritidis* (ATCC 13076) as Gram-negative strains. The strains were provided by the Industrial University of Ho Chi Minh City's Institute of Biotechnology and Food Technology.

2.3. Chemicals

Dimethyl sulfoxide (*DMSO*, ≥99.5%, Xilong, China), 2,2-diphenyl-1-picrylhydrazyl (*DPPH*, ≥97%, Sigma, USA), and 2,2'-azinobis (3-ethylbenzothiazoline-

6-sulfonic acid) (*ABTS*, ≥98%, Sigma, USA) were important chemicals used in the study. Absolute ethanol (C_2H_5OH, ≥99.7%, Xilong, China) and distilled water (local laboratory supply, Vietnam) were also employed. In addition, other analytical-grade chemicals, culture media, and antibacterial testing supplies, including Mueller-Hinton agar and nutrient broth (HiMedia, India), were used.

2.4. Evaluation of Physical Properties of CsEO

Measurements of relative density (*RD*), absolute density (*AD*), freezing point (*FP*), acid value (*AV*), ester value (*EV*), and saponification value (*SV*) were carried out based on the guidelines provided by ISO 279 [14], ISO 1041 [12], ISO 1242 [13], and ISO 7660 [15], respectively.

2.5. Determination of Fragrance Retention

To determine the fragrance retention (*FR*) of the essential oil (*EO*), concentration and FR duration were measured following a modified version of the Silina et al. [29] method. To assess fragrance retention, *CsEO* was mixed with 96% ethanol at varying concentrations (20, 40, 60, 80, and 100%, v/v). A maximum of three drops from each concentration were applied to scent test paper, ensuring even distribution. The time taken for the fragrance to disappear under controlled conditions was recorded.

2.6. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The *GC-MS* analysis of *CsEO* was performed using an Agilent 7890A gas chromatograph coupled to a 5977E mass spectrometer (Agilent Technologies, USA). A 1 µl sample was injected via autosampler into a Carbowax 20M capillary column (30 m × 0.25 mm × 0.25 µm, Agilent Technologies, USA) with helium as the carrier gas (constant flow: 10 $mL \cdot min^{-1}$; split ratio 10:1). The injector temperature was set to 250°C. The temperature program consisted of an initial hold at 50°C for 2 min, followed by a $10°C \cdot min^{-1}$ ramp to 250°C (held for 5 min), and a final increase to 280°C (3 min). Detection employed electron ionization (EI) at 70 eV.

2.7. Determination of Antioxidant Activity Using DPPH Assay

Following Quoc's [26] methodology with minor adjustments, the *DPPH* assay was used to assess the antioxidant activity of *CsEO* based on its capacity to scavenge free radicals. 96% ethanol was used to dilute the essential oil in order to produce solutions with varying concentrations. 0.3 ml sample of each solution was mixed with 2.7 ml of 0.1 mM *DPPH* solution and incubated in darkness at room temperature for 30 minutes. The absorbance reduction at 517 nm was recorded using a spectrophotometer (GENESYS™ 20, Thermo Fisher Scientific, USA), with vitamin C as the control. The percentage inhibition was calculated, and the IC_{50} value (concentration for 50% inhibition) was determined (Eq. 1). The antioxidant capacity (*AC*) was computed using a specific formula, where absorbance readings of the sample and the control were taken into account.

$$DPPH = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad (1)$$

where:

DPPH is the 2,2-diphenyl-1-picrylhydrazyl [%];

$A_{control}$ – the absorbance of the *DPPH* solution;

A_{sample} – the absorbance of the CsEO solution in the presence of the *DPPH* solution.

2.8. Determination of Antioxidant Activity Using ABTS Assay

The experiments were conducted based on the method described by Rezanejad et al. [27] with slight modifications. The $ABTS^{+}$ solution was prepared by mixing 7 mM *ABTS* with 2.45 mM potassium persulfate (1:1 ratio) in distilled water and incubating in darkness at room temperature for 16 h. The solution was then diluted to achieve an absorbance of 0.70 ± 0.02 at 734 nm. For the assay, 0.1 ml of *EO* solution was combined with 3 ml $ABTS^{+}$ solution, and the volume was adjusted to 5 ml with ethanol. After 6 min incubation in darkness, absorbance was measured at 734 nm. Vitamin C was used as the control. The inhibition percentage was calculated, and IC_{50} values were determined. Antioxidant capacity (*AC*) was quantified using Equation 2.

$$ABTS = \frac{\mathrm{A_{control} - A_{sample}}}{\mathrm{A_{control}}} \times 100 \qquad (2)$$

where:

ABTS is the 2,2'-azinobis [%];

$A_{control}$ – the absorbance of the *ABTS* solution;

A_{sample} – the absorbance of the CsEO solution in the presence of the *ABTS* solution.

2.9. Determination of the Antibacterial Activity of CsEO

The paper disc diffusion method, which was modified from Sadeghi et al. [28], was used to assess the antibacterial activity (*AA*). Using a sterile inoculation loop, a 100 µl bacterial suspension was evenly distributed on the surface of *MHA* medium at a concentration equal to the 0.5 McFarland standard (roughly 1.5×10^{8} $CFU.ml^{-1}$). Five microlitres of the *EO* were applied to sterile paper discs, each measuring six millimetres in diameter. The negative control was 5% (v/v) dimethyl sulfoxide (*DMSO*), and the positive control was gentamicin (10 µg/disc). After the plates were incubated for 24 hours at 37°C, the diameter of the inhibition zones surrounding the discs was measured to evaluate the antibacterial impact.

2.10. Analysis of Statistics

The ANOVA was used to evaluate variance in the statistical analysis, and the Statistics 20 software (IBM Corp., Armonk, NY, USA) was then used to compare means. At a 95% confidence level ($p \leq 0.05$), the least significant difference (LSD) method was used. All experiments were conducted in triplicate and the mean ± standard deviation (mean ± SD) is used to report the results.

3. Results and Discussion

3.1. Determination of Physicochemical Properties of Orange Essential Oil

The extracted essential oil exhibits a characteristic orange-yellow colour and is completely insoluble in water. The physicochemical analysis presented in

Table 1 shows that *CsEO* possesses notable properties, particularly its freezing point, which is lower than -18°C, allowing it to remain in a liquid state even at low temperatures. Additionally, the oil has a relative density (*RD*) of 0.8449 and an absolute density (*AD*) of 0.8423 g.ml^{-1}, which are higher than the values reported in previous studies. Specifically, Felicia et al. [7] recorded a *RD* of 0.738 and an *AD* of 0.7436 g.ml^{-1} in Malaysia, while Kamal et al. [16] reported an *AD* of 0.815 g.ml^{-1} in Pakistan. These variations in physicochemical properties may be influenced by geographical factors, age, or the extraction methods employed (e.g., Soxhlet extraction, distillation, supercritical fluid extraction, etc.).

Physicochemical properties of orange peel essential oil Table1

No.	Physicochemical properties	Value
1.	pH	4.83±0.82
2.	Freezing point [*FP*, °C]	< −18°C
3.	Relative density [*RD*]	0.8449 ± 0.0028
4.	Absolute density [*AD*, g.ml^{-1}]	0.8423 ± 0.0032
5.	Acid value [*AV*, mg KOH.g^{-1} *EO*]	3.5610 ± 0.0193
6.	Saponification value [*SV*, mg KOH.g^{-1} *EO*]	8.3929±0.0330
7.	Ester value [*EV*, mg KOH.g^{-1} *EO*]	4.8319 ± 0.0418
8.	Fragrance retention [*FR*, h]:	
	20% *EO*	31.23 ± 3.64
	40% *EO*	43.25 ± 4.82
	60% *EO*	52.87 ± 4.91
	80% *EO*	61.28 ± 6.15
	100% *EO*	69.37 ± 7.28

The acid value (*AV*) in this study (3.561 mg KOH.g^{-1}) is comparable to that of the CsEO from Nigeria, as reported by Fakayode and Abobi [6] using the Soxhlet extraction method (3.71 mg KOH.g^{-1}), but lower than that of the Malaysian *CsEO* extracted using supercritical fluid extraction (*SFE*) (4.13 mg KOH.g^{-1}), as reported by Felicia et al. [7]. This suggests that the extraction method may influence the free fatty acid content in the *EOs*, with *SFE* potentially extracting a greater variety of compounds, leading to a higher acid value [4].

The saponification value (*SV*) in this study (8.3929 mg KOH.g^{-1}) is significantly lower than those reported in the aforementioned studies, including the Nigerian *CsEO* (188 mg KOH.g^{-1}) and the Malaysian *CsEO* (121.67 mg KOH.g^{-1}) [6, 7]. Similarly, the ester value (*EV*, 4.8319 mg KOH.g^{-1}) is also considerably lower than that of the Malaysian *CsEO* (117.54 mg KOH.g^{-1}), reflecting differences in the chemical composition of essential oils obtained through different extraction methods [7]. The difference from other values may be due to methodological differences, for example, the solvent or supercritical extraction retains more of the non-volatile lipid components (triglycerides and esters), resulting in a higher *SV/EV*, whereas water-distilled essential oils like ours are mainly volatile terpenoids with negligible

saponified lipids, so much lower values are expected [24]. Plant variety geographic location, climate, and maturity stage can all affect the *AV* and *SV* of citrus essential oils. The *AV* and *SV* of the extracted *EO* can be affected by the maturity stage and altitude at which citrus plants are grown, per a study by Pradhan et al. [25].

Regarding fragrance retention, the scent duration of orange essential oil increases with concentration (20-100%). In this study, at 100% concentration (pure *CsEO*), the fragrance lasted up to 69.37 hours (approximately 2–3 days), indicating that although *CsEO* has a relatively fast evaporation rate, it can still maintain its aroma for several days at high concentrations.

3.2. Chemical Composition of Orange Peel Essential Oil

Table 2 presents the analysis of *CsEO*, showing that its *GC-MS* is primarily composed of D-Limonene, which dominates at 86.96%, followed by α-Pinene (5.15%), β-Myrcene (4.69%), Sabinene (2.21%), and Octanal (0.98%). Overall, monoterpene hydrocarbons account for 99.02% of the total *EO* content, with Limonene playing a crucial role in defining the characteristic citrus aroma and bioactive properties of the oil. Monoterpenes, especially limonene, have been shown to have antioxidant and antibacterial qualities, as well as possible uses in the food, medicine, and cosmetics sectors [5]. β-Myrcene, a monoterpene hydrocarbon, is also known for its anti-inflammatory, analgesic, and sedative effects, with applications in pharmaceuticals, fragrances, and as a flavouring agent [30]. Similarly, Zheljazkov et al. [35], pointed out that Sabinene possesses strong antioxidant and antimicrobial activities, supporting its potential use in natural preservatives and cosmetic formulations.

In contrast, Octanal (0.98%), classified as a carbonyl compound (aldehyde), contributes to the pleasant fruity and citrus-like aroma. Although present in a small proportion, aldehydes such as Octanal play a significant role in the sensory attributes of *EOs,* helping to balance the fragrance and enhance the commercial value of the product [8].

Chemical composition of orange peel essential oil Table 2

No.	Compounds	RT. [min]	Content [%]
1.	α-Pinene	3.27	5.15
2.	Sabinene	4.00	2.21
3.	β-Myrcene	4.24	4.69
4.	D-Limonene	4.61	86.96
5.	Octanal	5.24	0.98
Monoterpene			99.02
Aldehyde			0.98

When compared to previous studies, the chemical composition of this *EO* remains relatively consistent. For example, Hosni et al. [11] analysed the *CsEO* from Tunisia and reported a Limonene content ranging from 89.3 to 94.7%, which is slightly higher than

the 86.96% found in this study. Similarly, Kamal et al. [16] in Pakistan reported α-Pinene levels of only 1.17–1.43% and β-Myrcene at 1.37–1.82%, significantly lower than 5.15 and 4.69%, respectively, in this study. Such variations may arise from differences in plant variety, environmental conditions, or extraction techniques (e.g., Soxhlet extraction, distillation, supercritical fluid extraction, etc.).

3.3. Evaluation of Orange Peel Essential Oil's Antioxidant Capacity (*AC*)

The IC_{50} values for *DPPH* and *ABTS* in this study were 96.69 and 78.47 $mg.ml^{-1}$, respectively, both significantly higher than those of Vitamin C (6.17 and 3.12 $\mu g.ml^{-1}$, respectively), as shown in Table 3. This suggests that compared to the control (Vitamin C), the *AC* of *CsEO* is significantly lower.

Compared to previous studies, the $IC_{50\text{-}DPPH}$ of the *CsEO* in this study was higher than that of the *CsEO* from Malaysia (7.74 $mg.ml^{-1}$), as reported by Felicia et al. [7], and the $IC_{50\text{-}ABTS}$ was also higher than that reported in a study from Cameroon (4.27 $mg.ml^{-1}$), according to the study of Boris et al. [2]. This suggests that the *AC* of the *CsEO* in this study is lower than those in some previous studies.

These differences may be attributed to various factors such as environmental conditions, extraction methods, and the chemical composition of the essential oil. According to Çelik et al. [3], *CsEO* primarily contains monoterpenes (especially D-Limonene), which are non-polar compounds that may affect their ability to react with free radicals in different antioxidant assays. Additionally, as noted by Akbari et al. [1], the antioxidant potential of *EOs* is also related to the electron-donating capacity of their bioactive compounds, which helps neutralize free radicals and prevent oxidative processes.

Antioxidant activity of orange peel essential oil Table 3

Sample	$IC_{50\text{-}DPPH}$	$IC_{50\text{-}ABTS}$
Vitamin C [$\mu g.ml^{-1}$]	$6.17^{a} \pm 0.84$	$3.12^{b} \pm 0.29$
CsEO [$mg.ml^{-1}$]	$96.69^{b} \pm 2.28$	$78.47^{a} \pm 1.45$

Note: Values with different letters (a, b) in the same column differ significantly ($p \leq 0.05$).

Furthermore, Geng et al. [9] reported that certain components in *CsEO* may participate in metal chelation mechanisms, reducing the catalytic role of metal ions in the formation of reactive oxygen variety (*ROS*). This contributes to cellular protection against oxidative damage and may explain the differences in *AC* observed across studies.

Thus, these discrepant results suggest that the antioxidant capacity of essential oils is not a fixed value, but is greatly influenced by a combination of factors, including the assessment method, the specific chemical composition, and the diverse mechanisms of action of the compounds in the essential oils. Therefore, the application of essential oils as natural antioxidants should be considered based on the specific context and purpose of use, and at the same time open up further research on the mechanisms of action and potential synergies between the components.

3.4. Evaluation of Orange Peel Essential Oil's Antibacterial Activity (*AA*)

The study results indicate that *CsEO* exhibits significant *AA*, particularly against Gram-positive bacteria such as *B. cereus* (20.82 mm) and *S. aureus* (19.58 mm) (Table 4). Notably, *S. aureus* tested (ATCC 33591) is a methicillin-resistant strain, highlighting the clinical relevance of *CsEO* in combating antibiotic-resistant pathogens [31]. Significant action against Gram-negative bacteria, such as *S. enteritidis* (14.16 mm) and *E. coli* (15.68 mm), was also shown by *CsEO*. These inhibition zones were considerably larger than those reported by Felicia et al. [7] in Malaysia, where *CsEO* only exhibited inhibition zones of 10.3 mm for *S. aureus* and 11.0 mm for *E. coli*.

Antibacterial zones of orange essential oil Table 4

Bacterial strains	Diameter of the inhibitory zones of gentamicin [mm]	Diameter of the inhibitory zones of *CsEO* [mm]
Salmonella enteriditis (ATCC 13076)	$22.24^{Cb} \pm 1.26$	$14.16^{Aa} \pm 0.21$
Staphylococcus aureus (ATCC 33591)	$21.98^{Bb} \pm 0.22$	$19.58^{Ca} \pm 1.97$
Bacillus cereus (ATCC 11778)	$23.69^{Db} \pm 0.65$	$20.82^{Da} \pm 0.71$
Escherichia coli (ATCC 25922)	$18.74^{Ab} \pm 0.31$	$15.68^{Ba} \pm 1.68$

Note: Significant differences ($p \leq 0.05$) between samples or microorganisms are indicated by different letters within a row (a–b) or a column (A–D), respectively.

When compared to other citrus *EOs*, *Citrus medica* L. essential oil showed *AA* against *S. aureus* (19.2 mm) and *E. coli* (11.2 mm) [18]. Additionally, the *Citrus japonica* essential oil from China exhibited an inhibition zone of 8.2 mm against *S. aureus* and 21.59 mm against *E. coli* [20]. These results imply that the *AA* of essential oils belonging to the *Citrus* genus can differ greatly based on the specific chemical composition.

Such antibacterial performance can be correlated with the chemical profile obtained from the *GC-MS* analysis, which revealed that *CsEO* is overwhelmingly dominated by D-limonene (86.96%), followed by α-pinene (5.15%), β-myrcene (4.69%), and sabinene (2.21%). Monoterpene hydrocarbons, accounting for 99.02% of the total oil, are well known for their lipophilic nature, enabling them to disrupt bacterial membranes and enhance permeability. D-limonene, in particular, has been extensively documented for its antimicrobial effects, and its dominance in *CsEO* likely explains the strong inhibition zones observed, especially against Gram-positive bacteria. Minor constituents such as α-pinene and β-myrcene may also act synergistically with limonene, further potentiating antibacterial activity [34].

In addition, Okoye et al. [22] also pointed out that *CsEO* has the ability to penetrate the lipid bilayer of bacterial cell membranes, causing leakage of intracellular components and impairing

their viability. Although Gram-negative bacteria have an outer membrane containing lipopolysaccharide that acts as a protective barrier, *CsEO* can still interact with these lipophilic components, compromising the integrity of the cell membrane and inhibiting the growth of the microorganism.

4. Conclusions

The physicochemical characteristics, chemical makeup, and antioxidant activity of *CsEO* are all thoroughly examined in this work. The results confirm that *CsEO* has a high D-Limonene content (86.96%) and exhibits physicochemical characteristics similar to those reported in previous studies. While *CsEO* demonstrates moderate fragrance retention and stability, its antioxidant activity is relatively weak compared to Vitamin C ($IC_{50\text{-DPPH}}$ = 96.69 $mg.ml^{-1}$ and $IC_{50\text{-ABTS}}$ = 78.47 $mg.ml^{-1}$). Antibacterial activity tests revealed inhibition zone diameters ranging from 14.16 to 20.82 mm, depending on the tested bacterial strain. These results suggest that *CsEO*, with its antibacterial action against foodborne pathogens, could serve as a natural preservative in food products; its pleasant citrus aroma and moderate fragrance retention make it suitable for incorporation into cosmetics and personal care formulations; and its volatile nature rich in limonene supports its use in aromatherapy for mood-enhancing and relaxation purposes.

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