Antimicrobial and Antioxidant Properties of Essential Oils from Orange Peels and Eucalyptus Leaves Wastes

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Oranges and eucalyptus trees are abundant sources of waste and pruning, generating secondary streams that can be converted into valuable products. Both species are broadly cultivated in Mexico. Essential oils from orange peels and eucalyptus leaves possess antimicrobial and antioxidant properties, making them useful in various applications. In this study, the essential oils antioxidant potential was determined through radical scavenging activity and ferric reducing capacity, and the total phenolic content was measured. These essential oils also demonstrated inhibition capacity against *Escherichia coli* and *Staphylococcus aureus*. GC-MS analysis of the oils revealed the composition of representative compounds, with D-limonene constituting almost 75% of the orange essential oil and 1,8-cineol comprising 15.2% of the eucalyptus oil. The antioxidant test results between essential oils showed that they are similar, except for the FRAP test, where eucalyptus essential oil obtained a value three times higher than orange essential oil. The findings suggest that these essential oils can serve as natural and sustainable alternatives to synthetic antimicrobial and antioxidant agents.

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INTRODUCTION

The sweet orange, whose name comes from a Dravidian term meaning "essence," "aroma" or "perfume" (Oxford 2022), is the fruit of the orange tree. This tree is originally from Asia, specifically Pakistan, India, China, and Vietnam, and was introduced to Europe by Arabs and Persians before spreading to the Americas during the 16th century (SanJose 2022). The Americas, particularly Brazil, the United States of America (USA), and Mexico, have proven to be excellent regions for adapting this hybrid fruit. They are ranked first, fourth, and fifth, respectively, in global orange production, according to FAO (FAOSTAT 2022).

The *Eucalyptus* genus includes over 700 species of trees, ranging from shrubs to specimens over 70 meters tall. They are primarily native to Australia, with some species also found in New Guinea, nearby Indonesian islands, and the Philippines (Department of agriculture, water and the environment of Australia 2013). After European expeditions,

mainly British and Dutch, different species of this genus spread worldwide, reaching the Mediterranean region, Africa, and the Americas. In this last continent, suitable climates were found in Argentina, Mexico, and the United States.

During the second half of the 19th century, the *Eucalyptus* genus was introduced to Mexico, specifically to the country's central region, where malaria outbreaks were expected due to unsanitary conditions. Drying out the land was believed to help combat the disease (Hinke 2020). After its introduction to the country, eucalyptus became a precious and profitable forest crop in Mexico (Martinez *et al.* 2006). According to SEMARNAT, the Eucalyptus genus accounts for 19.6% of Mexico's timber species. In the state of Baja California, located in northwest Mexico and bordering California, USA, the presence of *E. camaldulensis*, also known as red gum eucalyptus, has been reported (SEMARNAT 2018).

Both the orange and eucalyptus trees are introduced species that found suitable climates in Mexico and are cultivated for their fruit in the case of the orange tree and wood in the case of the eucalyptus species. In Mexico, the average orange production from 2012 to 2021 was 4.5 million t, while the reported eucalyptus wood production in the same period was 4.6 tons (SIAP 2022). As with almost all crops, there are waste products that, if adequately valued, can yield valuable byproducts. The data is summarized in Table 1.

Table 1. Annual Production of Orange and Eucalyptus Wood in Mexico

Year	Orange Production (Mt)	Eucalyptus Wood Production (t)
2017	4.630	8.16
2018	4.738	-
2019	4.737	9.03
2020	4.649	4.76
2021	4.595	-

The orange juice production process yields a substantial waste volume, accounting for up to 70% of the fruit total mass (Ayala *et al*. 2017). This waste comprises a blend of various fruit tissues, which include the central core; albedo, a white filamentary tissue composed of tubular cells; the oils sacks, protected by a waxy epidermis; and the flavedo, the outer layer of the fruit known also as exocarp. Some orange peel waste could contain juice sacks (Fontana 2021). The outer tissues are rich in compounds of interest such as waxes, and proteins. Based on an assumption that 70% (w/w) of the orange turns into waste during juice extraction, it is estimated that approximately 3.27 Mt of orange waste was generated in Mexico. This estimation assumes that the entire orange production was allocated to this industry.

In a similar context, 77% of the biomass produced by eucalyptus comprises leaves and small branches, while the remaining 23% is firewood (SEMARNAT 2018). Given this premise, it can be inferred that the eucalyptus leaves production, which are suitable for essential oil extraction, amounts to 24.5 t per year in Mexico, considering only the officially recorded production (SIAP 2022).

Orange and eucalyptus crops yield a variety of byproducts, but their essential oils are particularly valuable and versatile. Volatile fractions of terpenes and related compounds can be extracted from orange peels and eucalyptus leaves. Both essential oils share common compounds, such as 1,8-cineol, D-limonene, menthol, and α-pinene, among others (Bendaoud *et al.* 2009; Ayala *et al.* 2017).

Essential oils are highly valued for their diverse uses. In addition to their intrinsic properties such as antioxidant, bactericide, fungicide, and repellent, they are fundamental ingredients in various products such as cleaning and personal hygiene articles, cosmetics, or as additives for foods, fuels, vegetable oils, polymers, among others (Bai *et al*. 2022; Couteau *et al*. 2022; Labiad *et al*. 2022; Wangrawa *et al*. 2022).

Currently, only a small portion of orange peel waste is utilized for essential oil production. The orange peel waste generated outside the industrial juice production ends up in landfills. Similarly, eucalyptus leaves are considered waste and are often disposed of through open burning, with no recorded essential oil production in the country. Harnessing these biomass resources presents a potential for essential oil production, which could be directed towards domestic consumption, export, or incorporation into value-added products such as antimicrobial or antioxidant agents.

Therefore, essential oils were obtained from orange peel waste and residual eucalyptus leaves from the state of Baja California, in northwest Mexico. The objective of the study was to evaluate the antimicrobial activity and antioxidant capacity of these essential oils. The effectiveness of these oils as antimicrobial agents was assessed using the disk diffusion method for the bacteria *Escherichia coli* and *Staphylococcus aureus*. The antioxidant capacity of both essential oils was evaluated using the methods of total phenols, ferric reducing capacity using FRAP, and free radical scavenging activity using DPPH. Currently, the literature studied the application of these essential oils in both antimicrobial activity and antioxidant capacity; however, the novelty of this work consisted in linking the composition of the essential oils and its extraction method with the results of both applications. Since extraction methods alter the composition of essential oils, the composition of each oil was measured by GC-MS.

EXPERIMENTAL

Reagents

2,4,6-tripyridyl-s-triazine (TPTZ), 2,2-diphenyl-2-picrylhydrazyl free radical (DPPH), gallic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Methanol, Folin-Ciocalteu reagent, sodium carbonate, sodium phosphate dibasic, sodium phosphate monobasic, sodium chlorine, dimethyl sulfoxide, hydrochloric acid and ferric chloride hexahydrate were purchased from Merck (Darmstadt, Germany). Distilled water was obtained by a local supplier.

Orange Peel Essential Oil Extraction

For this extraction, orange peel was considered as the total residues of oranges, which included endocarp, mesocarp, exocarp, and in some cases, residues of pulp that had not been used. The orange peel was obtained from fresh Valencia orange fruit (*Citrus sinensis*) from a local farmer in Mexicali, Baja California. For the extraction process, 65 g of orange peel were blended with 300 mL of distilled water. The mixture was then transferred to a round-bottomed flask and combined with 300 mL of water. Then a distillation of one stage was employed at atmospheric pressure using a heating mantle Glas-col series O versatile fabric, 115 W and 380 V) with a Basic PL120 control. Under these conditions the temperature of the gas phase flask was 106 °C, which included essential oil and water. In the condensation step, water as a cooling stream was used at a rate of 3.95 L/min and 10 °C for 50 min, the condensate temperature was 39 °C. The resulting

condensate was transferred to a 500 mL Wilmad-LabGlas VWR glass decanter to separate it from the water. The essential oil (EO) yield was measured using a 5 mL graduated cylinder and stored in an amber vial at 4 °C for further analysis. The complete extraction process lasted 1 h (Ayala *et al.* 2017).

Eucalyptus Leaves Essential Oil Extraction

For this extraction, eucalyptus leaves were considered as leaves from the trees without wood branches. The essential oil in eucalyptus leaves was extracted with a stainless-steel extractor. The extractor was formed by a horizontal cylindrical body of 10 L volume, with three steam inlets in the inferior part, an outlet on the upper part. The steam source was a Sussman MBA20F3 Electric Steam Generator with a power source of 3 kW, 110 V, maximum working pressure of 586 kPa, and maximum flow of 4 kg/h of steam. The extraction process required 1 kg of leaves sourced from the gardens of the Autonomous University of Baja California's Mexicali Campus. The extraction process used whole and fresh leaves rinsed with distilled water. The steam used for the extraction was at 206.8 kPa (30 psi). The resulting output was condensed and separated from water using a custom made heat exchanger constructed from stainless steel. The condenser was made using 2 concentric tubes of 5.08 cm and 2.54 cm of diameter, with a length of 60 cm. At the end of the condenser the essential oil was separated from water using a 250 mL StonyLab water oil separator; this last stream was at 41 °C. The essential oil yield was measured and stored in an amber vial at 4 °C until further analysis (Lainez *et al.* 2021).

GC-MS Analysis

Gas chromatography (GC) (Elkousy *et al.* 2022) analysis was performed using an Agilent 7890 A instrument equipped with an FID detector and an HP-INNOWAX polyethylene glycol column (60 mm x 0.25 mm, film thickness of 0.25 µm). The detector temperature was initially set at 70 °C for 10 min and then increased at a rate of 5 °C/min until it reached 240 °C. Helium was used as the carrier gas at a constant flow rate of 1 mL/min, and the injector had a split ratio of 50:1 at 250 °C.

Antimicrobial Test

The antimicrobial activity of these essential oils was evaluated against two bacterial strains: *Escherichia coli* and *Staphylococcus aureus*. These strains were isolated from spoiled minced meat and identified at the species level by the Department of Biochemistry and Microbiology. All strains were stored in the Microbial Culture Collection belonging to the Department of Biochemistry and Microbiology at "P. Hilendarski" University of Plovdiv. The susceptibility test was carried out by the agar diffusion method. First, 20 mL of sterilized nutrient medium was poured into sterile plastic Petri dishes and allowed to solidify at room temperature. Each dish was then inoculated with 100 µL of microbial inoculum, which was prepared with isolated bacteria diluted in 0.85% NaCl solution, adjusted to 0.5 turbidity read at 530 nm wavelength with a Grant bio-DEN-1 (Grant Instruments USA, Beaver Falls, Arizona, USA).

Whatman Number 1 paper discs of 6 mm were soaked with 20 µL of essential oil or extract solutions (10 mg/mL in dimethyl sulfoxide (DMSO)) and placed in the center of the inoculated Petri dish. One dish had only DMSO as a positive control for essential oils. The dishes were incubated at 37 °C for 48 h. After incubation, any inhibition zones were measured to determine the bioactivity of the essential oils and extracts against the bacterial strains, and it was classified as susceptible or not susceptible to the essential oils.

Antioxidant Capacity

Sample preparation

To assess the antioxidant capacity, essential oils were diluted in methanol. Solutions of 10%, 1%, and 0.1% concentrations were prepared for both orange peel and eucalyptus leaves essential oils. This was done to identify the working dilution for each antioxidant assay. The resulting solutions were referred to as working samples.

Total phenols determination

Total phenols in the extract were determined using the Singleton and Rossi technique on a 300 µL microplate (Singleton and Rosi 1965). This technique consists of reacting 37 µL of Folin-Ciocalteu reagent with 15 µL of the working sample and 128 µL of water inside a sample cell. After 5 min, the sample cell was brought to a volume of 300 µL using a 7% w/v solution of Na_2CO_3 and allowed to react for 1 h in the dark. Absorbance was measured at 760 nm using a Thermo Scientific Multiskan Spectrum® plate reader (Thermo Scientific, Rochester, NY, USA). Results were expressed as mg of gallic acid equivalent per gram of extract (GAE), and the test was performed by quadruplicate.

Free radical scavenging activity determination using the DPPH assay.

The free radical scavenging activity was measured using Brand-Williams' technique (Brand-Williams *et al.* 1995), with modifications (Sagaste *et al*, 2019). The test involved reacting 990 µL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) methanol solution at 6 µM with 10 µL of the working sample. After stirring, the mixture was left in a dark room for 30 min. Absorbance was measured at 517 nm using a Thermo Fisher Scientific® Genesys 20 spectrophotometer (Thermo Scientific, Rochester, NY, USA). Results were expressed as µmol of Trolox® equivalent antioxidant capacity per gram of extract (TEAC), and it was performed in quadruplicate.

The antioxidant effect of essential oils was represented by calculating the percentage reduction in absorbance compared to the blank *(%inh*), as in Eq. 1,

$$\%inh = (1- (A_s - A_b)/A_r)*100 \quad (1)$$

where *%inh* is the inhibition percentage of the DPPH radical, and A_s, A_b, and A_r are the 517 nm absorbance of the working sample, blank, and reference, respectively.

Ferric reduction capacity using FRAP

The extract's reducing capacity was measured using the FRAP technique, based on Benzie and Strain's methodology (Benzie and Strain 1996), with some modifications (Sagaste *et al*. 2019). The FRAP reagent was prepared by mixing 10 µM of 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl with an equal volume of a 20 µM aqueous solution of $FeCl_3$. The pH was adjusted to 3.6 using a sodium acetate buffer.

The assay was set up in a 300 µL well plate by mixing 15 µL of the working sample, 15 µL of buffer, and 270 µL of FRAP reagent. After incubating for 30 min in a dark room, absorbance was measured at 590 nm using a Thermo Scientific Multiskan® Spectrum plate reader (Thermo Scientific, Rochester, NY, USA). Results were expressed as µmol of Trolox® equivalent antioxidant capacity per gram of extract (TEAC), performed in quadruplicate. Higher absorbance indicates greater reducing capacity.

RESULTS AND DISCUSSION

Orange Peel and Eucalyptus Leaves Essential Oil Extraction

The volume of orange essential oil extracted from orange peel ranged from 2.6 to 3.1 mL, yielding 4 to 4.7% (v/w). This yield was within the upper range reported by various sources, which ranged from 0.81 to 5.3% (v/w) (Strano *et al*. 2014; Liu *et al.* 2019; Razola *et al.* 2021), depending on extraction conditions and methods. The authors of this work have also reported similar yields under different conditions.

The yield of eucalyptus essential oil was significantly lower at no more than 0.52% (v/w), or a maximum of 5.2 mL per 1 kg of tree leaves. Other authors have reported yields up to 3.4% (v/w) using hydrodistillation (Lainez *et al.* 2021; Sadraoui *et al*. 2022).

GC-MS Analysis of Essential Oils from Orange Peel and Eucalyptus Leaves

D-limonene was the most abundant compound in eucalyptus essential oil, accounting for over 27% of its composition. Other significant compounds included 1,8-cineol, α-pinene, and β-pinene. The terpenes identified in the oil are presented in Table 2.

D-limonene made up nearly 75% of orange essential oil, as reported by the authors of this work in a previous publication (Ayala *et al.* 2017). An additional 20 compounds were identified (Table 2). These findings are consistent with those reported in the literature, where D-limonene typically accounts for 72 to 96% of orange essential oil (do Evanglho *et al.* 2019; Padilla *et al.* 2022).

Table 2. *E. camaldulensis* and *C. sinensis* Essential Oil Composition

Name	*E. camal-dulensis*	*C. sinensis*	Name	*E. camal-dulensis*	*C. sinensis*
	Relative abundance	**Relative abundance**		**Relative abundance**	**Relative abundance**
D-limonene	**27.91**	**74.43**	Myrtenal	**0.55**	**-**
1.8-cineol (Eucalyptol)	**15.15**	**0.16**	α-Bulnesene	**0.53**	**-**
β-Pinene	**8.75**	**1.54**	Camphene	**0.42**	**0.99**
α-Pinene	**7.69**	**1.45**	p-Myrcene	**-**	**4.27**
(-)-Spathulenol	**5.15**	**-**	Sabinene	**-**	**3.26**
Longipinocarvone	**4.39**	**-**	Linalool	**-**	**1.54**
Caryophyllene oxide	**3.42**	**-**	Z-Carveol	**-**	**1.12**
α-Terpinolene	**1.72**	**-**	Germacrene	**-**	**1.12**
Germacrene B	**1.67**	**-**	β-Caryophyllene	**-**	**0.89**
Allo-aromadendrene	**1.61**	**-**	Citronellal	**-**	**0.87**
α-Phellandrene	**1.43**	**-**	Linalool oxide	**-**	**0.82**
β-Eudesmol	**0.97**	**-**	Citronellol	**-**	**0.78**
γ-Terpinene	**0.92**	**0.92**	β-Cubebene	**-**	**0.64**
(+)-Aromadendrene	**0.84**	**-**	Elemol	**-**	**0.36**
Viridiflorol	**0.68**	**-**	α-Terpinene	**-**	**0.33**
Terpinene-4-ol	**0.67**	**-**	p-Cymene	**-**	**0.27**
trans-Pinocarveol	**0.66**	**-**	Copaene	**-**	**0.26**
Cryptone	**0.56**	**-**	α-Humulene	**-**	**0.16**

The concentration of 1,8-cineol in eucalyptus essential oil was relatively low compared to values reported in the literature, which range from 5.8% to 54.29% (Lainez *et al.* 2021; Almas *et al.* 2021; Ebadollahi *et al.* 2022; Dev Sharma *et al.* 2022). The relative abundance of D-limonene in eucalyptus essential oil is between 5 and 15% (Sartoreli *et al.* 2007; Cheng *et al.* 2009). Factors such as tree age and life cycle can affect essential oil composition in *Eucalyptus* species. In this study, eucalyptus trees were planted on the Autonomous University of Baja California campus between 1979 and 1983, much older than those studied by Pagula (Pagula *et al*. 2000).

Antimicrobial Test

Figure 1 illustrates the results of antimicrobial tests on orange essential oil. Both orange and eucalyptus essential oils showed similar antimicrobial activity, with orange oil being more effective against *E. coli* and eucalyptus oil against *S. aureus*. The inhibition diameter was measured using the smallest circle that passes through the center of the Petri dish (ASTM Education 2009).

The inhibition zones observed on a Petri dish are a qualitative indicator of the antimicrobial activity of essential oils from orange peel waste (*Citrus sinensis*) and eucalyptus leaves (*Eucalyptus camaldulensis*) against *Escherichia coli* and *Staphylococcus aureus*. According to the Kirby-Bauer method manual published by the American Society of Microbiology (2009), inhibition zone diameters can be interpreted to classify bacteria as resistant, intermediate, or susceptible.

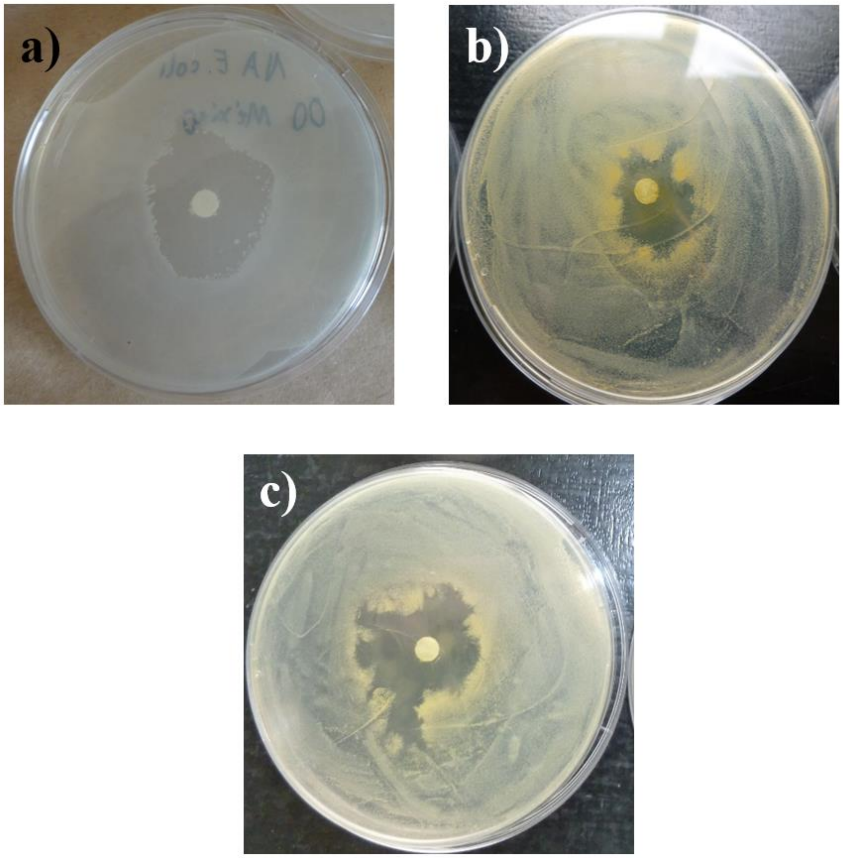

Fig. 1. a) susceptibility of *E. coli* to orange EO, b) intermediate to susceptibility of *S. aureus* to orange EO, c) susceptibility of *S. aureus* to eucalyptus EO

Orange essential oil inhibited the growth of *E. coli* with a diameter of 25 mm, while its inhibition diameter against *S. aureus* was 18 mm (do Evanglho *et al.* 2019). Both results are shown in Fig. 1 a) and Fig. 1 b). Sartorelli *et al.* (2007) reported that oil containing 95% D-limonene could not inhibit the growth of *E. coli* and other Gram-negative bacteria due to the poor permeability of their cell walls.

Eucalyptus essential oil inhibited the growth of *E. coli* with a diameter of 15 mm. For *S. aureus*, eucalyptus essential oil inhibited its growth by a diameter of 20 mm, as exhibited in Fig. 1c. These results are similar to those reported by Salem *et al.* (2018), who used eucalyptus essential oil containing 7 to 13% 1,8-cineol and obtained inhibition diameters of 8 to 20 mm in agar dilution.

Barth Reller (2009) and Schofield (2012) describe the disk diffusion method as qualitative, highlighting this classification. Ezzeldeen *et al.* (2016) also employed this methodology to assess the susceptibility of *Pseudomonas aeruginosa* in milk cultures, comparing the effectiveness of different extracts with antibiotics. For *S. aureus*, an inhibition zone larger than 19 mm is considered indicative of susceptibility, while for *E. coli*, the threshold for susceptibility starts at 17 mm. Although this method does not provide quantitative data such as the minimum inhibitory concentration (MIC), it offers a preliminary evaluation based on these ranges, allowing bacteria to be classified according to their susceptibility or resistance to the tested essential oils.

In this study, both bacterial strains showed susceptibility to orange peel essential oil, and only *S. aureus* showed susceptibility to eucalyptus essential oil. *E. coli* was considered to be intermediate to resistant against eucalyptus essential oil.

Antioxidant Capacity

Both orange and eucalyptus essential oils had similar concentrations of total phenols, with orange oil slightly higher. Results from the free radical scavenging assay revealed that orange essential oil had a higher antioxidant capacity than eucalyptus oil in proportion to their respective phenol contents.

These findings are consistent with those presented earlier, assuming that phenolic compounds are primarily responsible for antioxidant activity. However, eucalyptus essential oil presented better-reducing activity in the FRAP assay due to differences in reaction mechanisms. Results from antioxidant activity assays are summarized in Table 3.

Table 3. Orange peel and Eucalyptus Leaves Essential Oil Antioxidant Capacity

Method	Results	
	C. sinensis	*E. camaldulensis*
DPPH (TEAC/g)*	52.26±5.12	43.20±3.25
%Inh DPPH	22.29±2.32	18.19±1.47
FRAP (TEAC/g)*	9.72±0.42	26.53±2.74
Total phenols (GAE/g)**	149.14±8.51	144.01±5.60

* TEAC/g: Trolox equivalent antioxidant capacity (in milimoles of Trolox) per gram of sample.
** GAE/g: Gallic acid equivalent (in milimoles of Gallic acid) per gram of sample.

The results for *C. sinensis* are consistent with those reported by other authors. Elkhatim *et al.* (2018) found a correlation between citrus fruit peel phenolic content and its antioxidant capacity, while also noting that the phenolic content of citrus extracts was highest in the fruit peel. Similarly, Kadhom *et al.* (2020) reported a high antioxidant

capacity for citrus essential oil extracted from the fruit peel and flowers of *C. sinensis* and highlighted that the peel and flowers had the highest phenolic content.

Studies summarized in Table 4 revealed a small correlation between D-limonene content and TEAC measured by the DPPH method. One source reported that pure D-limonene had no radical scavenging activity (Torres-Alvarez *et al*. 2016). Some authors have suggested that D-limonene may not correlate with the antioxidant capacity of citrus oils (Guo *et al*. 2018), whereas others have reported synergistic effects with other compounds, such as flavonoids and phenolic acids (Fernandes *et al*. 2020; Lu *et al*. 2020).

Table 4. Other Antioxidant Capacity Results for Essential Oils from Citrus

Citrus Essential Oil	GAE/g	TEAC/g per DPPH	D-limonene Relative Abundance	Source
Microencapsulated orange essential oil	6.78	13.54	96.02 %	(Fernandes *et al*. 2020)
Cold press orange essential oil	-	21.24	91.12 %	(Torres-Alvarez *et al.* 2016)
D-limonene pure	-	0	100 %	(Torres-Alvarez *et al.* 2016)
Cold press orange essential oil	-	849.52	86.65	(Lu *et al.* 2020)
Commercial encapsulated *Citrus reticulata* essential oil	19.61	151.29	-	(Mahdi *et al.* 2021)
Citrus sinensis essential oil	-	42.785	71.06	(Guo *et al.* 2018)
Citrus sinensis peel extract	9.61	66.4	-	(Lagha *et al.* 2013)
Citrus sinensis peel oil	149.14	52.26	74.43	Present work

Other research suggests that water extraction yields higher phenolic content in *Eucalyptus* leaves than organic solvents (Singab *et al*. 2011; Lagha and Madani 2013; Dezsi *et al*. 2015; Mahdi *et al*. 2021). Bendaoud *et al.* (2009) found that hydrodistilled essential oil from *Eucalyptus* leaves rich in 1,8-cineol showed no significant antioxidant activity in DPPH assays but did in ABTS assays. Unlike this study, Bendaoud's essential oil was composed of 69.5% of 1,8-cineol and did not contain D-limonene (Bacanli *et al*. 2015; Youcef-Ettoumi *et al.* 2021). Although pure D-limonene has no significant antioxidant activity, it may have a synergistic effect with other compounds in eucalyptus essential oil. The compounds γ-terpinene and terpinolene, present in both essential oils used in this study, have been shown to have antiradical properties (Lu *et al*. 2020).

While the composition of essential oils varies depending on the extraction process, the results of Chahomchuen *et al.* (2020) show similar compounds to those identified in this study. Chahomchuen's data were used to plot four trends among minor compounds in the essential oils of orange peel and eucalyptus leaves, as illustrated in Fig. 2. The compounds selected from Chahomchuen's study were 1,8-cineol, α-pinene, α-phellandrene, and terpinene-4-ol. These compounds are represented on the horizontal axes of each graph, and their concentrations are compared with the effects on total phenols (GAE/g).

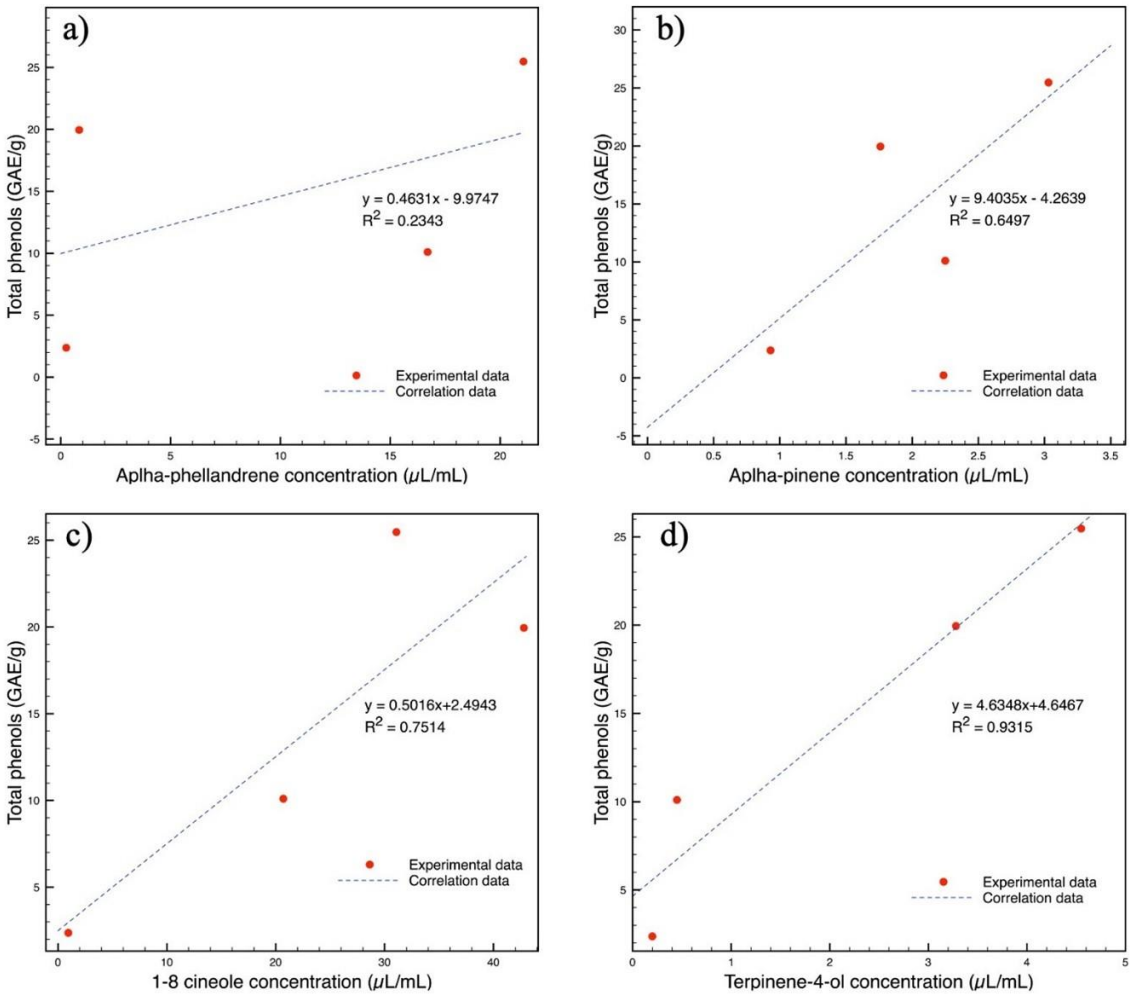

Fig. 2. Essential oil component concentration vs GAE: a) α-Phellandrene; b) α-Pinene; c) 1,8-cineol; d) Terpienen-4-ol

For statistical purposes, none of the four images in Fig. 2 exhibit a correlation exceeding R^2=0.95, indicating that the expressions in Fig. 2 cannot be used as predictors of antioxidant capacity based on the concentration of minor compounds. However, there was a noticeable trend between the increase in the concentration of these four compounds and the enhancement of antioxidant capacity, as measured by total phenols. The strongest trend was associated with terpinen-4-ol, while the weakest trend was associated with α-phellandrene. These values align with those reported by Aryal *et al.* (2019), who found an R^2 coefficient of 0.75 when correlating GAE with phenols, flavonoids, and other compounds in various plants. Piluzza and Bullitta (2011) observed similar correlations (up to 81%) between certain compounds with GAE and TEAC in their analysis of 44 plant species. This suggests that although D-limonene and 1,8-cineol do not possess high antioxidant capacity, other minor compounds within the essential oil contribute to this capacity.

Published research suggests that D-limonene concentration impacts antioxidant activity as measured by various mechanisms (Davicino *et al*. 2009). The correlation varies depending on the mechanism of the method used. Peroxide (H_2O_2) scavenging indicate a minimum when the concentration of this terpene is 50 µL/g, performing better with

concentrations higher or lower than that value (Goñi *et al*. 2009). In this study, however, the relative abundance of D-limonene was much greater than this critical value and may be decreasing the antioxidant activity of the essential oil's compound mixture.

The results of this study suggest that synergistic interactions between various compounds determine the overall properties of essential oils (Goñi *et al*. 2009; Sharma *et al.* 2020; Chraibi *et al.* 2021). Nguyen *et al.* (2014) found that lime essential oil had the lowest D-limonene concentration, but the highest antioxidant capacity compared to other citrus fruits such as orange and grapefruit.

Some research suggests an inverse relationship between TEAC antioxidant capacity and relative D-limonene abundance (Torres-Alvarez *et al.* 2016). For example, oil with 92% D-limonene had a TEAC value of 23.25, which increased almost sevenfold when the D-limonene concentration was reduced to 23%. In this study, however, both oils analyzed had similar antiradical activity despite *C. sinensis* having nearly three times the relative D-limonene abundance of *E. camaldulensis*.

Raspo *et al.* (2020) reports antioxidant activity by free radical scavenging, lower than those presented in this work, between 8 and 32 TEAC, for essential oils from different species of the *Citrus* genus with D-limonene concentrations of up to 98%. Singh *et al.* (2021) reported similar findings with the antioxidant activity of citrus oil between 6.1 and 8.1 TEAC/g as measured by the DPPH radical scavenging method. This result was attributed to terpenes such as citral, thymol, α-sinensal, α-terpineol, γ-terpinene, and citronellal. The last two terpenes are present in the oil used in this study.

CONCLUSIONS

1. The isolation process of essential oils from biomass waste did not adversely affect the antioxidant capacity or antimicrobial activity. Both essential oils were successfully derived from biomass waste.
2. The predominant compounds identified in each essential oil were D-limonene in orange peel oil and 1,8-cineol in eucalyptus leaf oil.
3. Although D-limonene and 1,8-cineol are not recognized as potent antioxidants individually, the antioxidant capacity of orange peel and eucalyptus leaves essential oils was increased due to the presence of other components in the oil such as α-pinene, α-phellandrene, and terpinene-4-ol.
4. The essential oil extracted from orange peel exhibited antimicrobial activity against *Escherichia coli*, whereas the essential oil from eucalyptus leaves demonstrated antimicrobial activity against *Staphylococcus aureus*.

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