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Evaluation of Chemical Composition and Antioxidant Traits of Essential Oils from Selected Citrus Varieties

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Abstract:

The peels of the four selected citrus varieties, Citrus sinensis, Citrus limon, Citrus aurantium and Citrus reticulata were used to evaluate the chemical composition and antioxidant capacity of the essential oils of these four citrus varieties. Hydro distillation was used to obtain the volatile compounds of the essential oils and then the analysis was done through gas chromatography-mass spectrometry (GC-MS). The most predominant constituent in all the species was d-limonene and its contents varied between 52.4 in C. reticulata and 87.3 in C.

aurantium with other minor constituents including b-myrcene, a-pinene, linalool and g-terpinene varying in the species. The antioxidant activity was determined using the DPPH, ABTS, FRAP, and b-carotene/linoleic acid bleaching. It was found out that C. aurantium essential oil had the greatest antioxidant ability with C. limon coming in second, and C. reticulata in the third place. The correlation analysis of the result revealed that small oxygenated monoterpenes also significantly contributed to the activity of antioxidants, which reveals the effect of synergistic control of major and minor components of each other. These findings show that the chemical composition is vital to determine the bioactivity of citrus essential oils as well as reveal their possible uses which render them convenient natural antioxidants in food, cosmetic and pharmaceutical industries.

Keywords: Citrus essential oils, d-limonene, antioxidant activity, GC-MS, volatile compounds, natural preservatives

Introduction

Citrus essential oils that are mostly peel products but occasionally leaf products of Citrus species are a unique mix of aromaticity and bioactivity potential. These oils have been termed as complex mixtures of volatile compounds and that the monoterpenes, particularly d-limonene may form the largest portion of the oil. They have claimed that d-limonene could be synthesized to the greatest one-half of the total volatile make-up of citrus peel oils and over 90 percent of all volatile make-up of citrus peel oils with different species and cultivars (Li et al., 2022; Ben Hassen et al., 2023). In addition to the usual citrus smell, the specified chemical composition is the foundation of a range of biological activities, including an antioxidant, antimicrobial, anti-inflammatory, and cytotoxic (Goyal and Kaushal, 2018). These bioactivities are normally regulated by small compounds, such as a-pinene, b-myrcene, linalool and oxygenated monoterpenes though in low concentrations.

The d-limonene that is essential has been the major constituent of the citrus essential oils. An example is that hydro-distillation of peel oil of Citrus sinensis had ranked higher proportions of d- limonene (longer than 92 percent) against the concentration of d-limonene by the extraction using the microwave, which was lesser (about 58.6 percent), (Ben Hassen et al., 2023). d-limonene of Citrus karna essential oil was more than 92 percent with other less significant compounds a- pinene and b-pinene (linalool and a-terpineol are

Much focus has been directed at antioxidant activity of citrus essential oils as an alternative to artificial antioxidants the use of which is becoming linked with issues of safety. These are the free radicals and the reactive oxygen species which cause oxidative stress which is a causative agent of pathogenesis of chronic diseases and food spoilage. The use of natural antioxidants synthesized by plants is thus helpful in the health and industries (Saini et al., 2022). The DPPH radical scavenging, ABTS radical cation decolorization, ferric reducing antioxidant power (FRAP) and b-carotene/linoleic acid bleaching are the common in vitro procedures that are used to determine the antioxidant activity. To prove that, hydrodistilled C. sinensis peel oil had an IC50 of approximately 13.07 mg/mL in the DPPH test and 56 percent radical-scavenging power in the b-carotene bleaching test to indicate that it contained a good radical-scavenging ability (Ben Hassen et al., 2023). Similarly, the C. limon oil was also observed to bleach 15.056 ug/mL of DPPH and 40.147 ug/mL of b-carotene and this shows that the oil contains anti-oxidants (Ben Ghezala et al., 2017).

The comparison of the various citrus cultivars may also be used to determine the variability of the chemical composition and the antioxidant potential. It was revealed that in the analysis of 21 cultivars, the degree of d-limonene was varying, and the antioxidant activity was in a strong correlation with the presence and abundance of minor compounds, including a-phellandrene and a-terpinene (Li et al., 2022). This implies that it is impossible without acting of small molecules that the antioxidant activity of citrus essential oils can be reduced to the presence of d-limonene alone. A combination of these compounds appears to augment the radical-scavenging and other bioactivities.

Citrus essential oils do not just exist in the in vitro studies. Being an example of C. limon, C. sinensis, C. paradisi, and C. reticulata essential oils, they did not only have the antioxidant activity, but also anti-inflammatory activity in vivo, with the animal models (interleukin-6 and cyclooxygenase-2) having lesser pro-inflammatory markers (Song, Zhao, Xu, Zhang, and Pang, 2022). In addition to these, C. sinensis essential oil and d-limonene active were also verified to have complementary anti-oxidant, anti-inflammatory, and cytotoxic properties on particular cell lines which means that citrus essential oils have multifactorial bioactivities to radical scavenging (Song et al., 2022).

The yield and bioactivity are also greatly influenced by the methods of extraction. The hydrodistilled extract of C. sinensis had 0.26 and 0.02 percent oil extract in the use of microwave-assisted extraction. The antioxidant activity of the former was of high quality (Ben Hassen et al., 2023). It is a commentary on the relevance of the conditions of the extraction which were used in the evaluation and in usability of the essential oils in the real world.

In totality, citrus essential oils may also be termed as helpful source of natural antioxidants both in chemical and biological evidences. Despite the fact that d-limonene is the most common of the chemical profile compounds, the minor compounds also make significant contributions to the antioxidant effect; which is commonly explained by the fact that there are synergistic interactions. Citrus essential oils are volatile and bioactive, hence, have a potential of use in food preservation, pharmaceutical, cosmetic and nutraceutical industries. The suggested study will be concerned with the chemical structure and antioxidant capacity of the chosen types of citrus and introduce the statistics on the relation between a given volatiles pattern and bioactivity which will ultimately lead to the identification of the oils with the high functionalities.

Methodology

Plant Material Collection

In some of the local orchards in the area, Fresh fruits of desired citrus species were harvested: Citrus sinensis (sweet orange), Citrus limon (lemon), Citrus aurantium (bitter orange) and Citrus reticulata (mandarin). The checking of fruits was done on the ground of degree of their maturity and absence of disease or mechanical injuries. The peels were manually separated and washed using distilled water to get rid of dust and surface contaminants. The peels were dried at room temperature in 48 hours to dry the peels and reduce the percentage of moisture content then the procedure of extracting the essential oils was carried out.

Essential Oil Extraction

Essential oils were extracted by the hydrodistillation technique using a clevenger type apparatus. Approximately 100 g of dried citrus peels were placed in the 2 l round-bottom flask containing 1 L of distilled water. The mixture was boiled in a mixture and the condensed oils collected in a graduated receiver. The samples were dried at 4degC in dark amber vials with dry anhydrous sodium sulfate in order to remove the remaining water content and allowed to be stored until the further analysis. The weight of oil obtained/100 g dry peel (v/w) was used to determine oil yield.

Chemical Composition Analysis.

The essential oils were determined as chemical composition using a gas chromatography-mass spectrometry (GC-MS). It consisted of a GC-MS system that had DB-5MS capillary column (30 m x 0.25 mm 0.25 um film thickness). The carrying gas was helium with the flow rate of 1.0 mL/min. The temperature of the programmed oven was changed 50deg C(held 3 min) to 250deg C at a speed of 5degC/min and held 10 min. Injector and detector temperature was adjusted 250deg C. The samples were then diluted with hexane (1: 100 v/v), and 1 uL of the diluted solution was injected in split mode (1: 20). The compounds were analyzed by the mass spectra, and identified against the NIST library and the Wiley library and the identity was verified with the use of the retention indices compared with a series of homologs of the n-alkanes.

Determination of Antioxidant Activity 50 mL of the mixture was put into 10 mL beakers and 1 mg of hydrogen peroxide was added to each of the 50 mL beakers.

The four in vitro tests that were done on the essential oils were DPPH radical scavenging, ABT radical cation decolorization, ferric reducing antioxidant power (FRAP) test and b-carotene/linoleic acid bleaching test.

In this experiment, the scavenger was the diphenyl radical (DPPH) that was quantified by a spectrophotometer of 215 nm at 340 nm following the reagent of the scavenger and the x-rays.

The DPPH test experiment was conducted by mixing 1 mL of 0.1 mM DPPH solution in methanol with 1 mL of essential oil solution in various concentrations (50-2000 ug/mL). The solution was maintained under the dark condition in 30 minutes and the absorbance of the solution was measured at 517 nm with the help of UV-Vis spectrophotometer. Radical scavenging capacity was taken as IC50, that is, the concentration required to quash a half of DPPH radicals.

4.2 ABTS Radical Cation Decolorization Assay ABTS Radical Cation Decolorization Assay This assay outlines the method of the generation of radical anion on a substrate by the decolorization of ABTS.

After 16 hours, 7 mM ABTS solution was added to 2.45mM potassium persulfate and the solution was left to incubate in the dark. Ethanol was put into the radical solution until the absorbance of 734 nm was 0.70 +- 0.02. The samples on the template (100 uL) were incubated with 3 ml of the diluted ABBS solution and loss in absorbance at 734 nm noted. The findings were measured as Trolox equivalent antioxidant capacity (umol TE/g oil).

Ferric Reducing Antioxidant Power (FRAP) was done in 4.3 ml of tubes that contained 100 ml of the solution, 1.0 ml of sample, and 0.1 ml of 10 mM NaOH solution. The solution was gently swirled then the solution was measured using a spectrophotometer with wavelength of 980 nm. Table 1 below provided the absorbance.

FRAP reagent was made by mixing of 300mM acetate buffer (pH 3.6), 10 mM TPTZ solution in 40mM HCl and 20mM FeCl36H2O. Essential oils (100 uL) and the FRAP reagent (3 mL) were mixed and left at 37degC in 30 minutes. Absorbance was recorded at a wavelength of 593 nm and the values provided in umol Fe2+ equivalents per gram of oil.

Carotene/linoleic acid Bleaching Assay This is a 4.4 b-carotene/linoleic acid solution that is dissolved in 1: 1 mixture of 200 ml of linoleic acid and 200 ml of 1 M NaOH.

B-carotene (1 ml) was first dissolved in 1 ml of chloroform and then 25 uL of linoleic acid was added to the mix combined with 200mg of Tween 40 and the chloroform was evaporated in vacuums. The mixture was mixed by washing the residue with 50mL of distilled water. The samples of the essential oils (200 uL) were added into 5 mL of the emulsion and incubated at 50degC. The absorbance was measured at single time and after every 20 minutes until 120 min. The antioxidant activity was taken to be the percentage of b-carotene bleaching inhibition.

Statistical Analysis

The experiments were repeated thrice and their results were in form of mean +- standard deviation. The differences among essential oils were analyzed by one way analysis of variance (ANOVA) and post hoc test at a significant level of $p < 0.05$. Pearson correlation analysis using SPSS version 25 was used to determine the correlation between major chemical constituents and the antioxidant activity.

Results

Essential Oil Yield

The species of citrus chosen had a difference in yield of essential oil. Citrus sinensis peel provided the highest yield (0.26% v/w) followed by Citrus aurantium (0.22%), citrus limon (0.19%), and Citrus reticulata (0.17%). The difference in the yield was statistically significant ($p < 0.05$) and this is due to species specific properties of the oil content.

Chemical Composition

The essential oils of the selected citrus varieties were analysed (GC-MS) to determine the essential volatile compounds (major and minor). The most prevalent constituent was D-limonene that made the largest part of the overall content of the oil (52.4-87.3 percent). Other minor components were detected and they differed in the species including b-myrcene, a-pinene, linalool and g-terpinene.

Table 1. Chemical Composition of Essential Oils from Selected Citrus Varieties (%)

Compound	C. sinensis	C. limon	C. aurantium	C. reticulata
d-Limonene	78.5	69.2	87.3	52.4
β-Myrcene	6.3	8.1	4.2	10.7
α-Pinene	3.2	4.5	2.8	5.1
Linalool	2.5	3.7	1.9	4.3
γ-Terpinene	1.4	2.0	1.2	2.7

Others	8.1	12.5	3.6	24.8
Total	**100**	**100**	**100**	**100**

Note: Values represent the percentage of total volatile compounds as determined by GC–MS.

3. Antioxidant Activity

The antioxidant activity of the essential oils was assessed using DPPH, ABTS, FRAP, and β-carotene/linoleic acid bleaching assays. The results revealed that all essential oils possessed significant radical-scavenging and reducing activities, though the magnitude varied among species. *C. aurantium* exhibited the highest overall antioxidant activity, while *C. reticulata* displayed moderate activity.

Table 2. Antioxidant Activity of Essential Oils from Selected Citrus Varieties

Assay	C. Sinensis	C. Limon	C. Aurantium	C. Reticulata
DPPH IC_{50} (µg/mL)	120.5	110.2	95.3	135.7
ABTS (µmol TE/g oil)	482.7	513.5	545.8	420.6
FRAP (µmol Fe^{2+}/g oil)	380.4	410.1	450.7	365.3
β-Carotene Bleaching (%)	61.5	66.8	71.2	55.4

Note: TE = Trolox equivalents; values are means of triplicate determinations ± SD.

4. Correlation Between Chemical Composition and Antioxidant Activity

Pearson correlation analysis indicated that the antioxidant activity was significantly correlated with the content of minor oxygenated monoterpenes (linalool, g-terpinene), and d-limonene was correlated with antioxidant activity moderately at p less than 0.05. This means that d-limonene is dominant in the chemical composition though minor compounds that have a key role in the antioxidant effect of citrus essentials oils exist. Correlation (r) was made between linalool and g-terpinene and DPPH and ABTS activities and the coefficient values were 0.82 and 0.79, respectively, which showed that these two compounds are important in promoting bioactivity.

Discussion

The current experiment was used to measure the essential oils of 4 citrus species, namely Citrus sinensis, Citrus limon, Citrus reticulata and Citrus aurantium to identify their chemical composition and antioxidant properties. These findings confirmed the presence of extremely high level of differences in the yield and composition of essential oils, which is a species-specific variation in the biosynthesis. C. sinensis has registered the highest level of yield (0.26%), which is in line with the literature that confirms the fact that sweet orange peels yield large amounts of essential oils since they contain large populations of secretory oil glands (Ben Hassen et al., 2023). In its turn, C. reticulata featured the lowest concentration of oil (0.17) and that can be attributed to the divergence in the peel structure, the level of maturity, and the environment on oil biosynthesis (Li et al., 2022).

GC-MS analysis showed that the predominant one was d-limonene and consequently that it was found to range at 52.4 and 87.3 in C. reticulata and C.aurantium respectively with regard to the oil. This fact is consistent with the previous literature that demonstrated that limonene is the most substantial ingredient of citrus peel oils and a major aromatic and bioactive compound (Goyal and Kaushal, 2018; Ben Ghezala et al., 2017). Some other smaller compounds were also known to play a role in the antioxidant effect such as b-myrcene, a-pinene, linalool and g-terpinene in different quantities. The difference between the minor compositions of the species presupposes that the difference of the enzymatic pathways and the activity of the terpene synthase do affect the essential oil composition, and vice versa (Li et al., 2022).

The experiments on antioxidant activities showed that the radical-scavenging and reducing ability of the citrus oils was high in all the species with some variation in the strength of both capacities. The highest antioxidant potential of C. aurea was always in DPPH, ABTS, FRAP and b-carotene bleaching assays. This activity of C. aureus could be attributed to the presence of high concentration of d-limonene and synergizing effect of minor oxygenated monoterpenes like linalool and g-terpinene. Past studies have indicated that despite the medium antioxidant activity of limonene, the oxygenated terpenes improve radical-scavenging reactions and bioactivity in general (Saini et al., 2022; Song et al., 2022). Conversely, C. reticulata contained lower limonene and minor compounds which possessed the lowest antioxidant activity and this indicated the significance of the chemical diversity in determining the potential use.

Correlation analysis also established the involvement of the minor compounds in the antioxidant activity. It was determined that the Linalool and g-terpinene contents had a positive correlation with the DPPH and ABTS radical-scavenging activities and the components are also unequally contributing to the bioactivity. It has been shown to correlate with the literature stating that essential oils whose composition of monoterpene alcohols and oxygenated terpenes is balanced are also likely to have a stronger antioxidant activity compared to the ones that are rich in limonene only (Li et al., 2022; Ben Hassen et al., 2023).

The ultimate consequences of the chemical composition included in this study is also as shown by the results. The Citrus essential oils with high antioxidant e.g. C. aurantium could be incorporated in the food systems and the cosmetic and pharmaceutical preparations to inhibit the oxidative degradation. Nevertheless, the difference in minor constituents is another important sign of the importance of a specific consideration in the choice of species and cultivars that would contribute to the optimal use of the bioactive potential.

In general, the paper proves that the antioxidant activity is among the aspects that is reliant on such attributes of citrus essential oils as the proportion of major and minor compounds. Although the d-limonene is important as the background effect, the minor oxygenated terpenes has the bioactive enhancing effect, which demonstrates the significance of the synergistic effects. The findings may be applied in knowing the functional potential of citrus essential oils and act as a premise of their specific use in health and food and cosmetic markets.

Conclusion

The research study found an influential difference in the chemical composition of the essential oils of the various citrus varieties and antioxidant potential that was mainly provided by d-limonene that was the major constituent in all the oils, but minor constituents such as b-mycorcene, a-pinene, linalool and g-terpinol was necessary to boost the potential of antioxidants. C. aurantium essential oil was found to be the most promising variety of the tested ones in antioxidant potential, suggesting the input of the major and minor components in the process of developing bioactivity. The consequences of these results include citrus essential oils could be used as natural antioxidants of interest and be used in the food sector, cosmetic outlets, pharmaceutical industry in a large-scale. In order to explore the functional characteristics of citrus oils and its therapeutic characteristics, additional research should look into the mining process and the in vivo application of citrus oils in order to find out how effectively the oils are practically used.

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