Analysis of Essential Oil Composition and Antioxidant and Antimicrobial Activities of Different Parts of Bitter Orange (Postprint)

Authors: Liu Yanan, Wen Yali, Chen Xiawei, Lin Jiaxun, Xu Yourui, Xu Yourui

Date: 2022-12-01T00:00:00+00:00

Abstract

To comprehensively investigate the chemical composition, antioxidant and antimicrobial activities of essential oils from the whole plant of bitter orange (Citrus aurantium var. amara), this study used its leaves, flowers, and young fruits as materials, analyzed their components by steam distillation (SD) combined with gas chromatography-mass spectrometry (GC-MS), and compared the antioxidant and antimicrobial activities of the three through in vitro testing methods. The results showed that: (1) A total of 94 compounds were isolated and identified from the essential oils of bitter orange leaves, flowers, and young fruits. The composition types and contents of the essential oils from the three parts exhibited certain differences, with 13 compounds being common to all. Thirty-four compounds were identified from the leaves, with higher contents of linalool (30.51%) and α-terpineol (14.78%); thirty-two compounds were identified from the flowers, with linalool (57.59%) and d-limonene (16.15%) as the main components; sixty-nine volatile compounds were identified from the young fruits, mainly containing d-limonene (25.55%) and γ-terpinene (10.48%). (2) The essential oils from the three parts of bitter orange exhibited varying degrees of antioxidant activity. The IC50 values of the essential oils from young fruits, leaves, and flowers against ABTS were 2.6, 5.1, and 8.2 $mg \cdot mL^{-1}$, respectively, and against DPPH radicals were 2.7, 4.3, and 5.0 $mg \cdot mL^{-1}$, respectively, with the young fruit essential oil showing the best antioxidant activity. (3) The essential oil from bitter orange young fruits also demonstrated stronger antimicrobial activity against Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa than those from leaves and flowers. These research results can provide a theoretical basis for the extraction and utilization of essential oils from bitter orange leaves, flowers, and young fruits.

Full Text

Abstract

This study analyzed the chemical constituents and compared the antioxidant and antibacterial activities of essential oils from different parts of *Citrus aurantium* L. var. *amara* Engl. Essential oils from leaves (L.O), flowers (F.O), and young fruits (YF.O) were extracted by steam distillation, and their chemical compositions were identified by GC-MS. The results showed that: (1) A total of 94 volatile components were isolated and identified from the three plant parts, with 13 common components among them. The leaf oil contained 34 components, with linalool (30.51%) and limonene (16.15%) as the major compounds. The flower oil contained 32 components, primarily linalool (57.59%) and limonene (16.15%). The young fruit oil contained 69 components, mainly limonene (25.55%) and γ-terpinene (10.48%). (2) The essential oils from different parts exhibited varying antioxidant activities. The IC_{50} values for ABTS radical scavenging were 2.6, 5.1, and 8.2 $mg \cdot mL^{-1}$ for young fruit, leaf, and flower oils, respectively, while the IC_{50} values for DPPH radical scavenging were 2.7, 4.3, and 5.0 $mg \cdot mL^{-1}$, respectively. The young fruit oil demonstrated superior antioxidant activity compared to the leaf and flower oils. (3) The young fruit oil also showed notable antibacterial activity against tested strains, outperforming the leaf and flower oils. These findings provide a theoretical basis for the extraction and utilization of essential oils from different parts of *C. aurantium* var. *amara*.

1. Materials and Methods

1.3.2 GC-MS Analysis

1.3.2.1 Gas Chromatography Conditions The GC analysis was performed on an Agilent 7890B-5977B system equipped with an HP-5MS capillary column (30 m × 0.25 mm × 0.25 m). The oven temperature program started at 45°C, held for 3 minutes, then increased at 20°C $\cdot$ min^{-1} to 200°C, and finally held at 200°C for 10 minutes. The carrier gas was helium at a flow rate of 1.2 mL $\cdot$ min^{-1}. The injector temperature was set at 250°C with a pressure of 9.4667 psi, and the injection volume was 1 L.

1.3.2.2 Mass Spectrometry Conditions The MS operated in electron ionization (EI) mode at 70 eV with an ion source temperature of 250°C and a quadrupole temperature of 230°C. The scan range was 50–550 m/z.

1.3.3 Antioxidant Activity Assays

1.3.3.1 ABTS Radical Scavenging Assay The ABTS radical cation solution was prepared by mixing 10 mL of 7 mM ABTS with 10 mL of 2.45 mM potassium persulfate ($K_2S_2O_8$) and allowing the mixture to stand in the dark for 13 hours. The solution was then diluted to achieve an absorbance of 0.70 ±

0.02 at 734 nm. For the assay, 1 mL of ABTS solution was mixed with sample solution and measured at 734 nm after 6 minutes. BHT was used as a positive control. The scavenging percentage was calculated as:

$$\text{SA\%} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}\right) \times 100\%$$

1.3.3.2 DPPH Radical Scavenging Assay A 0.2 mM DPPH solution was prepared by dissolving 4 mg of DPPH in 50 mL of ethanol. The assay mixture consisted of 2.0 mL DPPH solution and sample solution in a total volume of 4.0 mL. After incubation for 30 minutes in the dark, absorbance was measured at 517 nm. BHT served as the positive control. The scavenging percentage was calculated as:

$$\text{SD\%} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}\right) \times 100\%$$

1.3.4 Antibacterial Activity Assay

1.3.4.1 Bacterial Strains and Culture The test strains included *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853. Bacterial suspensions were adjusted to 1$×10^{8}$ CFU · mL^{-1} using a UV-PC1800 spectrophotometer.

1.3.4.2 Minimum Inhibitory Concentration (MIC) Determination The MIC was determined by the broth microdilution method following Zhang et al. (2017). Briefly, bacterial suspensions were diluted to 1$×10^{7}$ CFU · mL^{-1}, and 20 L aliquots were added to 96-well plates containing two-fold serial dilutions of essential oils (1–32 g · mL^{-1}) dissolved in DMSO. After incubation at 37°C for 24 hours, the MIC was defined as the lowest concentration showing no visible bacterial growth. Gentamicin was used as the positive control.

2. Results and Discussion

2.1 Chemical Composition of Essential Oils

GC-MS analysis identified a total of 94 volatile compounds across the three essential oils, with 13 common constituents. The leaf oil contained 34 identifiable compounds, with linalool (30.51%) and limonene (16.15%) as the predominant components. The flower oil comprised 32 compounds, dominated by linalool (57.59%) and limonene (16.15%). The young fruit oil contained the highest number of compounds (69), with limonene (25.55%) and γ-terpinene (10.48%) as the major constituents .

[FIGURE:1 shows the GC-MS total ion current chromatograms of L.O, F.O, and YF.O.]

2.2 Antioxidant Activity

2.2.1 ABTS Radical Scavenging Activity All three essential oils exhibited dose-dependent ABTS radical scavenging activity. At a concentration of 30 $mg \cdot mL^{-1}$, the young fruit oil achieved 99.00% scavenging activity, significantly higher than the leaf (92.8%) and flower (86.92%) oils. The IC_{50} values were 2.6, 5.1, and 8.2 $mg \cdot mL^{-1}$ for young fruit, leaf, and flower oils, respectively, compared to 0.1 $mg \cdot mL^{-1}$ for the BHT positive control [Figure 2: see original paper].

2.2.2 DPPH Radical Scavenging Activity Similarly, the essential oils showed concentration-dependent DPPH radical scavenging capacity. The young fruit oil demonstrated the highest activity with an IC_{50} value of 2.7 $mg \cdot mL^{-1}$, followed by leaf oil (4.3 $mg \cdot mL^{-1}$) and flower oil (5.0 $mg \cdot mL^{-1}$). At 20 $mg \cdot mL^{-1}$, the young fruit oil achieved 95.24% inhibition, while BHT at 0.1 $mg \cdot mL^{-1}$ reached 99.52% [Figure 3: see original paper].

2.3 Antibacterial Activity

The young fruit essential oil exhibited the strongest antibacterial activity among the three samples, with inhibition zone diameters ranging from 15.2–22.7 mm and MIC values of 8-16 $g \cdot mL^{-1}$ against the tested strains. The leaf and flower oils showed moderate activity with MIC values of 1-32 $g \cdot mL^{-1}$. The superior activity of the young fruit oil correlates with its higher content of limonene (25.55%) and γ-terpinene (10.48%), known antimicrobial compounds.

3. Conclusion

The essential oils from different parts of *Citrus aurantium* var. *amara* show significant variation in chemical composition and bioactivity. The young fruit oil, rich in limonene and γ-terpinene, exhibits the strongest antioxidant and antibacterial properties, suggesting its potential for applications in food preservation, nutraceuticals, and natural antimicrobial agents. These results provide a scientific foundation for the selective utilization of plant parts based on desired bioactive properties.

References

ALI, B., AL-WABEL, N.A., SHAMS, S., et al., 2015. Essential oils used in aromatherapy: A systemic review[J]. *Asian Pacific Journal of Tropical Biomedicine*, 5(8): 601-611.

CHEN, L.Y., CUI, Z.H., 2006. Research Progress on antibacterial activity of plant essential oils[J]. *Heilongjiang Medical Journal*, 19(3): 197-198.

CHOI, S.Y., KANG, P., LEE, H.S., et al., 2014. Effects of inhalation of essential oil of *Citrus aurantium* L. var. *amara* on menopausal symptoms, stress, and estrogen in postmenopausal women: a randomized controlled trial[J]. *Evidence-Based Complementary and Alternative Medicine*, 2014: 1-7.

DONG, X., LIU, X., XIAO, R.X., et al., 2022. Chemical constituents of essential oil from *Citrus aurantium* L. flowers and its antioxidant activities[J]. *Journal of Qingdao University of Science and Technology (Natural Science Edition)*, 43(3): 40-46.

FAN, R., 2011. Composition and antioxidant activity of the *Citrus maxima* peel essential oils obtained by three different extraction methods[D]. Guangzhou: South China University of Technology: 14-22.

HAJ AMMAR, A., BOUAJILA, J., LEBRIHI, A., et al., 2012. Chemical composition and in vitro antimicrobial and antioxidant activities of *Citrus aurantium* L. flowers essential oil (Neroli oil)[J]. *Pakistan Journal of Biological Sciences*, 15(21): 1034-1040.

HUANG, S.S., HOU, P.J., LI, X.L., et al., 2016. Analysis of volatile oil compounds of *Citrus aurantium* var. *amara* by supercritical fluid[J]. *Hubei Agricultural Sciences*, 55(12): 3182-3184.

JING, H., ZUO, J.F., 2005. Pharmacological research progress of volatile oil[J]. *Northwest Pharmaceutical Journal*, 20(2): 97-98.

KONG, Y.T., HE, H., AN, F.P., et al., 2021. Comparative study on antioxidant capacity of *Citrus aurantium* extracts[J]. *Food Research and Development*, 42(19): 28-35.

LI, H.M., GAO, Y., SHAO, X.F., et al., 2022. Study on total flavonoids content and comparison of antioxidant activity in different parts of *Citrus aurantium* from different provenances[J]. *China Food Additives*, 33(4): 211-217.

LIU, Q., ZHONG, L.Y., ZENG, J.H., et al., 2020. Study on the antibacterial activity of essential oils from seven species in the Rutaceae family[J]. *China Food Additives*, 31(5): 37-41.

ROTA, M.C., HERRERA, A., MARTÍNEZ, R.M., et al., 2008. Antimicrobial activity and chemical composition of *Thymus vulgaris*, *Thymus zygis*, and *Thymus hyemalis* essential oils[J]. *Food Control*, 19(7): 681–687.

STOHS, S.J., 2017. Safety, efficacy, and mechanistic studies regarding *Citrus aurantium* (bitter orange) extract and p-synephrine[J]. *Phytotherapy Research*, 31: 1463-1474.

SUN, W.Q., ZHANG, X.C., XIN, X.D., et al., 2022. Comparative study on extraction of volatile oil from *Citrus aurantium* Folium by steam distillation and water extraction coupling rectification[J]. *Academic Journal of Shanghai University of Traditional Chinese Medicine*, 36(1): 27-32.

SUNTAR, I., KHAN, H., PATEL, S., et al., 2018. An overview on *Citrus aurantium* L.: its functions as food ingredient and therapeutic agent[J]. *Oxidative Medicine and Cellular Longevity*, 2018: 7864269.

WANG, D.M., CHEN, X.F., MAI, L.W., et al., 2021. Study on the relationship between antioxidant capacity and total flavonoids contents of cassava leaves[J]. *Food Research and Development*, 42(2): 37-43.

WEI, Q., TANG, L.J., LOU, X.Y., et al., 2022. GC-MS analysis of constituents of volatile oil in different parts of *Citrus aurantium* var. *amara*[J]. *Science and Technology of Food Industry*, 43(12): 310-316.

YANG, Y., GAO, J.F., 2018. Volatile components and their antioxidant activities in different parts of *Citrus aurantium*[J]. *Guihaia*, 38(7): 943-952.

YE, Y.D., ZHANG, N., LI, Y.H., et al., 2019. Chemical composition and anti-inflammatory effect of essential oil from leaves of *Citrus aurantium* L.[J]. *Natural Product Research and Development*, 31(5): 760-765.

ZHA, Y.F., ZHAN, Y., LI, T., et al., 2022. Study on antioxidant effect of extracts from different parts of *Panax notoginseng* (Radix Notoginseng)[J]. *Acta Chinese Medicine*, 37(1): 142-148.

ZHANG, H.Y., GAO, Y., LAI, P.X., 2017. Chemical composition, antioxidant, antimicrobial and cytotoxic activities of essential oil from *Citrus aurantium* L. var. *amara*[J]. *Molecules*, 22(3): 381.

ZUO, A.X., LIU, J.G., GAO, Y.M., et al., 2022. GC-MS analysis and comparison of antioxidant activities of volatile oils from different parts of *Citrus aurantium* Thunb[J]. *Food Research and Development*, 43(8): 146-151.

Note: Figure translations are in progress. See original paper for figures.