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Research Article

GC, GC/MS analysis, and biological effects of *Citrus aurantium amara* essential oil

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Citrus aurantium amara (sour orange) belongs to one of the largest genera of the Rutaceae family. The species of this genus are consumed worldwide as fresh or in form of beverages. They include well-known crops lemons, oranges, mandarins, grapefruits, and limes. The industrial processing of these fruits produces high amounts of waste (around 50%) which is a valuable source of essential oils. Since they are produced mainly from peel, considered waste, these essential oils have great economic value. In that regard, the aim of this study was to evaluate the chemical composition of the essential oil obtained from the peel of *Citrus aurantium amara*, as well as to assess the biological effects by the means of antioxidant and antibacterial activity. Results of GC and GC/MS analysis characterized this EO as a valuable source of limonene found in the amount of 90.4% of the total. Results of antioxidant activity indicate better inhibition of $ABTS^{\cdot+}$ (44.93 ±1.45%) compared to the $DPPH^{\cdot}$ (11.03 ±1.08%). Moreover, the results of the antimicrobial assessment using the disc diffusion method displayed low inhibition potency of this essential oil towards G^+ and G^- bacteria and yeast strains.

Keywords: essential oil, sour orange, chemical composition, antioxidant activity, antimicrobial activity

1 Introduction

Aromatic plants are a well-known source of essential oils (EOs), that are regarded as natural, volatile liquids or semiliquids rich in biologically active secondary metabolites. EOs are produced in more than 17,500 aromatic species in different parts of plants like roots, flowers, leaves, peels, fruits, seeds, etc. (Baptista-Silva et al., 2020; Falleh et al., 2020). Generally, these volatile liquids correspond to less than 5% of plants' dry mass (Falleh et al., 2020). Mainly they are responsible for plants' specific scents. From the chemical point of view, their composition consists of different classes of terpenes, terpenoids, phenolics, phenylpropanoids, etc. formed by various biogenetic pathways (Baptista-Silva et al., 2020; Falleh et al., 2020; Pavela et al., 2019). The production of these compounds is mainly affected by their role in plant life, as well as the ripening stage, harvesting time, and environmental and plant genotype factors. In industry, they are mainly used as flavouring agents in food products and beverages, cosmetics, agriculture, sanitary products, and aromatherapy (Bourgou et al., 2012; Bousbia et al., 2009; Falleh et al., 2020; Pavela, 2005). Their industry use is supported by their proven biological effects such as antimicrobial, antitumor and proapoptotic, antioxidant, antiviral, antimycotic, antiparasitic, and insecticidal (Baptista-Silva et al., 2020; Falleh et al., 2020; Pavela, 2005; Popović-Djordjević et al., 2019).

Citrus aurantium amara or a sour orange belongs to the *Citrus* species of the Rutaceae family which is native to the tropical regions of Southeast Asia, China, Northern India, and Northern Myanmar (Bora et al., 2020). The cultivation of this species dates back to 2,100 BC (Dosoky & Setzer, 2018). Their fruits are important horticultural crops whose production is estimated at over 80 million tons per year (Bourgou et al., 2012). The industrial processing of these fruits produces high amounts of waste (around 50%), which is a valuable source of essential oils, flavonoids, polyphenols, carotenoids, soluble sugars, cellulose, hemicellulose, pectin, and other beneficial secondary metabolites (Dosoky & Setzer, 2018). Because of its economic value, many studies investigate the biological properties and chemical composition of this species'

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peel, leaf, and flower essential oils. Moreover, EOs are generally considered safe by the EPA (Environmental Protection Agency) as well as the US FDA (Food and Drug Administration), which encourages the investigations of this plant product. Regarding the biological effects of this species, they are characterized as beneficial in the prevention of many diseases (Bora et al., 2020). Current reports show their antioxidant, antimicrobial, anti-inflammatory, and insecticidal properties. Likewise, they are shown to be beneficial in preventing the development of mental health diseases (Bora et al., 2020; de Moraes Pultrini et al., 2006; Mannucci et al., 2018).

Investigations on the chemical composition of *Citrus* species essential oils characterized them as valuable sources of monoterpene hydrocarbons, especially limonene which in peel oils has a high abundance. Besides limonene, EOs from this species contains a wide variety of compounds, with a number of components that varies in the range from 20 to 60 (Ruiz & Flotats, 2014). In high amounts can also be found γ-terpinene, linalool, linalyl acetate, α-terpineol, β-ocimene, terpinolene, and β-pinene (Boussaada & Chemli, 2006; Dosoky & Setzer, 2018; Mancuso et al., 2019; Ruiz & Flotats, 2014). Since limonene is found to be a major compound whose concentration is in the range from 32% to 98%, depending on the species from which the EO is extracted, harvesting time, geographical origin, etc. the chemical, physical and biological properties of this compound can significantly affect the properties of the EO (de Araújo et al., 2020; González-Mas et al., 2019; Mahato et al., 2019; Ruiz & Flotats, 2014).

Bearing in mind the above-mentioned, the aim of this study was to evaluate the chemical composition of *Citrus aurantium amara* essential oil using GC, and GC/MS techniques, as well as to evaluate its biological effects. The antioxidant properties of the investigated essential oil were determined by the means of decolorization of DPPH radical and ABTS radical cation. Moreover, the antimicrobial activity of this EO towards Gram-positive and Gram-negative bacterial strains as well as yeast was evaluated.

2 Material and methods

2.1 Essential oil

Essential oil from *Citrus aurantium amara* was purchased from Hanus, s.r.o. (Nitra, Slovakia). This sample was obtained by steam distillation of peal. The sample was stored in the dark at 4 °C prior to the analyses.

2.2 Gas chromatography-mass spectrometry and gas chromatography analyses

The chemical composition of the volatile constituents of *Citrus aurantium amara* essential oil was carried out using a gas chromatograph (Agilent Technologies (Palo Alto, Santa Clara, CA, USA) 6890 N) which was equipped with a quadrupole mass spectrometer 5975 B (Agilent Technologies, Santa Clara, CA, USA). This gas chromatograph was operated with an interfaced HP Enhanced ChemStation software (Agilent Technologies). The separation of volatile compounds was executed by employing the HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm). Helium 5.0 has been used as a carrier gas, with a flow rate set at 1mL.min^{-1}. The sample of essential oil was diluted with hexane (10% solution), and the injection volume was 1 μL. The temperature of the split/splitless injector, the MS source, and the MS quadruple were set at 280 °C, 230 °C, and 150 °C respectively. The solvent delay time was 3.0 min, while the mass scan range was 35–550 amu at 70 eV. The conditions of the analysis (GC and GC-MS) were the following: temperature program of 60 °C to 150 °C (rate of increase 3 °C.min^{-1}), and 150 °C to 280 °C (rate of increase 5 °C.min^{-1}), held 4 min at 280 °C. The total run time was 60 min, and the split ratio was 40.8 : 1.

Volatiles present in the essential oil sample were identified by the means of their retention indices (RI) in comparison with the reference spectra reported in the literature and the ones stored in the MS library (Wiley7Nist) (Adams, 2007; van den Dool & Dec. Kratz, 1963). Using GC-FID with the same HP-5MS capillary column performed was semi quantification of the components taking into consideration amounts higher than 0.1%.

2.3 DPPH assay

The antioxidant screening of *Citrus aurantium amara* essential oil was performed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method (Galovičová et al., 2022). The 190 μl of DPPH (Sigma Aldrich, Schnelldorf, Germany) radical solution in methanol (prepared so absorbance is adjusted at 0.8 at 515 nm) was mixed with the 10 μl EO in a 96-well microtiter plate. The reaction mixture was incubated at room temperature for 30 min in dark with continuous shaking at 1,000 rpm after which the absorbance of the sample was measured spectrophotometrically at 517 nm. All measurements were performed in triplicate. Methanol (Uvasol® for spectroscopy, Merck, Darmstadt, Germany) was used as a control solution, whereas Trolox (Sigma Aldrich, Schnelldorf, Germany) was used as a reference compound. Antioxidant activity was expressed as a percentage of

DPPH· inhibition. The calculation was performed using the equation:

$$(A_0 - A_A)/A_0 \times 100 \quad (1)$$

where: A_0 – the absorbance of DPPH·; A_A – the absorbance of the sample

Radical scavenging activity was recalculated against a standard reference substance Trolox dissolved in methanol to a final concentration range of 1–5 mg.L^{-1}. The total radical scavenging capacity was expressed according to the calibration curve of Trolox (TEAC). From the Trolox calibration curve, the IC_{50} value was obtained. The results were presented as mean values ± standard deviation (SD) of three independent measurements.

2.4 ABTS assay

ABTS [2,20-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium] (Sigma Aldrich, Schnelldorf, Germany) radical cation was generated according to the already described procedure (Proestos et al., 2013). The prepared radical cation was diluted prior to the analysis up to an absorbance value of 0.7 at 744 nm. The 190 µl of this solution was mixed with 10 µl of EO (in a 96-well microtiter plate) for 30 min with continuous shaking at 1,000 rpm at room temperature in the dark. A decrease in absorbance at 744 nm was registered and the results are presented as a percentage of ABTS inhibition using the same equation (1) as in section 2.3. All measurements were performed in triplicate. As blank solution used was methanol, and as standard reference substance used was Trolox. Results were expressed as % of inhibition as well as according to the calibration curve of Trolox (TEAC). The IC_{50} value for Trolox was calculated from the calibration curve. The results were presented as mean values ± standard deviation (SD) of three independent measurements.

2.5 Tested microorganisms

Microorganisms including Gram-negative (G^-) bacteria *Yersinia enterocolitica* CCM 5671, Gram-positive (G^+) bacteria *Enterococcus faecalis* CCM 4224, and yeasts *Candida krusei* CCM 8271, *Candida albicans* CCM 8186, *Candida tropicalis* CCM 8223, and *Candida glabrata* CCM 8270 were obtained from the Czech collection of microorganisms.

2.6 Disk diffusion method

The antimicrobial activity of *C. aurantium amara* was determined by the means of disc diffusion method using bacteria that were cultivated on Tryptone Soya Agar (TSA, Oxoid, Basingstoke, method UK) at 37 °C for 24 h, while yeasts were cultivated on the Sabouraud dextrose agar (Oxoid, Basingstoke, UK) at 25 °C for 24 h. For inoculation of Mueller Hinton agar (MHA, Oxoid, Basingstoke, UK) used was 100 µL of prepared microorganisms inoculum with the optical density of 0.5 McFarland standard (that corresponds to $1.5 \cdot 10^8$ CFU.mL^{-1}). Blank discs with a diameter of 6 mm were saturated using 5 µL of tested EO and placed on prepared agar. Aerobic incubation of bacteria lasted 24 h at 37 °C, while yeasts were incubated at 25 °C for the same period. The inhibitory activity was detected in the following criteria: zone inhibition diameter above 5mm is considered as a weak inhibitory activity, above 10 mm – is moderate inhibitory activity, and above 15 mm – is a very strong inhibitory activity. Each test was performed in triplicate and the results were presented as mean values ± standard deviation (SD) of three independent measurements.

3 Results and discussion

3.1 GC and GC/MS analysis of C. aurantium amara essential oil

Results obtained by the means of GC and GC/MS analysis are presented in Table 1 as a percentage of identified volatile constituents in the EO sample (with literature and calculated retention indices). Table 2 shows the percentage of the abundance of different classes of compounds identified in the EO of *C. aurantium amara*. Corresponding chromatograms are presented in Fig. 1 and Fig. 2. As can be seen from Table 1, in this sample we have identified 20 compounds that represent 99.8% of the total, out of which monoterpene hydrocarbons represent the main class of compounds identified in this EO with the contribution of 96.1% of the total (Table 2). Previous results of *C. aurantium* essential oils obtained from dried and fresh peel show that 13–37 compounds were identified in different studies, which is in agreement with our results (Bendahaa et al., 2016). Moreover, monoterpene hydrocarbon limonene was the main component of this EO identified in the amount of 90.4%. Previously published results of *Citrus* species essential oils obtained from the peel are in agreement with the results obtained in this study that characterizes them as a valuable source of limonene. The differences between those previous reports are reflected in the content of limonene which is influenced by harvesting time, plant part and origin, as well as the extraction method, geographical, and environmental factors (Bendahaa et al., 2016; Bora et al., 2020; Boussaada & Chemli, 2006, 2007; González-Mas et al., 2019). Alongside limonene, the chemical composition of *C. aurantium amara* EO examined in this study was characterized by relatively high amounts of other monoterpene

Table 1 Chemical composition of essential oil from *C. aurantium* amara, with corresponding literature and calculated retention indices (RI)

No	Compound*	%	RI (lit.)	RI (calc.)**
1	α-pinene	0.9	939	938
2	sabinene	0.4	975	977
3	β-pinene	1.5	979	981
4	β-myrcene	2.6	990	991
5	octanal	tr***	998	1,004
6	limonene	90.4	1,029	1,038
7	α-terpinolene	0.3	1,088	1,100
8	*cis*-limonene oxid	tr	1,136	1,136
9	*trans*-limonene oxid	0.2	1,142	1,140
10	α-terpineol	1.7	1,188	1,196
11	*cis*-dihydro carvone	tr	1,192	1,199
12	decanal	tr	1,201	1,206
13	*trans*-carveol	tr	1,216	1,219
14	*cis*-carveol	tr	1,229	1,232
15	neral	tr	1,238	1,238
16	carvone	tr	1,243	1,244
17	linalool acetate	1.6	1,257	1,249
18	geranial	tr	1,267	1,268
19	neryl acetate	0.2	1,361	1,378
20	(*E*)-caryophyllene	tr	1,419	1,422
Total (%)		99.80		

* – identified compounds; ** – values of retention indices on HP-5MS column; *** tr – compounds identified in amounts less than 0.1%

Table 2 Percentage composition of each class of identified compounds

Class of compounds	%
Monoterpenes	99.8
Monoterpene hydrocarbons	96.1
Oxygenated monoterpenes	3.7
Monoterpene epoxide	0.2
Monoterpene alcohols	1.7
Monoterpene ketones	tr
Monoterpene esters	1.8
Monoterpene aldehyde	tr
Sesquiterpenes	tr
Sesquiterpene hydrocarbons	tr
Non-terpenic	tr
Aldehydes	tr
Total (%)	99.8

hydrocarbons β-myrcene (2.6%), and β-pinene (1.5%), followed by monoterpene alcohol α-terpineol (1.7%) and monoterpene ester linalool acetate (1.6%). Other components were identified in amounts lower than 1.0%.

3.2 Antioxidant activity

The antioxidant potential of essential oil obtained from the peel of *C. aurantium amara* was determined by the means of its capacity to neutralize stable DPPH radical and ABTS radical cation and the obtained results

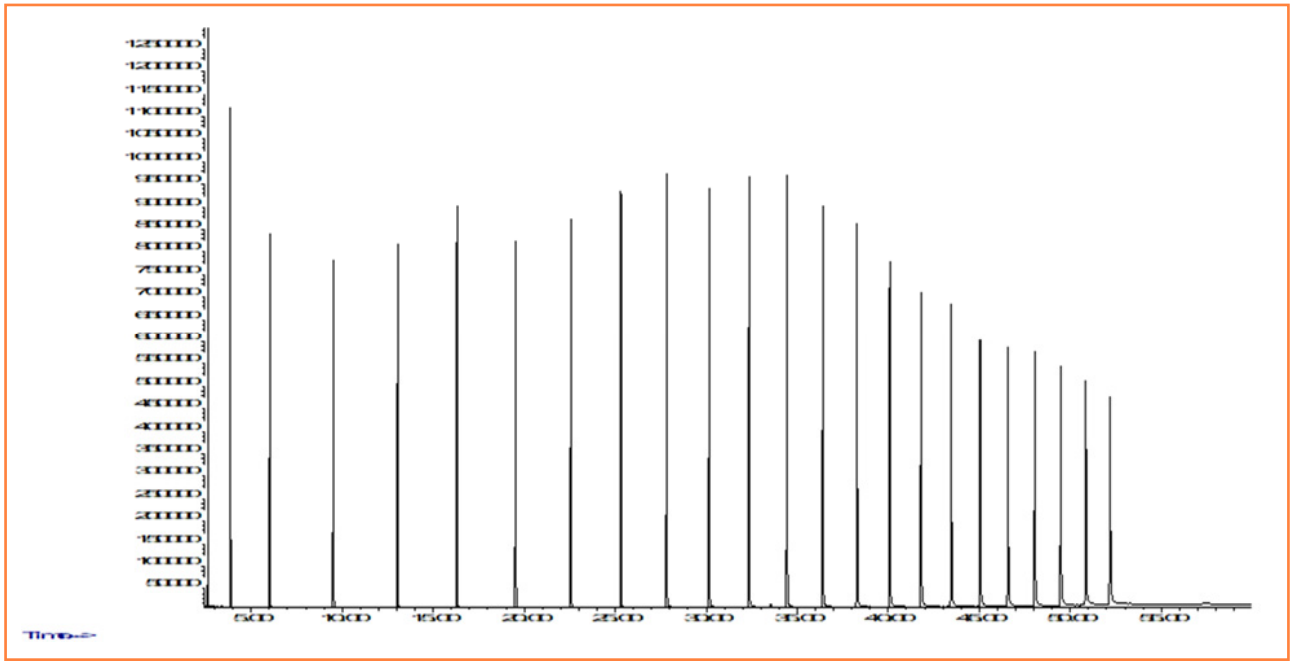


Figure 1 GC/MS chromatogram of series of n-alkanes (C7–C35) for calculation of Kovats retention indices for *C. aurantium amara* EO

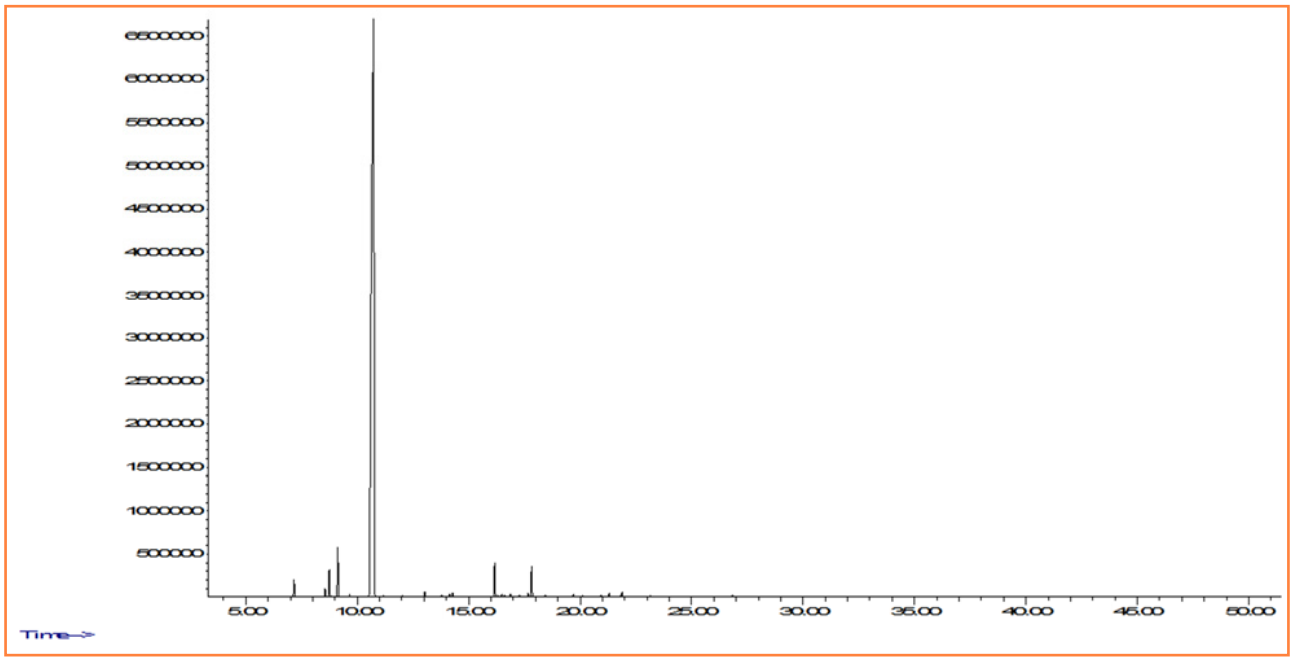


Figure 2 GC/MS chromatogram of *C. aurantium amara* EO for calculation of Kovats retention indices

are presented in Table 3. As a positive control in this experiment, the standard compound Trolox was used. The obtained results indicate better neutralization of $ABTS^{\bullet+}$ compared to $DPPH^{\bullet}$ by both, the EO sample and Trolox standard. Previous reports demonstrated that the examination of plant foods containing hydrophilic, lipophilic, and high-pigmented antioxidant compounds shows the superiority of the ABTS assay compared to the DPPH assay, which can explain the results obtained in this study (Floegel et al., 2011).

The used amount of 10 µL of essential oil per sample was not able to reach the inhibition percentage of DPPH radical and ABTS radical cation up to 50%. In the case of $DPPH^{\bullet}$, the TEAC value was determined at 1.32 ±0.10 $mg.L^{-1}$ which corresponds to 11.03 ±1.08% percentage of inhibition. For $ABTS^{\bullet+}$, the TEAC value was 2.72 ±0.01 $mg.L^{-1}$ which is equivalent to 44.93 ±1.45% inhibition percentage. Previous results obtained by Sarrou et al. (2013) show that EO from *C. aurantium* obtained from the peel inhibited DPPH radical at 19.29%. Other reports indicate that *C. aurantium* essential oils obtained from flavedo display inhibition of DPPH radical with IC_{50} values in the range of 33.01% to 40.74%, and ABTS radical cation with IC_{50} values in the range of 25.31% to 36.22% (Badalamenti et al., 2022). Benadahaa et al. (2016) also reported weak effects on DPPH neutralization of *C. aurantium* ranging from 7–15%. Differences in the obtained results can be explained by the method used for the evaluation of neutralization of stable $DPPH^{\bullet}$ radical and $ABTS^{\bullet+}$ radical cation, as well as the chemical composition of tested EO, and the part of the plant used for EO extraction. The low antioxidant capabilities of the tested EO can be a result of a high abundance of limonene which is considered to not play the principal role in the determination of the scavenging activity for radicals (Bendahaa et al., 2016).

3.3 Antimicrobial activity

The results of the antimicrobial potential of *C. aurantium amara* EO against yeasts and G^+ and G^- bacteria are presented in Table 4. Overall, obtained results show the weak antimicrobial activity of tested EO. The strongest inhibition was observed towards yeast strain *C. albicans* with an inhibition zone of (4.67 ±0.58 mm). However, the activity of this EO towards other yeast strains was considerably lower, and no inhibition was observed on *C. tropicalis*. As for the bacterial strains, obtained results show similar inhibition potential for G^+ *E. faecalis* (3.00 ±0.01 mm) and G^- bacteria *Y. enterocolitica* (2.67 ±0.58 mm). Previous findings indicate that a high abundance of hydrocarbons may be responsible for

Table 3 *In vitro* antioxidant activity of *C. aurantium amara* essential oils

	% of inhibition	TEAC ($mg.L^{-1}$)	Trolox (IC_{50})($mg.L^{-1}$)
DPPH•	11.03 ±1.08	1.32 ±0.10	4.39 ±0.13
ABTS•+	44.93 ±1.45	2.72 ±0.01	2.96 ±0.01

the lower antibacterial effects of EOs, which can be an explanation of the results obtained in this study since the pathways of antimicrobial mechanisms of these volatile liquids are not yet well understood (Kalemba et al., 2012).

Table 4 Disk diffusion method in the *C. aurantium amara* EO against G^+, G^- bacteria, and yeasts (inhibition zones in mm)

Microorganism	Zone inhibition (mm)
Yersinia enterocolitica	2.67 ±0.58
Enterococcus faecalis	3.00 ±0.01
Candida albicans	4.67 ±0.58
Candida glabrata	2.67 ±0.58
Candida krusei	1.33 ±0.58
Candida tropicalis	0.00 ±0.00

4 Conclusions

In this work, the chemical composition of essential oil from the peel of *C. aurantium amara* was determined using GC and GC/MS analysis. The obtained results showed the presence of twenty constituents with the highest limonene abundance of 90.4%. Moreover, noticeable amounts of α-pinene, sabinene, β-pinene, β-myrcene, α-terpineol, and linalool acetate were detected (0.9%, 0.4%, 1.5%, 2.6%, 1.7%, and 1.6%, respectively). The antioxidant investigations revealed lower activity of EO against DPPH$^{\bullet}$ in comparison to Trolox (11.03 ±1.08%; TEAC = 1.32 ±0.10 mg.L^{-1}). In the ABTS assay, EO reached the inhibition percentage of 44.93 ±1.45% which corresponds to 2.72 ±0.01 mg.L^{-1} TEAC. Antimicrobial investigations were performed on yeasts, G^+, and G^- bacteria. The obtained results show weak inhibition of microorganisms in the treatment with *C. aurantium amara* essential oil which can be attributed to the low antimicrobial effects of limonene.

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References

Adams, R. P. (2007). *Identification of Essential Oil Components By Gas Chromatography/Mass Spectroscopy*. Allured Publishing Corporation (4 th ed.).

Badalamenti, N., Bruno, M., Schicchi, R., Geraci, A., Leporini, M., Gervasi, L., Tundis, R., & Loizzo, M. R. (2022). Chemical Compositions and Antioxidant Activities of Essential Oils, and Their Combinations, Obtained from Flavedo By-Product of Seven Cultivars of Sicilian *Citrus aurantium* L. *Molecules*, 27(5), 1580. https://doi.org/10.3390/molecules27051580

Baptista-Silva, S., Borges, S., Ramos, O. L., Pintado, M., & Sarmento, B. (2020). The progress of essential oils as potential therapeutic agents: a review. *Journal of Essential Oil Research*, 32(4), 279–295. https://doi.org/10.1080/10412905.2020.1746698

Bendahaa, H., Bouchalb, B., el Mounsia, I., Salhic, A., Berrabehd, M., el Bellaouib, M., & Mimounia, M. (2016). Chemical composition, antioxidant, antibacterial and antifungal activities of peel essential oils of *Citrus aurantium* grown in Eastern Morocco. *Der Pharmacia Lettre*, 8(4), 239–245

Bora, H., Kamle, M., Mahato, D. K., Tiwari, P., & Kumar, P. (2020). *Citrus* Essential Oils (CEOs) and Their Applications in Food: An Overview. *Plants*, 9(3), 357. https://doi.org/10.3390/plants9030357

Bourgou, S., Rahali, F. Z., Ourghemmi, I., & Saïdani Tounsi, M. (2012). Changes of Peel Essential Oil Composition of Four Tunisian *Citrus* during Fruit Maturation. *The Scientific World Journal*, 2012, 1–10. https://doi.org/10.1100/2012/528593

Bousbia, N., Vian, M. A., Ferhat, M. A., Meklati, B. Y., & Chemat, F. (2009). A new process for extraction of essential oil from *Citrus* peels: Microwave hydrodiffusion and gravity. *Journal of Food Engineering*, 90(3), 409–413. https://doi.org/10.1016/j.jfoodeng.2008.06.034

Boussaada, O., & Chemli, R. (2006). Chemical Composition of Essential Oils from Flowers, Leaves and Peel of *Citrus aurantium* L. var. *amara* from Tunisia. *Journal of Essential Oil Bearing Plants*, 9(2), 133–139. https://doi.org/10.1080/0972060X.2006.10643484

Boussaada, O., & Chemli, R. (2007). Seasonal Variation of Essential Oil Composition of *Citrus aurantium* L. var. *amara*. *Journal of Essential Oil Bearing Plants*, 10(2), 109–120. https://doi.org/10.1080/0972060X.2007.10643528

de Araújo, J. S. F., de Souza, E. L., Oliveira, J. R., Gomes, A. C. A., Kotzebue, L. R. V., da Silva Agostini, D. L., de Oliveira, D. L. V., Mazzetto, S. E., da Silva, A. L., & Cavalcanti, M. T. (2020). Microencapsulation of sweet orange essential oil (*Citrus aurantium* var. *dulcis*) by liophylization using maltodextrin and maltodextrin/gelatin mixtures: Preparation, characterization, antimicrobial and antioxidant activities. *International Journal of Biological Macromolecules*, 143, 991–999. https://doi.org/10.1016/j.ijbiomac.2019.09.160

de Moraes Pultrini, A., Almeida Galindo, L., & Costa, M. (2006). Effects of the essential oil from *Citrus aurantium* L. in experimental anxiety models in mice. *Life Sciences*, 78(15), 1720–1725. https://doi.org/10.1016/j.lfs.2005.08.004

Dosoky, N., & Setzer, W. (2018). Biological Activities and Safety of *Citrus* spp. Essential Oils. *International Journal of Molecular Sciences*, 19(7), 1966. https://doi.org/10.3390/ijms19071966

Falleh, H., ben Jemaa, M., Saada, M., & Ksouri, R. (2020). Essential oils: A promising eco-friendly food preservative. *Food Chemistry*, 330, 127268. https://doi.org/10.1016/j.foodchem.2020.127268

Floegel, A., Kim, D.-O., Chung, S.-J., Koo, S. I., & Chun, O. K. (2011). Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. *Journal of Food Composition and Analysis*, 24(7), 1043–1048. https://doi.org/10.1016/j.jfca.2011.01.008

Galovičová, L., Čmiková, N., Vukovic, N., Vukic, M., Kowalczewski, P. Ł., Bakay, L., & Kačániová, M. (2022). Chemical Composition, Antioxidant, Antimicrobial, Antibiofilm and

Anti-Insect Activities of *Jasminum grandiflorum* Essential Oil. *Horticulturae*, 8(10), 953. https://doi.org/10.3390/horticulturae8100953

González-Mas, M. C., Rambla, J. L., López-Gresa, M. P., Blázquez, M. A., & Granell, A. (2019). Volatile Compounds in *Citrus* Essential Oils: A Comprehensive Review. *Frontiers in Plant Science*, 10. https://doi.org/10.3389/fpls.2019.00012

Kalemba, D., Matla, M., & Smętek, A. (2012). *Antimicrobial Activities of Essential Oils. In Dietary Phytochemicals and Microbes* (pp. 157–183). Springer Netherlands. https://doi.org/10.1007/978-94-007-3926-0_5

Mahato, N., Sharma, K., Koteswararao, R., Sinha, M., Baral, E., & Cho, M. H. (2019). *Citrus* essential oils: Extraction, authentication and application in food preservation. *Critical Reviews in Food Science and Nutrition*, 59(4), 611–625. https://doi.org/10.1080/10408398.2017.1384716

Mancuso, M., Catalfamo, M., Laganà, P., Rappazzo, A. C., Raymo, V., Zampino, D., & Zaccone, R. (2019). Screening of antimicrobial activity of citrus essential oils against pathogenic bacteria and *Candida strains*. *Flavour and Fragrance Journal*, 34(3), 187–200. https://doi.org/10.1002/ffj.3491

Mannucci, C., Calapai, F., Cardia, L., Inferrera, G., D'Arena, G., di Pietro, M., Navarra, M., Gangemi, S., Ventura Spagnolo, E., & Calapai, G. (2018). Clinical Pharmacology of *Citrus aurantium* and *Citrus sinensis* for the Treatment of Anxiety. *Evidence-Based Complementary and Alternative Medicine*, 2018, 1–18. https://doi.org/10.1155/2018/3624094

Pavela, R. (2005). Insecticidal activity of some essential oils against larvae of *Spodoptera littoralis*. *Fitoterapia*, 76(7–8), 691–696. https://doi.org/10.1016/j.fitote.2005.06.001

Pavela, R., Benelli, G., Pavoni, L., Bonacucina, G., Cespi, M., Cianfaglione, K., Bajalan, I., Morshedloo, M. R., Lupidi, G., Romano, D., Canale, A., & Maggi, F. (2019). Microemulsions for delivery of Apiaceae essential oils – Towards highly effective and eco-friendly mosquito larvicides? *Industrial Crops and Products*, 129, 631–640. https://doi.org/10.1016/j.indcrop.2018.11.073

Popović-Djordjević, J., Cengiz, M., Ozer, M. S., & Sarikurkcu, C. (2019). *Calamintha incana*: Essential oil composition and biological activity. *Industrial Crops and Products*, 128, 162–166. https://doi.org/10.1016/j.indcrop.2018.11.003

Proestos, C., Lytoudi, K., Mavromelanidou, O., Zoumpoulakis, P., & Sinanoglou, V. (2013). Antioxidant Capacity of Selected Plant Extracts and Their Essential Oils. *Antioxidants*, 2(1), 11–22. https://doi.org/10.3390/antiox2010011

Ruiz, B., & Flotats, X. (2014). *Citrus* essential oils and their influence on the anaerobic digestion process: An overview. *Waste Management*, 34(11), 2063–2079. https://doi.org/10.1016/j.wasman.2014.06.026

Sarrou, E., Chatzopoulou, P., Dimassi-Theriou, K., & Therios, I. (2013). Volatile Constituents and Antioxidant Activity of Peel, Flowers and Leaf Oils of *Citrus aurantium* L. Growing in Greece. *Molecules*, 18(9), 10639–10647. https://doi.org/10.3390/molecules180910639

van den Dool, H., & Dec. Kratz, P. (1963). A generalization of the retention index system including linear temperature programmed gas – liquid partition chromatography. *Journal of Chromatography A*, 11, 463–471. https://doi.org/10.1016/S0021-9673(01)80947-X