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# Metabolic profiling and enzyme inhibitory activity of the essential oil of *Citrus aurantium* fruit peel

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## Abstract

**Background** Bitter orange (*Citrus aurantium*) is a fruiting shrub native to tropical and subtropical countries around the world and cultivated in many regions due to its nutraceutical value. The current study investigated the metabolic profiling and enzyme inhibitory activities of volatile constituents derived from the *C. aurantium* peel cultivated in Egypt by three different extraction methods.

**Methods** The volatile chemical constituents of the peel of *C. aurantium* were isolated using three methods; steam distillation (SD), hydrodistillation (HD), and microwave-assisted hydrodistillation (MAHD), and then were investigated by GC-MS. The antioxidant potential was evaluated by different assays such as DPPH, ABTS, FRAP, CUPRAC, and phosphomolybdenum and metal chelating potential. Moreover, the effect of enzyme inhibition of the three essential oils was tested using BChE, AChE, tyrosinase, glucosidase, as well as amylase assays.

**Results** A total of six compounds were detected by GC/MS analysis. The major constituent obtained by all three extraction methods was limonene (98.86% by SD, 98.68% by HD, and 99.23% by MAHD). Differences in the composition of the compounds of the three oils were observed. The hydrodistillation technique has yielded the highest number of compounds, notably two oxygenated monoterpenes: linalool (0.12%) and  $\alpha$ -terpineol acetate (0.1%).

**Conclusion** In our study differences in the extraction methods of *C. aurantium* peel oils resulted in differences in the oils' chemical composition. *Citrus* essential oils and their components showed potential antioxidant, anticholinesterase, antimelanogenesis, and antidiabetic activities. The presence of linalool and  $\alpha$ -terpineol acetate may explain the superior activity observed for the oil isolated by HD in both radical scavenging and AChE inhibition assays, as well as in the enzyme inhibition assays.

**Keywords** *Citrus aurantium*, Essential oil, GC-MS, Antioxidants, Enzyme inhibition

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## Introduction

The *Citrus* genus (Rutaceae) comprises approximately 140 genera and 1300 species [1]. This diverse group of tropical fruits includes well-known species like orange (*C. sinensis*), mandarin (*C. reticulata*), limes (*C. aurantium*), lemon (*C. limon*), and grapefruit (*C. paradisi*) [2]. *Citrus* fruits are not only delicious but also include nutritional value, containing significant levels of bioactive compounds, including citric acid [3] flavonoids [3, 4], limonoids [5], essential oils (EOs), vitamins, especially vitamin C and carotenoids [6] and alkaloids [7] all of which are responsible for various health benefits. Numerous studies have demonstrated the diverse health benefits and biological activities of *Citrus* and its constituents [8]. Additionally, different parts of the *Citrus* plant, including the leaves, flowers, fruits, and peels, are rich sources of essential oils. *Citrus* EOs have been reported for their antioxidant, anticancer [9], antibacterial [10], antidiabetic [11], insecticidal [12], and antifungal [13]. These properties have significant applications in various industries, including cosmetics, agriculture, food production as well as pharmaceutical formulations [14, 15]. *C. aurantium* species known as bitter orange is a fruiting shrub native to tropical and subtropical countries around the world and cultivated in many regions due to its nutraceutical value as well as economic importance [16]. Several studies reported the biologically active essential oils extracted from peels, leaves (petitgrain), and flowers (neroli) of *C. aurantium* [17–20]. Peel volatile oil particularly has been notified in literature for its significant economic value and market demand, being used in flavor, fragrance, medicine, and other fields [21]. In our previous study [23], we investigated the chemical profile of *C. aurantium* leaf oil that was extracted by 3 different methods as well as its biological *in-vitro* activities. However, despite the existing knowledge on the biological activities of *Citrus* EOs, there remains a need to further explore and compare the antioxidant and enzyme inhibition activities of peel EOs extracted through different methods, which is the primary focus of this study.

## Experimental

### Plant collection

The fruits of *C. aurantium* were obtained in March 2023 from Shibin Al Kawm City in a private garden, Al Minufiyah, Egypt. Authentication of the plant was done by Dr. Usama K. Abdel Hameed, Department of Botany, Faculty of Science, Ain Shams University, Cairo, Egypt. A voucher specimen under the code PHG-P-CA-461 was preserved in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt.

### Essential oil preparation

#### Hydrodistillation

Essential oil was extracted from the dried peels of *C. aurantium* by hydrodistillation by Clevenger-type glass apparatus for four hours with a solid-liquid ratio of 250 g/800 mL. The extracted oil (1.20 mL) was preserved in sealed dark vials at 4 °C. A triplicate of the experiment was conducted.

#### Steam distillation

*C. aurantium* (dried peels, 250 g) was subjected to steam distillation for 3 h using the apparatus of steam distillation. The oil (1.00 mL) was dried using Na<sub>2</sub>SO<sub>4</sub> (anhydrous) and stored in a sealed dark vial at 4 °C. A triplicate of the experiment was conducted.

#### Microwave-assisted hydro-distillation

The dried peels of *C. aurantium* (250 g) were homogenized and soaked in distilled water (500 mL) and placed for 35 min. in the microwave at the radiation of 80% to yield 0.7 mL of the extracted essential oil.

### GC/MS analysis of essential oils

The essential oil compositions obtained from the three isolation procedures were examined using GC coupled with MS. The Shimadzu GC/MS-QP 2010 (Koyoto, Japan) was connected to a mass spectrometer (SSQ 7000 quadrupole: Thermo-Finnigan, Bremen, Germany). A capillary column (model number Rtx-5MS, length 30 m, internal diameter 0.25 mm, film thickness 0.25 µm; Restek, USA) was utilized. After two minutes of gentle temperature adjustment to 45 °C, the temperature was increased to 300 °C (rate of 5 °C/minute for 5 min). A temperature of 250 °C was maintained for the injector and 280 °C for the detector, respectively. Before the analysis, samples of the essential oil were diluted (1% v/v) using n-hexane. A 1:15 split ratio was used for the automatic injection of 1 µL of sample.

### Identification of the oil components

The GC peaks' mass spectra have been identified by searching libraries (NIST and WILEY). Confirmation of the identification has been achieved by calculating the peaks' retention indices (RI) in relation to (C<sub>6</sub>–C<sub>22</sub>) n-alkanes and comparing the results with published data [22–27]. Peak area percent was used for quantification, and the average of three measurements was presented [28].

### Antioxidant and enzyme inhibitory assays

We examined the antioxidant potential of the essential oils by conducting six distinct spectrophotometric tests. This consisted of the 2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) and

2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assays, that assess the antioxidants' aptitude for free radicals' neutralization. Through the ferric-reducing antioxidant power (FRAP) and cupric-reducing antioxidant capacity (CUPRAC) tests, the reduction activity of the extract was examined. We evaluated the total antioxidant and metal chelating activities using the phosphomolybdenum and ferrozine assays, respectively. All assays, except Metal Chelating Activity (MCA), were standardized with a Trolox standard. MCA was compared in terms of Ethylenediaminetetraacetic acid (EDTA) equivalent per gram of essential oil, as outlined in our earlier publications [29, 30].

### Enzyme inhibition assays

In order to assess the inhibitory effects of the oils under investigation on different enzymes, we employed acetylcholinesterase (AChE), butyrylcholinesterase (BChE), tyrosinase, amylase, and glucosidase. Detailed experimental procedures can be found in our previously published research [29, 31]. In terms of mg of galanthamine equivalents (GALAE) per gram of oil, we measured the inhibition of AChE and BChE, whereas tyrosinase inhibition was expressed as mg of kojic acid equivalents (KAE) per gram of oil. Additionally, inhibition of both  $\alpha$ -amylase and  $\alpha$ -glucosidase was quantified as millimoles of acarbose equivalents (ACAE) per gram of oil.

### Statistical analysis

The procedures were carried out in triplicate, and differences between the methods were analyzed using ANOVA and Tukey's test ( $p < 0.05$ ). The statistical analysis was carried out using Graph Pad Prism (version 9.2).

## Results and discussion

### Essential oil yield and composition

*C. aurantium* peels essential oils were isolated using three methods; steam distillation (SD), hydrodistillation (HD), and microwave-assisted hydro-distillation (MAHD), the chemical constituents of the oils were analyzed using GC-MS. The GC-MS chromatograms of the essential oil isolated using the three extraction procedures are represented in Fig. 1. This entailed in the identification of a total of six different compounds, which are listed in Table 1. All essential oils obtained by the three extraction techniques were composed mainly of monoterpenoid compounds. The major constituent obtained by all three methods applied was limonene (98.86% by SD, 98.68% by HD, and 99.23% by MAHD). In our study, some differences were observed in the essential oil composition obtained by the three different extraction techniques. HD has yielded the greatest number of compounds in which two oxygenated monoterpenes namely, linalool and  $\alpha$ -Terpineol acetate have been obtained with a percent

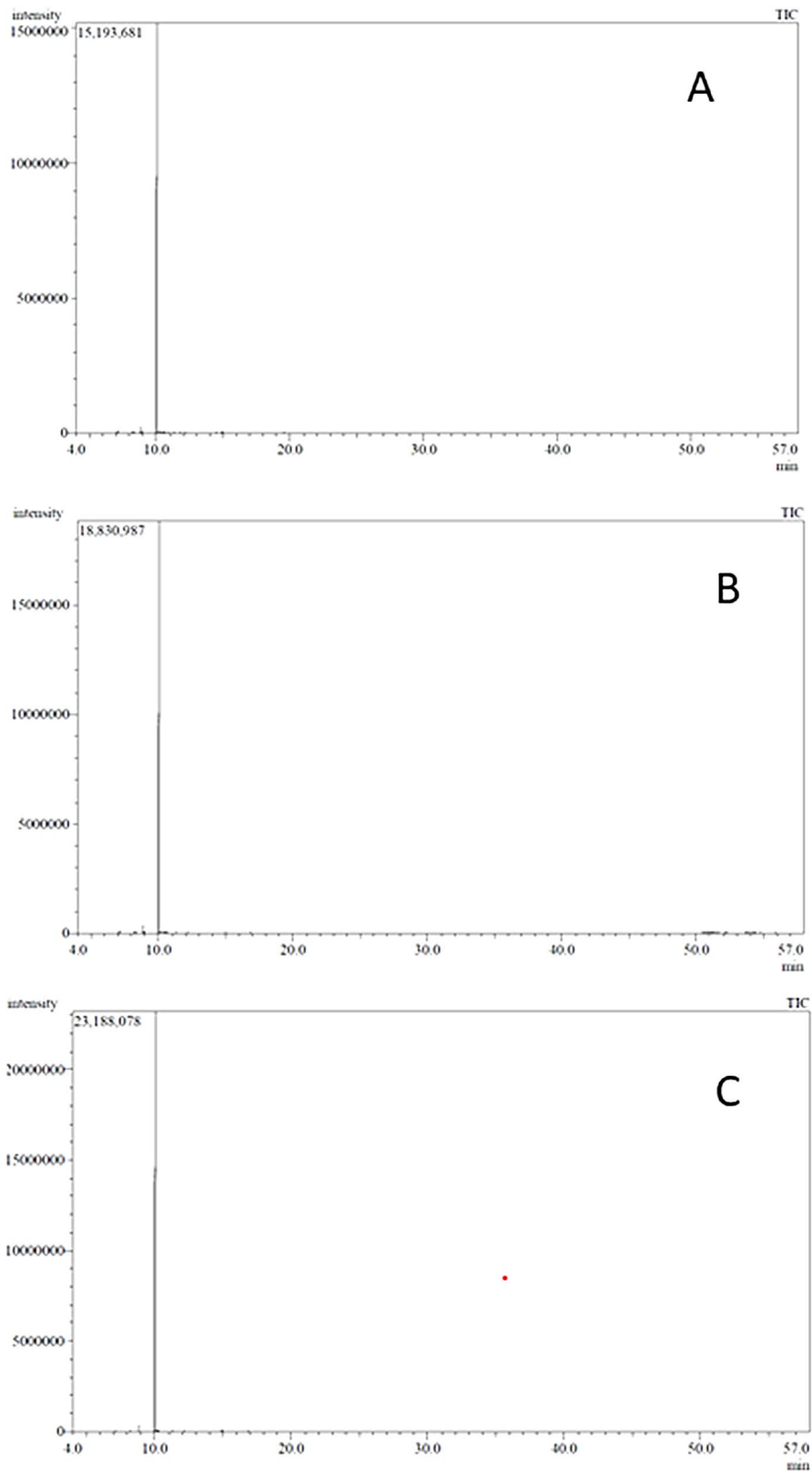
composition of 0.12% and 0.1%, respectively. MAHD has yielded only one oxygenated monoterpene (linalool) with a percent composition of 0.06% from the isolated oil. The SD method showed the absence of oxygenated compounds and resulted in the isolation of only monoterpene hydrocarbons ( $\alpha$ -pinene, sabinene, and  $\beta$ -myrcene). Both  $\alpha$ -pinene and  $\beta$ -myrcene were obtained in all essential oil samples extracted by the three methods, while sabinene was absent in the oil obtained by MAHD as displayed in Table 1.

The isolation of *C. aurantium* peel EO using the HD method previously reported the identification of twenty chemical compounds [20]. In our current investigation, by employing the same technique only six compounds were identified from the *C. aurantium* peel EO. Our analysis revealed limonene as the major constituent, comprising 98.68% of the isolated EO, which is in line with the previous findings, though the previous study reported a slightly lower abundance of limonene (94.67%). We attribute this variation to geographical differences in the origin of the fruit samples.

EOs isolated from various parts of *C. aurantium* using our study's isolation methods have consistently shown the presence of limonene, though not as a major constituent. For instance, when isolated from the leaves using the HD method, limonene comprised only 0.71% of the total components [32]. While, major components identified in the leaves' EO obtained by the HD method were linalool, linalyl acetate, and  $\alpha$ -terpineol constituting greater than 73% of the total compounds [33]. Limonene was also detected, comprising 11.67% of the total EO composition from the buds of *C. aurantium* using the SD method, compared to 98.86% obtained in our study from peel EO using the same technique. These findings suggest that EOs isolated from different plant parts using the same technique will lead to different chemical compositions. This is clear also regarding linalool, when extracted using the HD technique in our study, appeared in minor amounts (0.12%), contrasting with the higher composition percentage of 30.62% reported from the *C. aurantium* leaves using the same technique [32].

On the other hand, differences in extraction methods result in variations in the chemical profiles of the EOs. For example, sabinene and  $\alpha$ -pinene were identified previously from leaves EOs of *C. aurantium* by SD method only and were absent in oils obtained by reflux extraction and ultrasound-assisted extraction technique [33].

From the above, it is concluded that, in our study, although differences in the extraction methods of *C. aurantium* peel oils resulted in differences in the composition of the EOs, limonene remained the major component in all samples. Comparing our findings with the previous reports showed the differences in the essential



**Fig. 1** GC-MS chromatograms of (A) HD; (B) SD; (C)MAHD

**Table 1** Chemical composition of the essential oils using GC/MS analysis

No.	Rt* (min)	Compound <sup>a</sup> name	Composition % <sup>b</sup>			RI <sup>c</sup> <sub>exp</sub>	RI <sub>rep</sub>	Molecular formula	Iden- tifica- tion
			C. auran- tium SD	C. auran- tium HD	C. au- rantium MAHD				
1	7.083	$\alpha$ -Pinene	0.27	0.25	0.17	929	932	C <sub>10</sub> H <sub>16</sub>	MS, RI
2	8.259	Sabinene	0.04	0.09	-	968	969	C <sub>10</sub> H <sub>16</sub>	MS, RI
3	8.815	$\beta$ -Myrcene	0.8	0.67	0.54	987	987	C <sub>10</sub> H <sub>16</sub>	MS, RI
4	10.083	Limonene	<b>98.86</b>	<b>98.68</b>	<b>99.23</b>	1029	1029	C <sub>10</sub> H <sub>16</sub>	MS, RI
5	12.155	Linalool	-	0.12	0.06	1096	1095	C <sub>10</sub> H <sub>18</sub> O	MS, RI
6	19.54	$\alpha$ -Terpineol acetate	-	0.1	-	1346	1346	C <sub>10</sub> H <sub>20</sub> O <sub>2</sub>	MS, RI
Monoterpene hydrocarbons			99.66	99.69	99.98				
Oxygenated compounds			-	0.22	0.06				
Total identified compounds			99.66	99.91	100				

<sup>a</sup> Compounds are scheduled according to their elution

<sup>b</sup> Average of three analyses MS, identification based on mass spectral data

<sup>c</sup>RI, Kovats retention, exp (experimental), rep (reported)

\*Rt: Retention time

(-) Unidentified in the samples, (SD) (Steam Distillation), HD (Hydrodistillation), MAHD (Microwave-Assisted Hydrodistillation)

**Table 2** Antioxidant activities of the three extracted *C. aurantium* essential oils

Methods	DPPH (mg TE/g)	ABTS (mg TE/g)	CUPRAC (mg TE/g)	FRAP (mg TE/g)	PBD (mmol TE/g)	MCA (mg EDTAE/g)
HD	3.44 ± 0.28 <sup>b</sup>	4.09 ± 0.83 <sup>a</sup>	57.50 ± 2.01 <sup>b</sup>	53.92 ± 0.79 <sup>b</sup>	42.30 ± 0.03 <sup>c</sup>	16.20 ± 0.29 <sup>a</sup>
SD	4.74 ± 0.20 <sup>a</sup>	1.56 ± 0.13 <sup>b</sup>	69.30 ± 1.13 <sup>a</sup>	55.84 ± 2.42 <sup>ab</sup>	49.45 ± 0.34 <sup>a</sup>	15.86 ± 1.98 <sup>a</sup>
MV	2.51 ± 0.68 <sup>b</sup>	1.24 ± 0.18 <sup>b</sup>	70.75 ± 1.02 <sup>a</sup>	59.92 ± 1.72 <sup>a</sup>	47.89 ± 0.03 <sup>b</sup>	12.89 ± 1.59 <sup>b</sup>

\*Values are reported as mean ± SD of three parallel experiments. DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); CUPRAC: Cupric reducing antioxidant capacity; FRAP: Ferric reducing antioxidant power; PBD: Phosphomolybdenum; MCA: Metal chelating activity. TE: Trolox equivalent; EDTAE: EDTA equivalent. Different letters indicate significant differences in the methods ( $p < 0.05$ )

oils' composition between different organs of *C. aurantium* even when using the same isolation methods.

### Antioxidant activity

Oxidative stress refers to the disparity between the defensive capabilities of the antioxidant system and the generation of reactive oxygen species. Plant antioxidants are commonly acknowledged as nutraceuticals, serving to eliminate free radicals and functioning as antioxidants [34, 35]. Compounds with reductive properties have traditionally been employed in the food industry to protect products from oxidation [36]. Nonetheless, the current study's focus lies on their capacity to counteract oxidative stress.

Table 2 represents a comprehensive overview of antioxidant activities associated with the essential oil extracted through three distinct methods namely; HD, SD, and MAHD. The antioxidant activities were measured using various assays including; DPPH, ABTS, CUPRAC, FRAP, PBD, and MCA. When the antioxidant activities were compared across the methods, it was found that the essential oil extracted through the SD method displayed higher antioxidant activity in the DPPH (4.74 mg TE/g) and PBD (49.45 mmol TE/g) assays compared to the HD and MAHD methods. On the other hand, the essential oil extracted through the HD extraction method exhibited

higher radical scavenging activity in the ABTS assay (4.09 mg EDTAE/g) and MCA (16.20 mg EDTAE/g). In addition, in DPPH assay, HD (3.44 mg TE/g) and MAHD (2.51 mg TE/g) showed similar ability ( $p > 0.05$ ).

This may be attributed to the linalool and  $\alpha$ -Terpineol contents in this extraction method [37, 38]. However, the MAHD method, while exhibiting the lowest antioxidant activity in most assays, still showed notable values in the CUPRAC (70.75 mg TE/g) and FRAP (59.92 mg TE/g) assays. The results suggest that the choice of extraction method of essential oil significantly influences the antioxidant activity of the volatile oil, with each method yielding distinctive outcomes across various assays. This provides valuable insights for future research, emphasizing the importance of considering these findings when choosing an appropriate extraction method based on the desired antioxidant potential for specific applications. All the tested EOs demonstrated antioxidant activity with some variation. Numerous studies exploring the chemical constituents and bioactivity of different *Citrus* oils have consistently reported robust radical scavenging activity [39, 40]. In the current study, the essential oils (EOs) are primarily comprised of monoterpene hydrocarbons, notably limonene, and the antioxidant properties of limonene have been previously documented [41–43]. Investigations into the chemical composition of essential oils

extracted from *C. aurantium* align with our findings, suggesting that the primary components of the oil of the peel are monoterpene hydrocarbons such as limonene [20, 44]. A study conducted by Hsouna et al., reported that *C. aurantium* L. flower extract displayed superior DPPH scavenging activity, with an IC<sub>50</sub> of 1.85 µg/ml [45]. The EO of *C. aurantium* L. exhibited IC<sub>50</sub> values of 1040 µg/ml, 1580 µg/ml, and 140 µg/ml in the DPPH assay, reducing antioxidant power, and H<sub>2</sub>O<sub>2</sub> scavenging respectively [46].

### Enzyme inhibitory activities

In this current investigation, we investigated the therapeutic activity of EOs derived from *C. aurantium* species in Alzheimer's disease (AD) management, melanogenesis, and diabetes. This evaluation was conducted through the analysis of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibition, as well as tyrosinase and α-glucosidase inhibition assays. AChE and BChE are enzymes linked to AD development [47], tyrosinase is implicated in pigmentation processes [48], and α-glucosidase is associated with diabetes [49]. Previous studies have demonstrated that bioactive compounds found in plants play a significant role in inhibiting these aforementioned enzymes [50, 51].

Table 3 provides a detailed overview of enzyme inhibition activities of EOs extracted through HD, SD, and MAHD. The enzyme inhibition activities are measured against key enzymes, namely acetylcholinesterase (AChE), butyrylcholinesterase (BChE), tyrosinase, and α-glucosidase.

When comparing enzyme inhibition activities across the extraction methods, it was observed that the essential oils extracted through the HD method exhibited notably higher AChE inhibition at 2.47 mg GALAE/g, closely followed by MAHD measuring 2.43 mg GALAE/g. Moreover, MAHD demonstrated superior BChE inhibition activity, measuring 4.21 mg GALAE/g, while SD essential oils exhibited BChE inhibition at 4.12 mg GALAE/g. Additionally, EO extracted through MAHD displayed the highest anti-tyrosinase activity, recording 83.39 mg KAE/g. In comparison, HD yielded moderate tyrosinase

inhibition at 41.28 mg KAE/g, whereas MAHD showed the lowest inhibition at 36.76 mg KAE/g. Furthermore, the essential oils extracted through the SD method exhibited 2.66 mmol ACAE/g, comparable α-Glucosidase inhibition to essential oils extracted through HD, which measured 2.62 mmol ACAE/g. These results highlight the potential of *Citrus* EOs and their components as promising candidates with anti-cholinesterase, anti-melanogenesis, and anti-diabetic activities. They are well-suited for applications in pharmaceuticals targeting neurodegenerative disorders, skin hyperpigmentation, and diabetes.

Previous research showed the enzyme-inhibitory properties of diverse *Citrus* species [52–56]. For instance, *C. aurantifolia* and *C. aurantium* EOs exhibited AChE inhibition, with respective IC<sub>50</sub> values of 139.3 and 147.5 µg/mL. While a consistent but comparatively lower bioactivity was noted against BChE, with IC<sub>50</sub> values ranging from 235.5 to 266.6 µg/mL for both species, respectively [52]. Various monoterpenoids identified in *Citrus* EOs have been investigated for their potential to inhibit cholinesterase [53]. Furthermore, a study involving thirteen different *Citrus* EOs and their volatile flavor constituents revealed significant tyrosinase inhibitory activity [54]. Essential oils from various *Citrus* species were reported to reduce the expression levels of Tyrosinase, suggesting their efficient inhibition of melanogenesis [55]. Moreover, the EO extracted from the peel of *C. aurantium* was found to inhibit α-glucosidase activity [56].

### Conclusion

The volatile components from the peel of *C. aurantium* were extracted using three distinct methods: HD, SD, and MAHD. These extracts were subsequently analyzed using GC/MS. Across all three methods, limonene was identified as the major compound, constituting 98.86% in SD, 98.68% in HD, and 99.23% in MAHD extracts. The chemical composition of the oils was varied, attributable to the extraction techniques employed. The antioxidant effects of the essential oils were tested by six different assays; DPPH, ABTS, CUPRAC, FRAP, PBD, and MCA. Results showed that the essential oil extracted using the SD method demonstrated superior antioxidant activity in the DPPH and PBD assays. While the HD extraction method showed higher radical scavenging activity in the ABTS assay and MCA. Comparing the enzyme inhibition activities among different extraction methods for essential oils, essential oils extracted through the HD method showed higher AChE inhibition. While MAHD-extracted oil demonstrated superior BChE and tyrosinase inhibition activities. Additionally, SD essential oils exhibited comparable α-Glucosidase inhibition to HD. Overall, these findings suggest the potential of *Citrus* EOs and their components for antioxidant, anti-cholinesterase, anti-melanogenesis, and anti-diabetic activities. And not

**Table 3** Enzyme inhibitory activities of the three extracted *C. aurantium* essential oils

Methods	AChE (mg GALAE/g)	BChE (mg GALAE/g)	Tyrosinase (mg KAE/g)	α-Glucosidase (mmol ACAE/g)
HD	2.47 ± 0.04 <sup>a</sup>	2.92 ± 0.12 <sup>b</sup>	41.28 ± 3.59 <sup>b</sup>	2.62 ± 0.08 <sup>a</sup>
SD	2.09 ± 0.01 <sup>a</sup>	4.12 ± 0.47 <sup>a</sup>	36.76 ± 2.63 <sup>b</sup>	2.66 ± 0.06 <sup>a</sup>
MV	2.43 ± 0.11 <sup>a</sup>	4.21 ± 0.42 <sup>a</sup>	83.39 ± 1.03 <sup>a</sup>	2.57 ± 0.05 <sup>a</sup>

\*Values are reported as mean ± SD of three parallel experiments. AChE: Acetylcholinesterase; BChE: Butyrylcholinesterase; GALAE: Galanthamine equivalent; KAE: Kojic acid equivalent; ACAE: Acarbose equivalent. Different letters indicate significant differences in the methods ( $p < 0.05$ )

## only the plant organ that can affect the biological activity but also the extraction method.

### Abbreviations

ABTS	2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid
ACAE	Acarbose equivalents
AChE	Acetylcholinesterase
AD	Alzheimer's disease
BChE	Butyrylcholinesterase
CUPRAC	Cupric-Reducing Antioxidant Capacity
DPPH	2,2-diphenyl-1-picryl-hydrazyl-hydrate
EDTA	Ethylenediaminetetraacetic acid
EO	Essential oils
FRAP	Ferric-Reducing Antioxidant Power
HD	Hydrodistillation
KAE	Kojic acid equivalents
MAHD	Microwave-Assisted Hydrodistillation
SD	Steam distillation

### Author contributions

Naglaa S. Ashmawy: Methodology, Writing original manuscript Nilofar Nilofar: writing the original manuscript, Gokhan Zengin: Conceptualization, Omayma A. Eldahshan: Conceptualization, Reviewing and editing. All the authors read and approved the final manuscript.

### Data availability

Data are available upon request from Omayma Eldahshan; oeldahshan@pharma.asu.edu.eg.

### Declarations

#### Ethics approval and consent to participate

All methods were performed in accordance with the relevant guidelines and regulations.

#### Consent for publication

Not applicable.

#### Competing interests

The authors declare no competing interests.

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### References

1. Agouillal F, Taher ZM, Moghrani H, Nasrallah N, El Enshasy H. A review of genetic taxonomy, Biomolecules Chemistry and Bioactivities of Citrus hystrix DC. *Biosci Biotechnol Res Asia*. 2017;14(1):285.
2. Alam F, Mohammadin K, Shafique Z, Amjad ST, Asad MHH. Citrus flavonoids as potential therapeutic agents: a review. *Phytother Res*. 2022;36(4):1417–41.
3. Mandalari G, Bennett RN, Bisignano G, Saija A, Dugo G, Lo Curto RB, et al. Characterization of flavonoids and pectins from bergamot (*Citrus bergamia* Risso) peel, a major byproduct of essential oil extraction. *J Agric Food Chem*. 2006;54(1):197–203.
4. Yang Y, Wang X, Zhao C, Tian G, Zhang H, Xiao H, et al. Chemical mapping of essential oils, flavonoids and carotenoids in citrus peels by Raman microscopy. *J Food Sci*. 2017;82(12):2840–6.
5. Gualdani R, Cavalluzzi MM, Lentini G, Habtemariam S. The chemistry and pharmacology of citrus limonoids. *Molecules*. 2016;21(11):1530.
6. Aschoff JK, Kaufmann S, Kalkan O, Neidhart S, Carle R, Schweiggert RM. In vitro bioaccessibility of carotenoids, flavonoids, and vitamin C from differently processed oranges and orange juices [*Citrus sinensis* (L.) Osbeck]. *J Agric Food Chem*. 2015;63(2):578–87.
7. Bora H, Kamle M, Mahato DK, Tiwari P, Kumar P. Citrus essential oils (CEOs) and their applications in food: an overview. *Plants*. 2020;9(3):357.
8. Khan UM, Sameen A, Aadil RM, Shahid M, Sezen S, Zarrabi A, et al. Citrus genus and its waste utilization: a review on health-promoting activities and industrial application. *Evidence-Based Complement Altern Med*. 2021;2021:1–17.
9. Ashmawy A, Mostafa N, Eldahshan O. GC/MS analysis and molecular profiling of lemon volatile oil against breast cancer. *J Essent Oil Bearing Plants*. 2019;22(4):903–16.
10. Eldahshan OA, Halim AF. Comparison of the composition and antimicrobial activities of the essential oils of green branches and leaves of Egyptian navel orange (*Citrus sinensis* (L.) Osbeck var. Malesy). *Chem Biodivers*. 2016;13(6):681–5.
11. Dang NH, Nhung PH, Mai Anh BT, Thu Thuy DT, Minh CV, Dat NT. Chemical composition and  $\alpha$ -glucosidase inhibitory activity of Vietnamese citrus peels essential oils. *Journal of Chemistry*. 2016;2016.
12. Khanikar B, Adhikari K, Rabha B. Citrus essential oils: a suite of insecticidal compounds. *Development and Biotechnology: Citrus—Research*; 2021.
13. Jing L, Lei Z, Li L, Xie R, Xi W, Guan Y, et al. Antifungal activity of citrus essential oils. *J Agric Food Chem*. 2014;62(14):3011–33.
14. Lin X, Cao S, Sun J, Lu D, Zhong B, Chun J. The chemical compositions, and antibacterial and antioxidant activities of four types of citrus essential oils. *Molecules*. 2021;26(11):3412.
15. Jugreet BS, Suroowan S, Rengasamy RK, Mahomoodally MF. Chemistry, bio-activities, mode of action and industrial applications of essential oils. *Trends Food Sci Technol*. 2020;101:89–105.
16. Suryawanshi JAS. An overview of Citrus aurantium used in treatment of various diseases. *Afr J Plant Sci*. 2011;5(7):390–5.
17. Gniewosz M, Kraśniewska K, Kosakowska O, Pobięga K, Wolska I. Chemical compounds and antimicrobial activity of petitgrain (*Citrus aurantium* L. var. amara) essential oil. *Herba Pol*. 2017;63(4):18–25.
18. Scandurra C, Mezzalana S, Cuttillo S, Zapparella R, Statti G, Maldonato NM, et al. editors. The effectiveness of neroli essential oil in relieving anxiety and perceived pain in women during labor: a Randomized controlled trial. *Healthcare: MDPI*; 2022.
19. Haj Ammar A, Bouajila J, Lebrihi A, Mathieu F, Romdhane M, Zagrouba F. Chemical composition and in vitro antimicrobial and antioxidant activities of Citrus aurantium L. flowers essential oil (Neroli oil). *Pak J Biol Sci*. 2012;15(21):1034–40.
20. Sarrou E, Chatzopoulou P, Dimassi-Theriou K, Therios I. Volatile constituents and antioxidant activity of peel, flowers and leaf oils of Citrus aurantium L. growing in Greece. *Molecules*. 2013;18(9):10639–47.
21. Liu K, Deng W, Cao S, Chun J. Chemical composition of peel, leaf and flower essential oils from 'Newhall' and 'Gannanza' navel oranges. *Am J Essent Oils Nat Prod*. 2018;6(3):35–9.
22. Azab SS, Abdel Jaleel GA, Eldahshan OA. Anti-inflammatory and gastro-protective potential of leaf essential oil of *Cinnamomum glanduliferum* in ethanol-induced rat experimental gastritis. *Pharm Biol*. 2017;55(1):1654–61.
23. Shahat EA, Bakr RO, Eldahshan OA, Ayoub NA. Chemical composition and biological activities of the essential oil from leaves and flowers of *Pulicaria incisa* sub. *Candolleana* (family Asteraceae). *Chem Biodivers*. 2017;14(4):e1600156.
24. Todirascu-Ciornea E, El-Nashar HA, Mostafa NM, Eldahshan OA, Boiangiu RS, Dumitru G et al. Schinus terebinthifolius essential oil attenuates scopolamine-induced memory deficits via cholinergic modulation and antioxidant properties in a zebrafish model. *Evidence-Based Complementary and Alternative Medicine*. 2019;2019.
25. Singab ANB, Mostafa NM, Eldahshan OA, Ashour ML, Wink M. Profile of volatile components of hydrodistilled and extracted leaves of *Jacaranda acutifolia* and their antimicrobial activity against foodborne pathogens. *Nat Prod Commun*. 2014;9(7):1934578X1400900731.
26. Abd El-Ghffar EA, El-Nashar HA, Eldahshan OA, Singab ANB. GC-MS analysis and hepatoprotective activity of the n-hexane extract of *Acrocarpus fraxinifolius* leaves against Paracetamol-induced hepatotoxicity in male albino rats. *Pharm Biol*. 2017;55(1):441–9.
27. Ashmawy NS, Gad HA, El-Nashar HA. Comparative study of essential oils from different organs of *Syzygium cumini* (Pamplesia) based on GC/MS chemical profiling and in Vitro Antiaging Activity. *Molecules*. 2023;28(23):7861.
28. Ashmawy NS, Hamoda AM, Gad HA, El-Keblawy AA, Soliman SS. Newly-sprouted leaves at the stem base differ anatomically and histochemically from the crown leaves in *Ficus Johannis*. *Bot Lett*. 2023:1–9.
29. Uysal S, Zengin G, Locatelli M, Bahadori MB, Mocan A, Bellagamba G, et al. Cytotoxic and enzyme inhibitory potential of two *Potentilla* species (*P. Speciosa* L. and *P. reptans* Willd.) and their chemical composition. *Front Pharmacol*. 2017;8:290.

30. Zengin G, Aktumsek A. Investigation of antioxidant potentials of solvent extracts from different anatomical parts of *Asphodeline Anatolica* E. Tuzlaci: an endemic plant to Turkey. *Afr J Tradit Complement Altern Med*. 2014;11(2):481–8.
31. Zengin G. A study on in vitro enzyme inhibitory properties of *asphodeline anatolica*: New sources of natural inhibitors for public health problems. *Ind Crops Prod*. 2016;83:39–43.
32. Oulebsir C, Mefti-Kortebay H, Djazouli Z-E, Zebib B, Merah O. Essential oil of *Citrus aurantium* L. leaves: composition, antioxidant activity, elastase and collagenase inhibition. *Agronomy*. 2022;12(6):1466.
33. Jiang MH, Yang L, Zhu L, Piao JH, Jiang JG. Comparative GC/MS analysis of essential oils extracted by 3 methods from the bud of *Citrus aurantium* L. var. *amara* Engl. *J Food Sci*. 2011;76(9):C1219–25.
34. Bakar K, Nilofar N, Mohamed A, Świątek Ł, Hryć B, Sieniawska E, et al. Evaluating phytochemical profiles, cytotoxicity, antiviral activity, antioxidant potential, and enzyme inhibition of *Vepris boiviniana* extracts. *Molecules*. 2023;28(22):7531.
35. Lizama C, Romero-Parra J, Andrade D, Riveros F, Bórquez J, Ahmed S, et al. Analysis of carotenoids in haloarchaea species from atacama saline lakes by high resolution uhplc-q-orbitrap-mass spectrometry: antioxidant potential and biological effect on cell viability. *Antioxidants*. 2021;10(8):1230.
36. Albu S, Joyce E, Paniwnyk L, Lorimer J, Mason TJ. Potential for the use of ultrasound in the extraction of antioxidants from *Rosmarinus officinalis* for the food and pharmaceutical industry. *Ultrason Sonochem*. 2004;11(3–4):261–5.
37. Seol G-H, Kang P, Lee HS, Seol GH. Antioxidant activity of linalool in patients with carpal tunnel syndrome. *BMC Neurol*. 2016;16:1–6.
38. Gouveia DN, Costa JS, Oliveira MA, Rabelo TK, e Silva AMdO, Carvalho AA, et al.  $\alpha$ -Terpineol reduces cancer pain via modulation of oxidative stress and inhibition of iNOS. *Biomed Pharmacother*. 2018;105:652–61.
39. Baik J-S, Kim S-S, Lee J-A, Oh T-H, Kim J-Y, Lee N-H, Hyun C-G. Chemical composition and biological activities of essential oils extracted from Korean endemic citrus species. *J Microbiol Biotechnol*. 2008;18(1):74–9.
40. Malhotra S, Suri S, Tuli R. Antioxidant activity of citrus cultivars and chemical composition of *Citrus Karna* essential oil. *Planta Med*. 2008:62–4.
41. Piccialli I, Tedeschi V, Caputo L, Amato G, De Martino L, De Feo V, et al. The antioxidant activity of limonene counteracts neurotoxicity triggered by  $\beta$ 1–42 oligomers in primary cortical neurons. *Antioxidants*. 2021;10(6):937.
42. Junior MRM, e Silva TAR, Franchi GC, Nowill A, Pastore GM, Hyslop S. Antioxidant potential of aroma compounds obtained by limonene biotransformation of orange essential oil. *Food Chem*. 2009;116(1):8–12.
43. Vieira AJ, Beserra FP, Souza M, Totti B, Rozza A, Limonene. Aroma of innovation in health and disease. *Chemico-Biol Interact*. 2018;283:97–106.
44. Choi H-S, Song HS, Ukeda H, Sawamura M. Radical-scavenging activities of citrus essential oils and their components: detection using 1, 1-diphenyl-2-picrylhydrazyl. *J Agric Food Chem*. 2000;48(9):4156–61.
45. Hsouna AB, Hamdi N, Halima NB, Abdelkafi S. Characterization of essential oil from *Citrus aurantium* L. flowers: antimicrobial and antioxidant activities. *J Oleo Sci*. 2013;62(10):763–72.
46. Majnooni M-B, Mansouri K, Gholivand M-B, Mostafaie A, Mohammadi-Motlagh H-R, Afzanzade N-S, et al. Chemical composition, cytotoxicity and antioxidant activities of the essential oil from the leaves of *Citrus aurantium* L. *Afr J Biotechnol*. 2012;11(2):498–503.
47. Lane RM, Potkin SG, Enz A. Targeting acetylcholinesterase and butyrylcholinesterase in dementia. *Int J Neuropsychopharmacol*. 2006;9(1):101–24.
48. Kim I-S, Kim ER, Nam HJ, Chin MO, Moon YH, Oh MR, et al. Activating mutation of *Gsa* in McCune-Albright syndrome causes skin pigmentation by tyrosinase gene activation on affected melanocytes. *Horm Res*. 1999;52(5):235–40.
49. Hossain U, Das AK, Ghosh S, Sil PC. An overview on the role of bioactive  $\alpha$ -glucosidase inhibitors in ameliorating diabetic complications. *Food Chem Toxicol*. 2020;145:111738.
50. Barrientos RE, Ahmed S, Cortés C, Fernández-Galleguillos C, Romero-Parra J, Simirgiotis MJ, Echeverría J. Chemical fingerprinting and biological evaluation of the endemic Chilean fruit *Greigia sphacelata* (Ruiz and Pav.) Regel (Bromeliaceae) by UHPLC-PDA-orbitrap-mass spectrometry. *Molecules*. 2020;25(16):3750.
51. Zengin G, Nilofar, Yildiztugay E, Bouyahya A, Cavusoglu H, Gevrenova R, Zheleva-Dimitrova D. A comparative study on UHPLC-HRMS profiles and Biological activities of *Inula sarana* different extracts and its Beta-cyclodextrin complex: effective insights for Novel Applications. *Antioxidants*. 2023;12(10):1842.
52. Tundis R, Loizzo MR, Bonesi M, Menichini F, Mastellone V, Colica C, Menichini F. Comparative study on the antioxidant capacity and cholinesterase inhibitory activity of *Citrus aurantifolia* Swingle, *C. Aurantium* L., and *C. Bergamia* Risso and Poit. Peel essential oils. *J Food Sci*. 2012;77(1):H40–6.
53. Miyazawa M, Watanabe H, Kameoka H. Inhibition of acetylcholinesterase activity by monoterpenoids with ap-menthane skeleton. *J Agric Food Chem*. 1997;45(3):677–9.
54. Matsuura R, Ukeda H, Sawamura M. Tyrosinase inhibitory activity of citrus essential oils. *J Agric Food Chem*. 2006;54(6):2309–13.
55. Yang J, Lee S-Y, Jang S-K, Kim K-J, Park M-J. Inhibition of melanogenesis by essential oils from the citrus cultivars peels. *Int J Mol Sci*. 2023;24(4):4207.
56. Heydari Koochi Z, Jahromi KG, Kavooosi G, Babaei S. Citrus peel waste essential oil: Chemical composition along with anti-amylase and anti-glucosidase potential. *Int J Food Sci Technol*. 2022;57(10):6795–804.

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