Original Research Article

GC-MS profile, α-glucosidase inhibition potential, antibacterial and antioxidant evaluation of peels Citrus aurantium (L), essential oil.

ABSTRACT

This study was designed to analyze the chemical composition of Citrus aurantium essential oil (CAEO) peels and to evaluate α-glucosidase inhibition potential, antioxidant and antibacterial activities. According to GC-MS analyses, 37 compounds were identified with limonene was the most abundant (62.2%). Majority of the identified compounds belong to hydrocarbon monoterpenes fraction (75.7%), followed by oxygenated monoterpenes (19.16%). CAEO α-glucosidase inhibition outlined an important activity with IC_{50} = 10±1 mg/mL. Moreover, antioxidant activity revealed that CAEO exhibited a potent scavenging effect through 2,2-diphenyl-1 picrylhydrazyl radical (DPPH˙) (IC_{50}=33.66 µg/mL) and an important ferric ion reducing antioxidant power (FRAP) activity (EC_{50}=98.67 µg/mL). Antimicrobial data demonstrate that CAEO was active against a panel of pathogenic bacteria and that CAEO was able to destroy bacterial cells (bactericidal) according to the MBC/MIC ratios towards Gram+ and Gram- tested strains.

Keywords: *Citrus aurantium, Essential oil, GC-MS, anti-α-glucosidase, Antioxidant, Antibacterial.*

1. INTRODUCTION

The genus *Citrus* belongs to the family Rutaceae, with important crops like orange, lemons, pummelos, grapefruits, limes, etc. [1]. *Citrus* fruits with high nutritional value, along with potential several secondary metabolites, including favones, favanones, favonols, favans, and anthocyanins, which are recognized to have beneficial and healthy effect for human. Among the most common citrus species, *Citrus aurantium* L., also known as Seville orange, sour orange, or bitter orange, originating in eastern Africa, and Syria, and cultivated in Spain, Italy, and North America [2]. In addition to the richness in bioactive molecules, have demonstrated several health effects such as antioxidant, antimicrobial, anti-inflammatory, anti-hypertensive, neuroprotective, antimutagenic, and antiallergic properties [3,4]. *Citrus* are sources of essential oils due to their aromatic compounds which are used in drinks, confectionery, cookies, desserts, cakes, and ice cream [5,6].

In general, *citrus* fruits essential oils have been recognized as an important natural resource, they possess considerable advantage and enjoy popularity thanks to the gradual discovery of their properties: antibacterial, anti-inflammatory, antiseptic, antidiabetic, antiviral,

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antifungal, antioxidant, stimulating, calming and relaxing [7-10]. Furthermore, essential oils have been extracted from the leaves, stem, roots, and peels of different species with Citrus essential oils (CEOs) containing various potent compounds like *α/β*-pinene, sabinene*, β*-myrcene, *d*-limonene, linalool, *α*-humulene, and *α*-terpineol belonging to the monoterpenes, monoterpene aldehyde/alcohol, and sesquiterpenes group, respectively.
Citrus essential oil is largely present in the peels compared to other parts. It represents an abundant and inexpensive source of terpenes and oxygenated terpenes which are of interest to many sectors, in particular; food industry, pharmaceuticals, cosmetics, the aroma and perfume industry; molecules, such as myrcene and linalool, are contained in small quantities in essential oils and which have high added value due to their particularly desirable sensory profile; although the non-oxygenated terpene, limonene is a major component of all essential oils in citrus fruits [11,12].

C. aurantium has been used in herbal medicine as a stimulant and appetite suppressant; it has also been used in traditional Chinese medicine to treat nausea, indigestion, and constipation. It is also used to treat cancer and certain cardiovascular diseases [13]. Furthermore, recent research showed immature peels and essential oils are used to treat intestinal diseases and antidiabetic effect [8,14,15]. These studies are focused on the search for potential inhibitors of the two enzymes α-glucosidase and α-amylase, in order to treat type 2 diabetes [16]. Furthermore, recent research has emphasized the importance of promoting safer and tolerable inhibitors for the two enzymes α-glucosidase and α-amylase that are naturally extracted from medicinal plants, fruits, and vegetables at a lower cost, particularly *Citrus* fruits. Many studies have shown that their consumption helps to treat a variety of chronic diseases, including type 2 diabetes. In this optic, the present study was conducted to explore CAEO chemical composition and its anti-α-glucosidase, antioxidant and antibacterial activities.

2. MATERIAL AND METHODS

2.1. Plant material and essential oil isolation

Citrus aurantium L. fruits were harvested from a garden of Faculty of Sciences and Technology of Sidi Bouzid (centre of Tunisia) and identified according to the flora of Tunisia. The EO extraction was carried out from the fresh peel of bigarade. The freshly harvested fruits were carefully washed to remove dust then peeled and cut into small pieces. An amount of 100 g of fresh peels was transferred to hydro-distillation for 3 hours with 500 mL distilled water using a Clevenger-type apparatus. The distilled EO was dried over anhydrous sodium sulfate, filtered, and stored at 4°C. The yield was calculated based on the dried weight of the sample.

2.2. Gas chromatography–mass spectrometry analyses of CAEO

2.2.1. Gas chromatography analysis.

Gas chromatograph: HP 5890-series II equipped with flame ionization detector (FID), HP-5 (30 m × 0.25 mm i.d., 0.25 µm film thickness) and the HP-Innowax column (polyethylene glycol column, 0.25 mm internal diameter, 30 m length and 0.25 µm film in thickness) fused silica capillary column, carrier gas nitrogen (1.2 ml per min). The oven temperature was programmed from 50°C (1 min) to 280°C at 5°C per minute. The injector and the detector temperatures were 250 and 280°C, respectively. Volume injected: 0.1 µl of 1% hexane solution. The identification of the components was performed by comparison of their

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retention times with those of pure authentic samples and by mean of their retention indices relative to the series of *n*-hydrocarbons.

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2.2.2. Gas chromatography-mass spectrometry analysis.

GC/MS analyses were performed with the Varian CP-3800 gas-chromatograph equipped with the HP-5 capillary column (30 m × 0.25 mm; coating thickness 0.25 μm) and the Varian Saturn 2000 ions trap mass detector. Analytical conditions: injector and transfer line temperatures 220 and 240°C respectively; oven temperature programmed from 60°C to 240°C at 3°C per min; carrier gas helium at 1 ml/min; injection of 0.2 μl (10% hexane solution); split ratio 1:30. The identification of the constituents was based on comparison to retention times with those of authentic samples, comparing their linear retention indices relative to the series of *n*-hydrocarbons, on computer matching against commercial and internal library mass spectra built up from pure substances, components of known oils and MS literature data [17]. Moreover, the molecular weights of all the identified substances were confirmed by GC/MS, using methanol as *CI* ionizing.

2.3. α-Glucosidase Inhibitory Assay

The α-glucosidase assay of the tested EOs was conducted according to the standard method with slight modification [18]. Inside the 96-well plate, 50 μL of phosphate buffer (100 mM, pH = 6.8), 10 μL α-glucosidase (1 U/mL), 20 μL of samples, and standard (acarbose) of different concentration were incubated for 15 min at 37°C. Briefly, 20 μL of 5 mM substrate (4-nitrophenyl β-d-glucopyranoside) was added to each well and left to incubate for 20 min at 37°C. The reacting mixture was stopped after incubation by adding 0.1 M sodium carbonate (50 μL). The release of p-nitrophenol of the reacting mixture relating to the activity of the enzyme was read at a wavelength of 405 nm using a multiplate reader (Multiskan, Thermo Scientific). The enzyme inhibition rate expressed as percentage of inhibition was calculated using the following formula:

Percentage inhibitory activity (%) = (1 − A/B) × 100

Where, A is the absorbance in the presence of test substance, and B is the absorbance in the presence of phosphate buffer (control). The results are expressed as IC_{50} values (μg/mL). All samples were prepared and analyzed in triplicate.

2.4. Antioxidant activity

2.4.1. Scavenging ability on DPPH radical

The DPPH· quenching ability of the EO was measured according to the method cited by Felhi et al. [19]. 1 ml of the oil, extracted at known concentrations, was added to 0.25 ml of a DPPH· methanolic solution. The mixture was shaken vigorously and left to rest at room temperature for 30 min in the dark. The absorbance of the resulting solution was then measured at 517 nm to determine the values corresponding to the ability of the extracts to reduce the stable radical DPPH to the yellow-coloured diphenylpicrylhydrazine. Antiradical activity was expressed as IC_{50} (μg/mL) values, reflecting the extract doses required to cause a 50% inhibition. A lower IC_{50} value corresponded to a higher antioxidant activity of plant extract. The ability to scavenge the DPPH radical was calculated using the following equation:

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$$DPPH^{\cdot} \text{ scavenging effect } (\%) = [(A_0 - A_1)/A_0]*100$$

Where A_0 refers to the absorbance of the control at 30 min, and A_1 to the absorbance of the sample at 30 min. All samples were analyzed in triplicate.

2.4.2. Reducing Power

The ability of the EO to reduce Fe^{3+} was assayed using the method cited by Hajlaoui et al. [20] and Bakari et al., [22]. Briefly, 1 mL of the EO was mixed with 2.5 mL of phosphate buffer (0.2M, pH 6.6) and 2.5 mL of 1% $K_3Fe(CN)_6$. Absorbance was measured at 700 nm. The mean of absorbance values was plotted against concentration values, and a linear regression analysis was performed. Increased absorbance of the reaction mixture indicated increased reducing power. The EC_{50} value (µg/mL) is the effective concentration at which absorbance was 0.5 for reducing power. BHT was used as positive control.

2.5. Antibacterial activity

2.5.1. Disc-Diffusion Assay

The bacterial strains tested in this study belonged to 8 references, which are presented in tables 3. The bacterial species consisted of 5 Gram-positive and 3 Gram-negative bacterial strains. The disc-diffusion assay was performed according to the protocol cited by Hajlaoui et al. [21]. For the experiments, a loopful of the microorganisms working stocks were enriched on a tube containing 9 mL of Mueller-Hinton broth then incubated at 37°C for 18 to 24 h. The overnight cultures were used for the antibacterial activity of the EO used in this study, and optical density was adjusted at 0.5 McFarland turbidity standards with a DENSIMAT (Biomérieux®). The inoculums of the bacteria were streaked onto MH plates using a sterile swab. Sterile filter discs (diameter 6 mm, Whatman paper No. 3) were impregnated with 10 µL of EO placed on the appropriate agar media. Gentamycin (10 µg/disc) was used as positive reference standards to determine the sensitivity of one strain/isolate to each of the tested microbial species. After incubation at 37°C for 18 to 24 h, the diameter of inhibition zone was measured with 1 mm flat rule, and diameters were interpreted according to the Committee of the French Society of the Antibiogram [23]. The dishes were incubated at 37°C for 18-24 h for microbial strains. The diameter of inhibition zones around each of the discs was taken as measure of antimicrobial activity. Each experiment was carried out in triplicate, and the mean diameter of the inhibition zone was recorded.

2.5.2. Micro-Well Determination of MIC and MBC

Minimal inhibition concentration (MIC) and minimal bactericidal concentration (MBC) values were determined for all bacterial strains used in this study as described by Hajlaoui et al. [21]. A 100 µL aliquot from the stock solutions of EO was added into the first wells. Then, 100 µL from the serial dilutions were transferred into eleven consecutive wells. The last well containing 195 µL of nutrient broth without EO and 5 µL of the inoculum on each strip was used as the negative control. The final volume in each well was 200 µL. The plates were incubated at 37°C for 18-24 h.

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The EO tested in this study was screened two times against each organism. The MIC value was defined as the lowest concentration of the compounds to inhibit the growth of the microorganisms. The MBC values were interpreted as the highest dilution (lowest concentration) of the sample, which showed clear fluid with no development of turbidity and without visible growth. MBC/MIC ratios were also calculated. All tests were performed once.

3. RESULTS AND DISCUSSION

3.1. Essential oil composition of CAEO

In this part, chemical composition identification of CAEO was carried out by calculating the retention index (IR) for each compound and their percentage. The constituents of this EO are listed according to their elution on an apolar column (HP-5) and a polar column. Results are shown in Table 1.

Table 1. Chemical composition, retention index (RI) and percentage composition of CAEO peels.

Sample	Compounds	RI a	RI b	Percentage (%)	Identification
1	Tricylene	1012	927	Tr	MS, RI
2	**α-thujene**	**1020**	**930**	**3,55**	MS, RI
3	α-pinene	1026	935	0,55	MS, RI
4	α-fenchene	1062	950	Tr	MS, RI
5	Camphene	1070	952	0,43	MS, RI
6	**Sabinene**	**1110**	**974**	**4,56**	MS, RI
7	β-pinene	1122	979	Tr	MS, RI
8	Myrcene	1161	995	0,85	MS, RI
9	**Limonene**	**1194**	**1033**	**62,2**	MS, RI
10	1,8-cineole	1215	1035	0,22	MS, RI
11	γ-terpinene	1245	1061	0,16	MS, RI
12	***o*-cymene**	**1260**	**1022**	**2,1**	MS, RI
13	*p*-cymene	1268	1026	1,3	MS, RI
14	*trans*-linalool oxide	1460	1092	Tr	MS, RI
15	**citronellal**	**1463**	**1157**	**2,35**	MS, RI
16	δ-elemene	1465	1332	Tr	MS, RI
17	α-copaene	1489	1380	0,62	MS, RI
18	**Linalool**	**1545**	**1102**	**8,2**	MS, RI
19	**linalyl acetate**	**1554**	**1260**	**3,2**	MS, RI
20	cis-sabinene hydrate	1558	1098	Tr	MS, RI
21	β-elemene	1587	1386	0,16	MS, RI
22	β-caryophyllene	1593	1424	0,51	MS, RI
23	terpinen-4-ol	1600	1178	0,7	MS, RI

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24	γ-elemene	1623	1491	1,21	MS, RI
25	α-humulene	1668	1461	Tr	MS, RI
26	**Neral**	**1671**	**1246**	**3,25**	MS, RI
27	α-terpineol	1690	1194	0,21	MS, RI
28	α-terpinyl acetate	1695	1351	Tr	MS, RI
29	neryl acetate	1720	1366	Tr	MS, RI
30	geranyl acetate	1750	1382	0,31	MS, RI
31	δ-cadinene	1754	1523	0,77	MS, RI
32	Nerol	1790	1232	0,72	MS, RI
33	2-phenylethyl acetate	1826	1256	Tr	MS, RI
34	caryophyllene oxide	1974	1588	Tr	MS, RI
35	nerolidol	2030	1568	0,51	MS, RI
36	farnesyl acetate	2194	1820	0,44	MS, RI
37	methyl anthranilate	2204	1360	0,22	MS, RI
Monoterpenes Hydrocarbons				75,7	
Oxygenated monoterpenes				19,16	
Sesquiterpene hydrocarbons				3,27	
Oxygenated Sesquiterpenes				0,95	
Others				0,22	
Total identification				99,3	

a: Polar column, b: apolar column, RI: retention index on polar and apolar columns; Tr : trace <0.1

GC-MS analysis of CAEO showed the presence of 37 compounds for 99.3% an identification percentage. The major compounds are: limonene (62.2%), α- thujene (3.55%), citronellal (2.35%), sabinene (4.56%), o-cymene (2.1%), linalool (8.2%), linalyl acetate (3.2%), neral (3.25%). The classification of these compounds according to organic families shows that CAEO peels is particularly rich in hydrocarbon monoterpenes (75.7%), followed by oxygenated monoterpenes (19.16%). While the percentage of hydrocarbon and oxygenated sesquiterpenes does not exceed 5%. This chemical composition remains specific and characteristic of bitter orange plants in the garden of the FST of Sidi Bouzid. This specificity was related to bioclimatic stage. In fact, each time the place of harvest changes, the chemical composition changes [24]. In addition, chemical composition of EO changes also according to the plant organs. Indeed, bnina et al. [24] study showed that EOs isolated from flowers and leaves of *C. aurantium* were particularly rich in oxygenated monoterpenes (59.02–69.21%) represented by linalool (41.82–37.24%) and linalyl acetate (13.75–7.87%), followed by hydrocarbon monoterpenes (24.61–32.28%). The most important hydrocarbon monoterpenes were α-thujene (6.15–10.65%) and β-pinene (9.21–9.68%). While the EO isolated from the peels was dominated by limonene (monoterpene hydrocarbon) (73.60%). But oxygenated monoterpenes only made up 11.68% of the total oil. Comparative studies of the chemical composition of this oil obtained from different origins of the Mediterranean

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basin have shown natural differences in chemical composition due to harvest season, fruits degree of maturity, plant species and geographical location (latitude, longitude, altitude, relative humidity, soil physicochemical parameters and winds) [25-29]. EO most studies of bitter orange peel have shown the dominance of limonene as the predominant compound (Table 2). It should therefore be noted that limonene is characteristic of bark even for other species of *Citrus* [30].

Table 2. Limonene percentage in the CAEOs peels from different provenances.

Country	Limonene (%)	References
Tunisia (Zaghouan)	96.90	Hosni *et al.* 2010
Tunisia (Monastir)	73.60	Bnina *et al.* (2019)
Egypt	69.50	Dugo *et al.* 2011)
Greece	94.7	Sarrou *et al.* 2013
Italy	93.40	Dugo *et al.* 2011)
Turkey (Antalya)	94.40	Kirbas *et al.* 2003
Cuba	86.20	Pino et Rosado 2000
Bulgaria	85.22	Desislavateneva 2018

3.2. α-Glucosidase Inhibitory Assay

In this part, Fig.1 showed the inhibitory effect of different concentrations of CAEO peels on α-glycosidase activity compared with Acarbose.

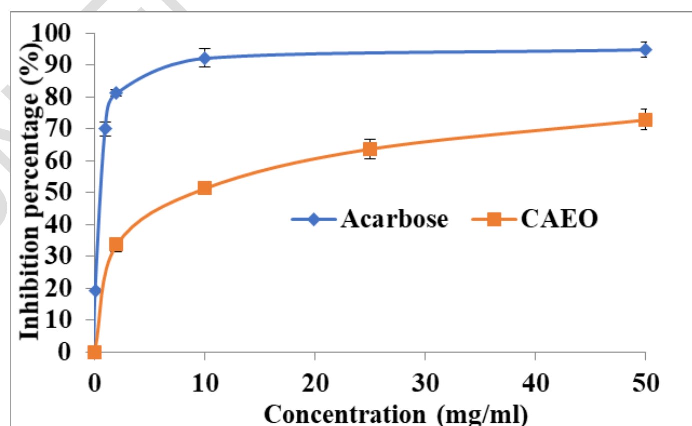

Fig. 1: inhibition percentage of α- glycosidase by CAEO peels and Acarbose.

Based on these results, EO and Acarbose exert an inhibitory effect on α-glycosidase. This inhibition increases in proportion to the increase in the concentration of the samples. The inhibition of Acarbose is found to be greater than EO. Indeed, a low concentration of Acarbose can cause maximum inhibition. The IC_{50} obtained (Fig. 2) with Acarbose (0.7 ± 0.1 mg / ml) is almost 14 times lower than that obtained with EO (10 ± 1 mg / ml). These results are in agreement with other studies showing an efficacy of EOs in inhibiting the enzymatic activity of α-glycosidase which remains lower than that of Acarbose. The percentages of inhibitions found by Benayad et al. [31] study are 22% and 65% respectively for EO of *C. aurantium* and Acarbose for the same concentration of 332 µg / ml. Likewise, Hajlaoui et al. [21] study focused on EO of two spices caraway and coriander showed that IC_{50} were around 6.83 ± 0.76; 6.24 ± 0.86; 7.07 ± 0.75 and 0.73 ± 0.1 mg / ml respectively for caraway, coriander, their mixture and Acarbose.

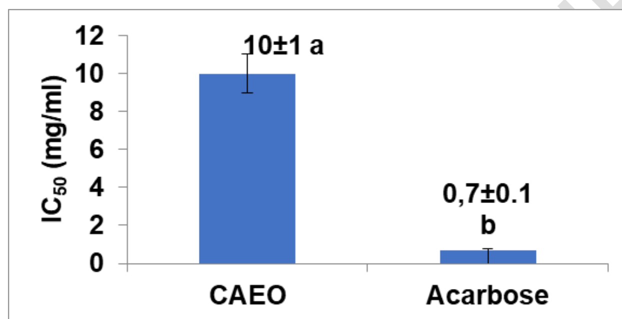

Fig. 2. The 50% inhibition concentration of α-glycosidase (IC_{50} mg / ml) of the CAEO peels compared with Acarbose.

Several antidiabetic trials, with a wide range of extracts and EOs from plants, inhibit the enzymatic activity of α-glucosidase and α-amylase. But the effectiveness of this inhibition depends on several parameters, including the composition of the bioactive mixture, the structure-function relationship, and type and stability degree of established links between enzyme and inhibitor molecule. Moreover, it has been shown that terpenes represent a good antidiabetic potential [32]. Among the active monoterpenes, p-cymene and -terpinene have revealed a powerful inhibitory effect [32,33]. The strongest α-glucosidase inhibitory effect was also displayed by EO *Sideritis galactic* containing a high level of α-pinene (32.2%) and all the activity was attributed to the high level of monoterpene hydrocarbons. In our study, this fraction is of 75.7% of total CAEO.

3.3. Antioxidant Activity

3.3.1. Scavenging Ability on DPPH Radical

The antiradical activity profile of CAEO compared to the synthetic antioxidant BHT is shown in Fig.3. This result revealed that EO has a significant antiradical activity but it is lower than that obtained by BHT. In fact, 100% inhibition is achieved for a 100

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µg/ml of BHT concentration. This percentage was not reached even 200µg/ml concentrations for EO.

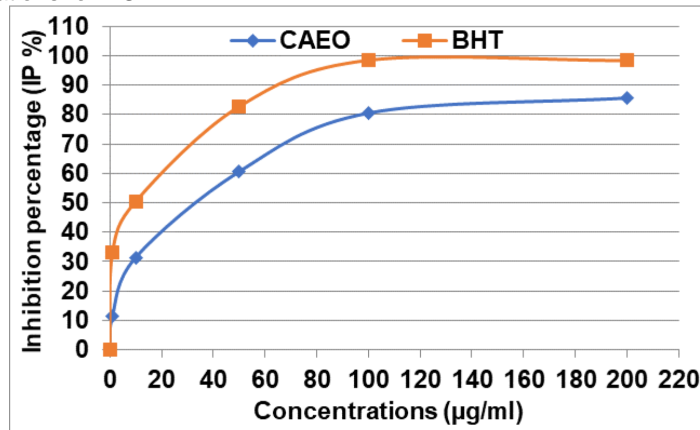

Fig. 3. Inhibition percentage curve of DPPH radical by CAEO peels and synthetic antioxidant (BHT).

The (IC_{50}) values (Fig. 4) shows that EO has a significant capacity for scavenging free radicals with an IC_{50} = 33.66 µg/ml. This activity is 3 times less than BHT (10.33% g / ml).

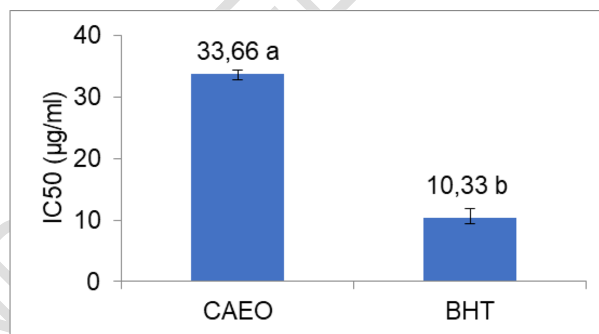

Fig. 34. Anti- radical activity (DPPH) (IC_{50} in µg.ml^{-1}) of the CAEO peels compared with synthetic antioxidant BHT. The means followed by the same letters are not significantly different at the 5% level.

The antioxidant properties of *citrus* fruits have been described by several authors. Choi et al. (2000) studying free radical scavenging activities of 34 types of *citrus* EOs by DPPH assay. Only four CAEO peel showed scavenging effects between 17.7% and 34, 1%. In addition, Hamdani (2018) comparing antiradical activity of the CAEOs from 4 sites in Algeria showed that the strongest antioxidant activity was characterized by CAEO from Boujlida region with IC_{50} of 32.9 mg/ml, while the lowest activity was expressed by CAEO from Ouzidane region with IC_{50} of 59.55

mg/ml. Results obtained from the IC_{50} showed that all samples of *C. aurantium* have a significant antioxidant power compared to limonene (IC_{50} of 258.74 mg/ml). These results are different from our study. This difference could be explained by chemical composition variation which is related to several factors namely the methodology used to obtain the extracts, the region of harvest, stage of fruit ripening, climate and fruits maturity [34,35].

3.3.2. Reducing Power

Reducing power capacity of CAEO was shown in fig. 5. Results indicate an increase in absorbance (OD) which refers to the increase in reducing capacity. CAEO reducing activity comparison with BHT showed a significant difference ($P<0.05$) for different tested concentrations. These results show significant antioxidant activity of CAEO, but it is weaker than BHT.

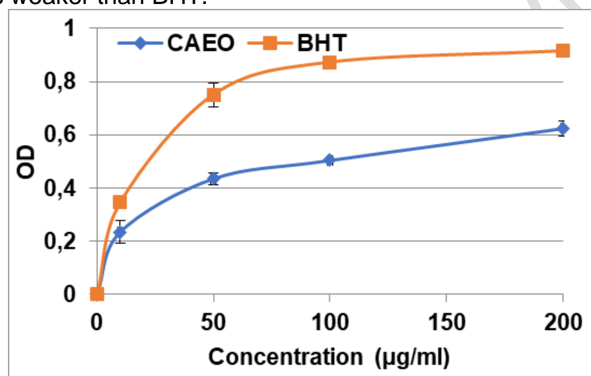

Fig. 5. Iron reduction capacity by CAEO peels compared with BHT.

Determination of EC_{50} values (Fig. 6) shows that iron reducing capacity of BHT exceeds four times the EO capacity. Values obtained are 22.67 and 98.67 µg/ml respectively for BHT and HE.

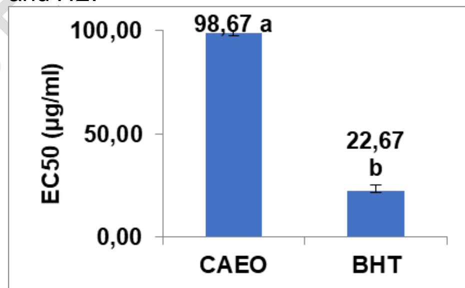

Fig. 6: Reducing power (EC_{50} in µg.ml-1) of CAEO peels compared with synthetic antioxidant (BHT). The means followed by the same letters are not significantly different at the 5% level.

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In the present study, the CAEO peels showed significant antioxidant activity which was supported by both tests; DPPH radical scavenging capacity and iron reduction (FRAP). This activity turns out to be more interesting than others in previous work. For example, the results found by Hamdani et al. [36], working on 4 samples of *C. aurantium*, showed that the IC_{50} values vary from 32.9 to 59.55 mg/ml and the EC_{50} values ranges from 1.369 to 2.204 mg/ml. However, limonene, the major compound, showed a low antioxidant activity, but this activity obtained could be due to the appreciable percentage of myrcene or its combination with limonene which appears to be effective. But in our study, the activity of EO is closely related to its composition, and the association of α-thujene, sabinene, linalool, linalyl acetate and neral with limonene may be responsible for this activity.

3.4. Antibacterial activity evaluation

Inhibition diameters values of CAEO against all studied strains presented in table 3, were ranged from 8.66±1.15 to 12±0 mm. These values are relatively high showing the inhibitory activity of bacterial growth of this EO despite being lower than those of gentamicin (from 20.33±0.57 to 32.67±0.58mm). Statistical analysis revealed a significant difference (P <0.05) in bacterial strains sensitivity to CAEO and gentamicin. But there is unclear difference between Gram+ and Gram- strains susceptibility to EO. However, Gram+ strains appear to be more sensitive to gentamicin than Gram- strains.

Table. 3. Zones of growth inhibition (IZ mm±SD), showing the qualitative antibacterial activity of peels CAEO against human pathogenic bacteria compared to standard antibiotic (gentamycin).

	CAEO (10µl/disque)	Gentamycin (10 µg/disque)
$Gram^{+}$ Bacteria		
S. epidermidis	$10±1b^{cB}$	$21.33±0.58^{dA}$
S. aureus	$12±0^{aB}$	$32,67±0,58^{aA}$
E. feacalis	$11±1^{bB}$	$26 ±1^{Ba}$
B. cereus	$9.33±0.57^{cB}$	$26 ±1^{bA}$
M. luteus	$11±1.73^{bB}$	$27,67±1,53^{bA}$
$Gram^{-}$ Bacteria		
S. typhimurium	$8.66±1.15^{cB}$	$20.33±0.57^{dA}$
L. monocytogenes	$11±1.73^{bB}$	$23±0^{cA}$
E. coli	$12±0^{aB}$	$22±1^{dA}$

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SD: Standard deviation; IZ: Inhibition zone diameter (mm) around the discs (6mm) impregnated with 10 µl of CAEO and 10 µg/disc for Gentamycin (Gent). a,b,c,d, A,B: Each value represents the average of 3 repetitions. Means followed by the same letters are not significantly different at P= 0.05 based on Duncan's multiple range test. Small letters are used to compare IZ CAEO and IZ Gentamycin means between different strains, while capital letters are used to compare means between IZ CAEO and IZ Gentamycin for the same strain

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The MIC and MBC values found showed that CAEO is effective against tested strains (Table 4). The concentrations obtained were ranged from 0.097 to 0.390 mg/ml and from 0.195 to 1.562 mg/ml respectively for MIC and MBC. However, this activity remains less effective than gentamicin which values were ranged from 0.004 to 0.019 mg/ml for MIC and 0.019 to 0.078 mg/ml for MBC. Based on these results, Gram+ strains appear to be less sensitive than Gram- strains to EO and gentamicin effects. This agrees with other previous work [37-39]. Explanation for this resistance is related to Gram- bacteria structure wall, which makes unable EO hydrophobic compounds to diffuse, unlike Gram+ bacteria [40]. Furthermore, to better underline the capability of CAEO in destroying bacterial cells (bactericidal), the MBC/MIC ratios have been determined for each strain. As shown, CAEO was found to be bactericidal against all tested strains.

Table. 4. Minimal inhibition concentration (MIC), minimal bactericidal concentration (MBC) and ratio MBC/MIC showing quantitative antibacterial activity of CAEO against human pathogenic bacteria compared to standard antibiotic (gentamycin).

	CAEO (10µl/disque)			**Gentamycin (10 µg/disque)**		
	MIC	MBC	MBC/MIC (Interpretation)	MIC	MBC	MBC/MIC (Interpretation)
Gram$^+$ Bacteria						
S. epidermidis	0.097	0.390	4 (Bactericidal)	0,009	0,039	4(Bactericidal)
S. aureus	0.097	0.390	4 (Bactericidal)	0,004	0,019	4(Bactericidal)
E. feacalis	0.097	0.195	2 (Bactericidal)	0,004	0,019	4(Bactericidal)
B. cereus	0.195	0.390	2 (Bactericidal)	0,004	0,039	8(Bacteriostatic)
M. luteus	0.097	0.195	2 (Bactericidal)	0,004	0,019	4(Bactericidal)
Gram$^-$ Bacteria						
S. typhimurium	0.390	1.562	4 (Bactericidal)	0,019	0,039	2(Bactericidal)
L.monocytogenes	0.195	0.781	4(Bactericidal)	0,019	0,078	4(Bactericidal)

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E. coli	0.390	0.781	2 (Bactericidal))	0,009	0,039	4(Bactericidal)

The antimicrobial activity of EOs is closely related to their chemical composition. Actually, the mechanism of terpenes action is not fully understood, but it is believed that these compounds are involved in the damage and stability of plasma and the subsequent membrane disruption by lipophilic compounds [31,34]. Limonene and linalool, which were found to be abundant in this study, were reported as compounds with significant antimicrobial property [41]. It has also been shown that limonene, the major compound of EOs of *Citrus* genus, has a weaker antibacterial effect than antifungal activity. But the antimicrobial activity of *Citrus* EO is enhanced by the presence of bioactive alcohol, linalool, a monoterpene alcohol, known to be a potent antimicrobial [41]. On the other hand, EO activity of *C. aurantium* peel may be the result of a synergistic effect between these different compounds, especially since the fraction of oxygenated monoterpenes is relatively high (19.16%).

4. CONCLUSION

In this study, CAEO peels exhibited potent anti-diabetic effect explained by a good capacity of α–glucosidase inhibition. Moreover, this EO has an important antioxidant and antibacterial activities. These potentialities are related to the chemical profiling which shows a composition rich in hydrocarbon and oxygenated monoterpenes known by their capacity to treat chronic diseases such as type 2 diabetes. In addition, this EO can be used as a food additive for its antibacterial activity.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

NOTE:

THE STUDY HIGHLIGHTS THE EFFICACY OF "HERBAL MEDICINE" WHICH IS AN ANCIENT TRADITION, USED IN SOME PARTS OF INDIA. THIS ANCIENT CONCEPT SHOULD BE CAREFULLY EVALUATED IN THE LIGHT OF MODERN MEDICAL SCIENCE AND CAN BE UTILIZED PARTIALLY IF FOUND SUITABLE.

COMPETING INTERESTS DISCLAIMER:

AUTHORS HAVE DECLARED THAT NO COMPETING INTERESTS EXIST. THE PRODUCTS USED FOR THIS RESEARCH ARE COMMONLY AND PREDOMINANTLY USE PRODUCTS IN OUR AREA OF RESEARCH AND

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COUNTRY. THERE IS ABSOLUTELY NO CONFLICT OF INTEREST BETWEEN THE AUTHORS AND PRODUCERS OF THE PRODUCTS BECAUSE WE DO NOT INTEND TO USE THESE PRODUCTS AS AN AVENUE FOR ANY LITIGATION BUT FOR THE ADVANCEMENT OF KNOWLEDGE. ALSO, THE RESEARCH WAS NOT FUNDED BY THE PRODUCING COMPANY RATHER IT WAS FUNDED BY PERSONAL EFFORTS OF THE AUTHORS.

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