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GC-MS Profile, α-glucosidase Inhibition Potential, Antibacterial and Antioxidant Evaluation of Peels *Citrus aurantium* (L), Essential Oil

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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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 ($DPPH^{\cdot}$) (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, and so on [1]. *Citrus* fruits with high nutritional value, along with potential several secondary metabolites, including flavones, flavanones, flavonols, flavans, and anthocyanins are recognized to have beneficial and healthy effects 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 was cultivated in Spain, Italy, and North America [2]. In addition to the richness in bioactive molecules, they have demonstrated several health effects such as antioxidant, antimicrobial, anti-inflammatory, antihypertensive, 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 (EOs) have been recognized as an important natural resource. They possess considerable advantage and enjoy popularity thanks to their antibacterial, anti-inflammatory, antiseptic, antidiabetic, antiviral, antifungal, antioxidant, stimulating, calming and relaxing properties [7-10]. Furthermore, essential oils have been extracted from the leaves, stem, roots, and peels of different species with *Citrus* EOs 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 is represent 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].

Citrus aurantium (L), 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 as well as cancer and cardiovascular diseases [13]. Furthermore, recent studies have been improved the efficiency of EOs and extracts as well as their secondary metabolites from as antimicrobials and antidiabetics gent [14-19]. Also, immature peels and EOs are used to treat intestinal diseases and antidiabetic effect [8,20,21]. 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 [22]. 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.

In this optic, the present study was conducted to explored 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 essential oil 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 *Citrus aurantium* Essential Oil

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 as ascribed by Hajlaoui et al. [23].

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 [23].

2.3 α-Glucosidase Inhibitory Assay

The α-glucosidase assay of the tested EO was conducted according to the standard method with slight modification [24].

2.4 Antioxidant Activity

2.4.1 Scavenging ability on 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical

The DPPH quenching ability of the EO was measured according to the method cited by Felhi et al. [25].

2.4.2 Reducing power

The ability of the EO to reduce Fe^{3+} was assayed using the method cited by Hajlaoui et al. [26] and Bakari et al., [27]. Butylated hydroxytoluene (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 Table 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. [20].

2.5.2 Micro-Well Determination of minimal inhibition concentration (MIC) and minimal bactericidal concentration (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. [20].

3. RESULTS AND DISCUSSION

3.1 Essential Oil Composition of *Citrus aurantium* Essential Oil

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 in Table 1.

GC-MS analysis of CAEO showed the presence of 37 compounds accounting for 99.3% of the EO. 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 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 also

[28]. In addition, the chemical composition of EO changes also according to the plant organs. Indeed, Bnina et al. [29] reported 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%), with the most important hydrocarbon monoterpenes were *α*-thujene (6.15–10.65%) and β-pinene (9.21–9.68%). In contrast, the EO isolated from the peels was dominated by limonene (monoterpene hydrocarbon) (73.60%), with oxygenated

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
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 column; Tr : trace <0.1

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)

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 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) [29-33]. On the other hand, EO from bitter orange peel have shown the dominance of limonene as the major compound. It should therefore be noted that limonene is characteristic of bark even for other species of *Citrus* [34].

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 to Acarbose.

Based on these results, EO and Acarbose exert an inhibitory effect on α-glycosidase. This inhibition increases in proportion with 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 percentage of inhibitions found by Benayad et al. [35] are 22% and 65%, respectively, for CAEO and Acarbose using the same concentration of 332 µg/ml. Recently, Hajlaoui et al. [20] 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.

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 [36]. Among the active monoterpenes, p-cymene and -terpinene have revealed a powerful inhibitory effect [36,37]. 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 µg/ml of BHT concentration. This percentage was not reached even 200µg/ml concentrations for EO.

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).

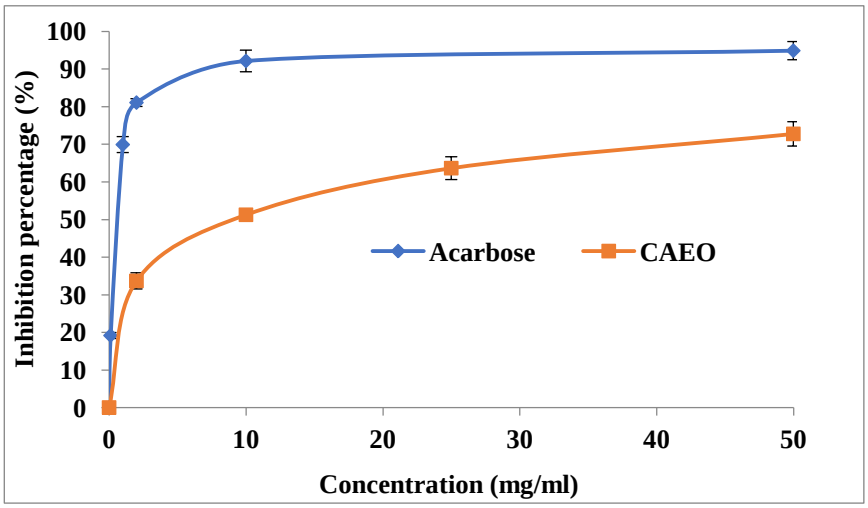

Fig. 1. Inhibition percentage of α- glycosidase by CAEO Peels and Acarbose

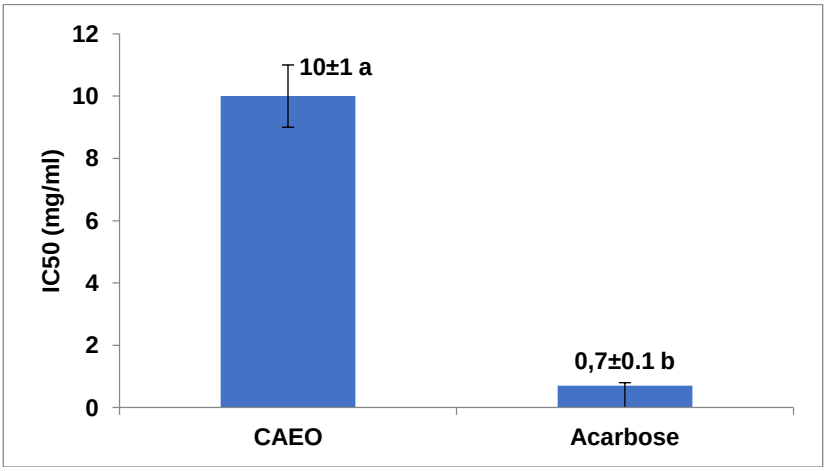

Fig. 2. The 50% Inhibition Concentration of α-Glycosidase (IC_{50} mg/ml) of the CAEO peels compared with Acarbose

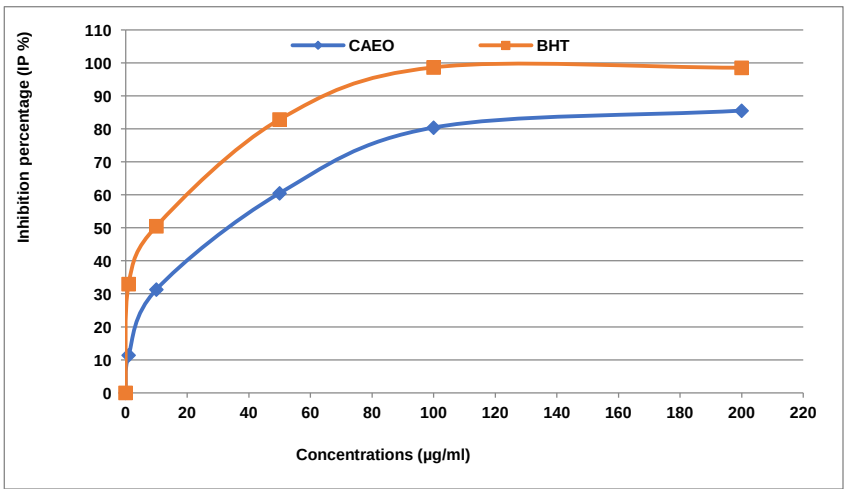

Fig. 3. Inhibition Percentage Curve of DPPH Radical by CAEO Peels and Synthetic Antioxidant (BHT)

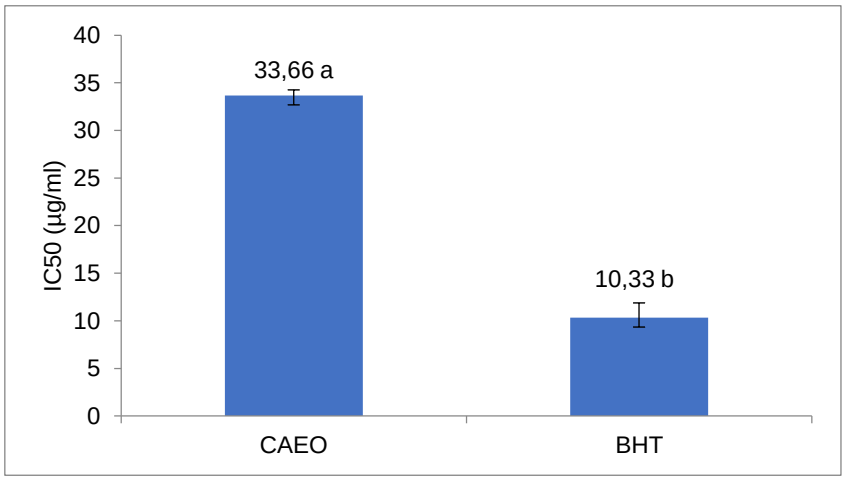

Fig. 3. Antiradical Activity (DPPH) (IC_{50} in µg.ml^{-1}) of the CAEO Peels Compared with Synthetic Antioxidant BHT

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. Hamdani and Allem [38] 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 [39,40].

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.

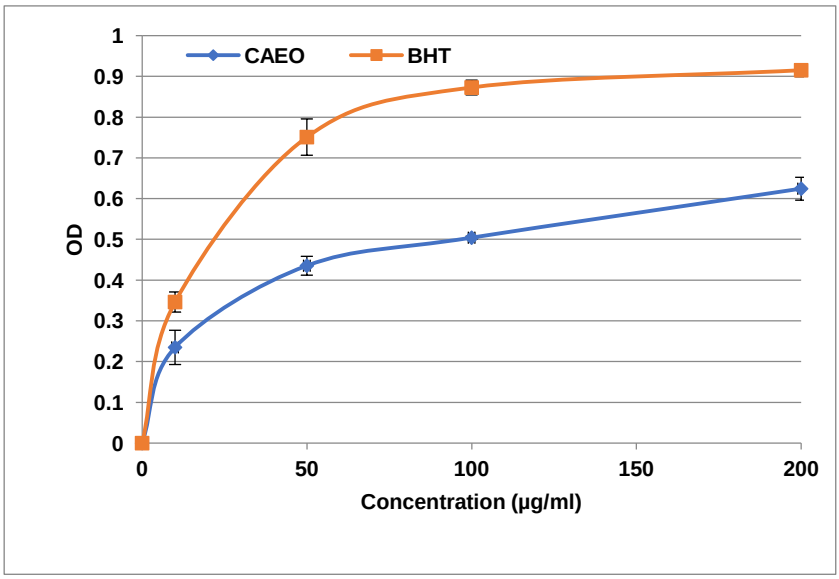

Fig. 5. Iron Reduction Capacity by CAEO Peels Compared with BHT

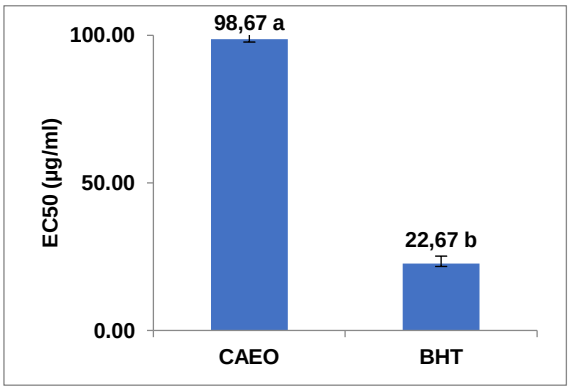

Fig. 6. Reducing power (EC_{50} in µg/ml) of CAEO peels compared with synthetic antioxidant (BHT). The means followed by the same letters are not significantly different at the 5% level

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

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. [38], working on 4 samples of CAEO, showed that 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, probably due to the appreciable percentage of myrcene or its combination with limonene which appears to be effective. As shown, 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 also 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.

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. 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 the EO and Gentamicin effects, which is in accordance with other previous work [41-43]. Explanation for this resistance is related to Gram-bacteria structure wall, which makes unable EO hydrophobic compounds to diffuse, unlike Gram+ Bacteria [44]. 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 towards all tested strains.

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 [35,39]. Limonene and linalool, which were found to be abundant in this study, were reported as compounds with significant antimicrobial property [45]. 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 [45]. 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%).

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 (Gentamicin)

	CAEO (10µl/disque)	Gentamicin (10 µg/disque)
Gram$^+$ Bacteria		
S. epidermidis	$10\pm1b^{cB}$	21.33 ± 0.58^{dA}
S. aureus	12 ± 0^{aB}	$32,67\pm0,58^{aA}$
E. feacalis	11 ± 1^{bB}	26 ± 1^{Ba}
B. cereus	9.33 ± 0.57^{cB}	26 ± 1^{bA}
M. luteus	11 ± 1.73^{bB}	$27,67\pm1,53^{bA}$
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}

SD: Standard deviation; IZ: Inhibition zone diameter (mm) around the discs (6mm) impregnated with 10 µl of CAEO and 10 µg/disc for Gentamicin (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 Gentamicin means between different strains, while capital letters are used to compare means between IZ CAEO and IZ Gentamicin for the same strain

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 (Gentamicin)

	CAEO (10µl/disque)			Gentamicin (10 µg/disc)		
	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)
E. coli	0.390	0.781	2 (Bactericidal))	0,009	0,039	4(Bactericidal)

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.

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.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and 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.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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