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

Hafedh Hajlaoui a, Soumaya Arraouadi b,c, Kaïss Aouadi d,e, Mejdi Snoussi f,g*, Emira Noumi i,h and Adel Kadri i,j

a Research Unit Valorization and Optimization of Resource Exploitation (UR16ES04), Faculty of Science and Technology of Sidi Bouzid, University of Kairouan, Campus University Agricultural City - Sidi Bouzid 9100 Tunisia.
b Regional Center of Agricultural Research (CRRA) Sidi Bouzid, Gafsa Road Km 6, PB 357, Sidi Bouzid 9100, Tunisia.
c Laboratory of Valorization of Unconventional Waters, INRGREF, University of Carthage, Tunisia.
d Department of Chemistry, College of Science, Qassim University, Buraidah 51452, Saudi Arabia.
e University of Monastir, Faculty of Sciences of Monastir, Avenue of the Environment, Monastir 5019, Tunisia.
f Department of Biology, Hail University, College of Science, P.O. Box 2440, 81451 Ha’il, Kingdom of Saudi Arabia.
g Laboratory of Genetic, Biodiversity and Valorization of Bioressources, Higher Institute of Biotechnology of Monastir, University of Monastir, Avenue Taher Hadded BP 74, 5000 Monastir, Tunisia.
h Laboratory of Bioressources: Integrative Biology and Recovery, High Institute of Biotechnology-University of Monastir, Monastir 5000, Tunisia.
i Faculty of Science and Arts in Baljurashi, Albaha University, P.O. Box (1988), Albaha, Saudi Arabia.
j Faculty of Science of Sfax, Department of Chemistry, University of Sfax, B.P. 1171, 3000, Sfax, Tunisia.

Authors’ contributions

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

Article Information

DOI: 10.9734/JPRI/2021/v33i60B34781

Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here:
https://www.sdiarticle5.com/review-history/81551

Received 15 October 2021
Accepted 20 December 2021
Published 23 December 2021
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 monoterpene fraction (75.7%), followed by oxygenated monoterpene (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, 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, anti-diabetic, 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, monoterpen 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%), α-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
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-Terpinol        | 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     | Neral             | 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 (Zaghoun)     | 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-glucosidase activity compared to Acarbose.

Based on these results, EO and Acarbose exert an inhibitory effect on α-glucosidase. 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 IC50 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-glucosidase, 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 IC50 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 monoterpen 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 (IC50) values (Fig. 4) shows that EO has a significant capacity for scavenging free radicals with an IC50 = 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\textsubscript{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)
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\textsubscript{50} of 32.9 mg/ml, while the lowest activity was expressed by CAEO from Ouzidane region with IC\textsubscript{50} of 59.55 mg/ml. Results obtained from the IC\textsubscript{50} showed that all samples of *C. aurantium* have a significant antioxidant power compared to limonene (IC\textsubscript{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.

![Graph showing antiradical activity (DPPH) of CAEO peels compared with synthetic antioxidant BHT.](image1)

**Fig. 3. Antiradical Activity (DPPH) (IC\textsubscript{50} in µg.ml\textsuperscript{-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*

![Graph showing iron reduction capacity by CAEO peels compared with BHT.](image2)

**Fig. 5. Iron Reduction Capacity by CAEO Peels Compared with BHT**
Determination of EC\textsubscript{50} values (Fig. 6) shows that iron reducing capacity of the oil exceeds four times its of BHT. EC\textsubscript{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\textsubscript{50} values vary from 32.9 to 59.55 mg/ml and the EC\textsubscript{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 monoterpen 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)

| Gram+ Bacteria  | CAEO (10 µl/disque) | Gentamicin (10 µg/disque) |
|-----------------|---------------------|---------------------------|
| S. epidermidis  | 10±1b1B             | 21.33±0.58aGA            |
| S. aureus       | 12±0a3B             | 32.67±0.58aGA            |
| E. faecalis     | 11±1c3B             | 26 ±1b3A                |
| B. cereus       | 9.33±0.57c3B        | 26 ±1b3A                |
| M. luteus       | 11±1.73c3B          | 27.67±1.53b3A           |
| Gram- Bacteria  |                     |                           |
| S. typhimurium  | 8.66±1.15c3S        | 20.33±0.57bGA           |
| L. monocytogenes| 11±1.73c3B          | 23±0c3A                 |
| E. coli         | 12±0a3B             | 22±1d3A                 |

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)

| Gram+ Bacteria  | MIC   | MBC   | MBC/MIC (Interpretation) | MIC   | MBC   | MBC/MIC (Interpretation) |
|-----------------|-------|-------|--------------------------|-------|-------|--------------------------|
| 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. faecalis     | 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 antidiabetic 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.

REFERENCES

1. Nieto G, Fernández-López J, Pérez-Alvarez J.A, Peñalver R, Ros G, Viuda-Martos M. Valorization of Citrus coproducts: Recovery of bioactive compounds and application in meat and meat products. Plants. 2021;10:1069.

2. Sharma K, Mahato N, Cho MH, Lee YR. Converting Citrus wastes into value-added products: Economic and environmentally friendly approaches. Nutrition. 2017;34:29–46.

3. Mahato N, Sharma K, Sinha M, Cho MH. Citrus waste derived nutraceuticals for health benefits: Current trends and future perspectives. J. Funct. Food. 2018;40:307–316.

4. Kuo PC, Liao YR, Hung HY, Chuang CW, Hwang TL, Huang SC, Shiao YJ, Kuo DH, Wu TS. Anti-Inflammatory and neuroprotective constituents from the peels of Citrus grandis. Molecules. 2017;22:967.

5. Hosni K, Kerkenni A, Meddei W, Brahim N, Sebei H. Volatile oil constituents of Rosa canali.: Quality as affected by the distillation method. Org Chem Inter. 2010;2010:7.

6. Geraci A, Di Stefano V, Di Martino E, Schillaci D, Schicchi R. Essential oil components of orange peels and antimicrobial activity. Natu Prod Res; 2016.

7. Siddique S, Shafique M, Parveen Z, Khan S, Khanum R. Volatil components, antioxidant and antimicrobial activity of Citrus aurantium var bitter orange peel oil. Pharmacology. Online. 2011;2:499–507.

8. Benayad O, Bouhrim M, Tiji S, Kharchoufa L, Addi M, Drouet S, Hano C, Lorenzo, JM, Bendaha H, Bouham M, et al. Phytochemical profile, α-glucosidase, and α-amylase inhibition potential and toxicity evaluation of extracts from Citrus aurantium (L) peel, a valuable by-product from Northeastern Morocco. Biomolecules. 2021;11:1555.

9. Kuo PC, Liao YR, Hung HY, Chuang CW, Hwang TL, Huang SC, Shiao YJ, Kuo DH, Wu TS. Anti-Inflammatory and neuroprotective compounds from the peels of Citrus grandis. Molecules. 2017;22:967.

10. Ferreira SS, Silva AM, Nunes FM. Citrus reticulata Blanco peels as a source of antioxidant and anti-proliferative phenolic compounds. Ind. Crops Prod. 2018;111:141–148.

11. Ouedrhiria W, Bohdida S, Balouiria M, El Ouali Lalamb A, Moja S, Chahid FO, Grechea H. Chemical composition of Citrus aurantium L. leaves and zest essential oils, their antioxidant, antibacterial single and combined effects. J Chemi Pharmac Res. 2015;7:78–84.

12. Periyanayagam K, Dhanalakshmi S, Karthikeyan V, Magesh M. Antibacterial activity of Citrus aurantium leaf essential oil against S. aureus and MRSA. J Drug Discov Therap. 2014;2:54–60.

13. Suryawanshi JAS. An overview of Citrus aurantium used in treatment of various diseases. Afr. J. Plant. Sci. 2011;5:390–395.

14. Alminderej F, Bakari S, Almundarji TI, Snoussi M, Aouadi K, Kadri A. Antibacterial activities of a new chemotype of Piper cubeba L. fruit essential oil (Methyleneugenol/Eugenol): In silico molecular docking and ADMET studies. Plants. 2020;9:1534.

15. Alminderej F, Bakari S, Almundarji TI, Snoussi M, Aouadi K, Kadri A. Antimicrobial and wound healing potential of a new chemotype from Piper cubeba L. essential oil and in silico study on S. aureus tyrosyl-tRNA Synthetase Protein. Plants. 2021;10:205.

16. Kadri A, Zarai Z, Ben Chobbba I, Gharsallah N, Damak M, Bekir A. Chemical composition and in vitro antioxidant activities of Thymelae ahirsuta L. essential oil from Tunisia. Afr. J. Biotechnol. 2011;15:2930–2935.

17. Mseddi K, Alimi F, Noumi E, Veettil VN, Deshpande S, Adnan M, Hamdi A, Elkahoui S, Alghamdi A, Kadri A, Patel M, Snoussi M. Thymus muslii Velen. as a promising source of potent bioactive compounds with its pharmacological properties: In vitro and in silico analysis. Arab. J. Chem. 2020;13:6782–6801.

18. Ben Mefteh F, Daoud A, Bouket AC, Thissera B, Kadri Y, Cherif-Silini H, Esheili M, Alenezi FN, Vallat A, Oszako T, et al.
Date palm tree’s root-derived endophytes as fungal cell factories for diverse bioactive metabolites. Int. J. Mol. Sci. 2018;19:1986.

19. Daoud A, Ben Metteh F, Mnafgui K, Turki M, Jmal S, Ben Amar, R, Ayadi F, El Feki A, Abid L, Rateb ME, Belbahri L, Kadri A, Gharsallah N. Cardioprotective effect of ethanolic extract of date palm pollen against isoproterenol induced myocardial infarction in rats through the inhibition of the angiotensin-converting enzyme. Exp Toxicicol Pathol. 2017;69:656–665. DOI: 10.1016/j.etp.2017.06.004

20. Ganiyu O, Tosin A O, Ayokunle O A. Inhibition of enzymes linked to type-2 diabetes and hypertension by essential oils from peels of orange and lemon. Inter j food prop. 2017;20:586-594. Available:https://doi.org/10.1080/10942912.2017.1303709.

21. Kim SS, Baik JS, Oh TH, Yoon WJ, Lee NH, Hyun CG. Biological activities of Korean Citrus abovides and Citrus natsuidaidai essentials oils against acne-including bacteria. Biosc Biotech Biochem. 2008;72:2507–2513.

22. Alu’Datt MH, Rababah T, Alhamad MN, Almahasneh MA, Ereifej K, Al-Karaki G, Al-Duais M, Andrade JE, Tranchant CC, Kubow S, et al. Profiles of free and bound phenolics extracted from: Citrus fruits and their roles in biological systems: Content, and antioxidant, anti-diabetic and anti-hypertensive properties. Food Funct. 2017; 8:3187–3197.

23. Hajlaoui H, Arraoudi S, Noumi E, Aouadi K, Adnan M, Khan MA, Kadri A, Snoussi M. Antimicrobial, antioxidant, anti-acyetylcholinesterase, anti-dietetic, and pharmacokinetic properties of Carum carvi L. and Coriandrum sativum L. essential oils alone and in combination. Molecules. 2021;26:3625. Available:https://doi.org/10.3390/molecules26123625.

24. Kadri A, Aouadi K. In vitro antimicrobial and α-glucosidase inhibitory potential of enantiopure cycloalkylglycine derivatives: Insights into their in silico pharmacokinetic, druglikeness, and medicinal chemistry properties. J. App. Pharm. Sci. 2020;10:107–115.

25. Felhi S, Saoudi M, Daoud A, Hajlaoui H, Ncir M, Chaabane R, El Feki A, Gharsallah N, Kadri A. Investigation of phytochemical contents, in vitro antioxidant and antibacterial behavior and in vivo anti-inflammatory potential of Ecballium elaterium methanol fruits extract. Food Sci. Technol. (Camp.) 2017;37:558–563.

26. Hajlaoui H, Arraoudi S, Mighri H, Chaibia M, Gharsallah N, Ros G, Nieto G, Kadri A. Phytochemical constituents and antioxidant activity of Oudneya africana L. leaves extracts: Evaluation effects on fatty acids and proteins oxidation of beef burger during refrigerated storage. Antioxidants. 2019;8:442.

27. Bakari S, Hajlaoui H, Daoud A, Mighri H, Ross-Garcia JM, Gharsallah N, Kadri A. Phytochemicals antioxidant and antimicrobial potentials and LC-MS analysis of hydroalcoholic extracts of leaves and flowers of Erodium glaucophyllum collected from Tunisian Sahara. Food Sci. Tech. (Campinas). 2018;38:310-317.

28. González-Mas MC, Rambla JL, López-Gresa MP, Blázquez MA, Granell A. Volatile compounds in citrus essential oils: A comprehensive review. Front. Plant Sci. 2019;10:12. DOI: 10.3389/fpls.2019.00012.

29. Bnina EB, Hajlaoui H, Chaieb I, Said MB, Jannet HB. Chemical composition, antimicrobial and insecticidal activities of the tunisian Citrus aurantium essential oils. Czech J Food Sci.; 2019.

30. Hosni K, Zahed N, Chrif R, Abid I, Medfiei W, Kallel M, et al. Composition of peel essential oils from four selected Tunisian Citrus species: Evidence for the genotypic influence. Food Chem. 2010;123(4):1098-1104.

31. Kirbaslar GF, Kirbaslar SI. Composition of cold pressed bitter orange peel oil from Turkey. J Essential Oil Resea. 2003;15:6-9.

32. Sarrou E, Chatzopoulou P, Dimassi-Therou K, Therios I. Volatile constituents and antioxidant activity of peels, flowers and leaf oils of Citrus aurantium L. growing in Greece Molec. 2013;18:10639–10647.

33. Dugo G, Bonaccorsi I, Sciarrone D, Costa R, Dugo P, Mondello L, et al. Characterization of oils from the fruits, leaves and flowers of the bitter orange tree. J Essen Oil Resea. 2011;23:45-59.

34. Bourgou S, Rahali F Z, Ourghemmi I, Saidani Tounsi M. Changes of Peel Essential Oil Composition of Four Tunisian Citrus during Fruit Maturation. The Scientific World Journal. 2012;Article ID 528593:10. DOI:10.1100/2012/528593.
35. Benayad O, Bouhrim M, Tiji S, Khrouchoufa L, Addi M, Drouet S, Hano C, Lorenzo JM, Bendaha H, Bnouham M, et al. Phytochemical profile, -glucosidase, and -amylase inhibition potential and toxicity evaluation of extracts from Citrus aurantium (L) peel, a valuable by-product from Northeastern Morocco. Biomolecules. 2021;11:1555.

36. Mahizan NA, Yang SK, Moo CL, Song AAL, Chong CM, Chong CW, Abusheiba A, Lim SHE, Lai KS. Terpene derivatives as a potential agent against antimicrobial resistance (AMR) Pathogens. Molecules. 2019;24:2631.

37. Majouli K, Hlila MB, Hamdi A, Flamini G, Jannet HB, Kenani A. Antioxidant activity and α-glucosidase inhibition by essential oils from Hertiacheirifolia (L.). Ind. Crops Prod. 2016;82:23–28.

38. Hamdani FZ, Allem R. Antifungal properties of leaf essential oils of Citrus against Alternaria alternata and Penicillium sp in vitro. Phytothérapie. 2017;15:263-266.

39. Gorinstein S, Martín-Belloso O, Park YS, Haruenkit R, Lojek A, ´Cíž M, Caspi A, Libman I, Traktenberg S. Comparison of some biochemical characteristics of different citrus fruits. Food Chem. 2001;74:309–315.

40. Radovi´c M, Milatovi´c D, Teši´c Z, Tosti T, Gaši´c U, Dojˇcinovi´c B, Zagorac DD. Influence of rootstocks on the chemical composition of the fruits of plum cultivars. J. Food Comp Anal. 2020;92:103480.

41. Bakkali F, Averbeck S, Averbeck, Idaomar M. Effets biologiques des huiles essentielles. Alimentaire et chimique Toxicology. 2008;46:446-475. (French)

42. Hussain AI, Farooq A, Shahzad AS, Chatha, Sajid L, Syed THS, Ashfaq A, Jenny W, Satyajit DS. Chemical composition and bioactivity studies of the essential oils from two Thymus species from the Pakistani flora. LWT - Food Sci Technol. 2013;50(1):185-192.

43. Ghannay S, Kadri A, Aouadi K. Synthesis. in vitro antimicrobial assessment and computational investigation of pharmacokinetic and bioactivity properties of novel trifluoromethylated compounds using in silico ADME and toxicity prediction tools. Monatsh. Chem. 2020;151:267-280.

44. Boukhatem MN, Ferhat MA, Kameli AK, Saïd F, Taib H, Tefahi D. Valorisation de l’essence aromatique du Thym (Thymus vulgaris L.) en aromathérapie anti-infectieuse. Inter J Innov Appl Stud. 2014;8(4):1418-1431.

45. Viuda-Martos M, Ruiz-Navajas Y, Fernandez-Lopez J, Perez-Alvarez J. Antifungal activity of lemon (Citrus lemon L.), mandarin (Citrus reticulata L.), grapefruit (Citrus paradisi L.) and orange (Citrus sinensis L.) essential oils. Food Control. 2008;19,1130–8.

© 2021 Hajlaoui et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/81551