Yield, Phytochemical Constituents, and Antibacterial Activity of Essential Oils from the Leaves/Twigs, Branches, Branch Wood, and Branch Bark of Sour Orange (Citrus aurantium L.)

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Abstract: In the present work, essential oils (EOs) extracted from different parts of sour orange Citrus aurantium (green leaves/twigs, small branches, wooden branches, and branch bark) were studied through gas chromatography coupled with mass spectrometry (GC/MS). Furthermore, the EOs in the amounts of 5, 10, 15, 20, and 25 µL were studied for their antibacterial activity against three pathogenic bacteria, Agrobacterium tumefaciens, Dickeya solani, and Erwinia amylovora. The main EO compounds in the leaves/twigs were 4-terpineol (22.59%), D-limonene (16.67%), 4-carvomenthene (12.84%), and linalool (7.82%). In small green branches, they were D-limonene (71.57%), dodecane (4.80%), oleic acid (2.72%), and trans-palmitoleic acid (2.62%), while in branch bark were D-limonene (54.61%), γ-terpinene (6.68%), dodecane (5.73%), and dimethyl anthranilate (3.13%), and in branch wood were D-limonene (38.13%), dimethyl anthranilate (8.13%), (−)-β-fenchol (6.83%), and dodecane (5.31%). At 25 µL, the EO from branches showed the highest activity against A. tumefaciens (IZ value of 17.66 mm), and leaves/twigs EO against D. solani and E. amylovora had an IZ value of 17.33 mm. It could be concluded for the first time that the wood and branch bark of C. aurantium are a source of phytochemicals, with D-limonene being the predominant compound in the EO, with potential antibacterial activities. The compounds identified in all the studied parts might be appropriate for many applications, such as antimicrobial agents, cosmetics, and pharmaceuticals.

Keywords: GC–MS; hydrodistillation; antibacterial activity; clevenger; Citrus aurantium; phytochemical; essential oils
1. Introduction

Natural extracts and essential oils (EOs) extracted from aromatic and indigenous plants have a broad spectrum of biological activities such as antibacterial, antifungal, antioxidant, anticancer [1–8]. EOs from Citrus spp., especially from peels, have been studied extensively in many research projects over the past few decades [9–11]. They have exhibited bioactivity potentials against the growth of pathogenic bacteria, fungi, and insects [12,13]. The main chemical compounds identified in the EOs from Citrus were limonene, α-pinene, β-pinene, citral, linalool, myrcene, γ-terpinene, eugenol methyl ether, neral, geranial, neryl acetate, and β-caryophyllene [14–18]. The Citrus plants have many biological and aromatic properties because of the occurrence of EOs, alkaloids, glycosides, flavonoids, tannins, and other compounds in its various parts [19,20].

*Citrus aurantium* L. (Rutaceae), known as sour or bitter orange, is extensively consumed in Mediterranean countries as marmalade and a flavoring agent [21]. The extracted oils have been recognized as safe for their wide uses as antibacterial, antifungal antioxidant, anti-inflammatory, and anxiolytic effects [22–25], and have analgesic activity [26].

Limonene was determined as the main component of bitter orange peel EO, followed by β-myrcene, linalool, β-pinene, and α-pinene [27]. The major compound in Tunisian neroli EO extracted from *C. aurantium* blossoms is 25.7% linalool [28]. The (R)-(-)-linalool was 59–64% in *Citrus* (south and south-central Brazil), whereas the hydrolate (orange water) of *C. aurantium* has nootkatone (17%), α-terpineol (10%), linalool (10%), and limonene (0.8%) [29].

At maturity, limonene exhibited the highest level, with several minor compounds, including linalool, myrcene, and α-terpinene, in the EOs from bitter orange peel [30]. Limonene (92–95%) with linalool and linalyl acetate (together 0.3–3.2%) were identified in the EOs from living (fruits that are still on the tree) bitter orange peel [31]. Shen et al. [32] showed the anti-inflammatory potential of EO from blossoms of *C. aurantium* L. var. *amara* Engl with major constituents of linalool, α-terpineol, (R)-limonene, and linalyl acetate [32]. *C. aurantium* zest EO is composed of limonene (85.22%), β-myrcene, and α-pinene as the main compounds [13]. EO of sweet orange zest consisted of limonene as the main compound, followed by myrcene, α-farnesene, and γ-terpinene [33,34], whereas the EO of sweet orange zest from Uganda and Rwanda contained limonene, myrcene, α-pinene, and linalool [35]. Using the hydrodistillation method, the linalool and terpenes were found to be the main compounds in Neroli blossom EO, whereas, in water recovered oils, linalool, linalyl acetate, geraniol, α-terpineol, and neral were the main compounds [36]. In flowers, the oil showed the presence of camphor, thymol, linalool, carvacrol, and borneol as main compounds with significant anti-oxidant effect [37].

The goal of the present work was to identify the aromatic chemical profile and antibacterial activity of the EOs from different parts of *C. aurantium* that could be suitable for different industrial purposes.

2. Materials and Methods

2.1. Plant Material of *C. aurantium*

Fresh branches of *C. aurantium* were collected in 2019, from Alexandria, Egypt, during pruning process for the trees. The resultant materials were separated to leaves/twigs, small green branches, branch wood, and branch bark. The wood and bark of branches were separated. All the materials were washed with tape water to remove the dust, then cut to small pieces by using scissors to facilitate the extraction process of essential oils (EOs).

2.2. Extraction of EOs

Approximately 100 g from each of leaves/twigs, branches, the wood of branches, and branch bark from *C. aurantium* were soaked in 2 L flasks with 1500 mL of water and hydrodistilled for 3 h in a Clevenger-type apparatus [38]. The distillates of the EOs were dried over anhydrous Na$_2$SO$_4$, filtrated, and measured with respect to the mass of fresh weight of raw material (Table 1). The EOs
from leaves/twigs (Petitgrain), branches (2–4 cm in diameter), the wood of branches, and branch bark were kept dry in sealed Eppendorf tubes and stored at 4 °C prior to chemical analyses.

Table 1. Oil yield from different parts of *Citrus aurantium*.

| Part Used          | Oil Yield (mL/100 g Material) |
|--------------------|-------------------------------|
| Leaves/twigs       | 3.45                          |
| Branches           | 1.55                          |
| Wood of branches   | 1.15                          |
| Branch bark        | 1.10                          |

2.3. Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

The chemical composition of the essential oils was determined using a Trace GC Ultra-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m × 0.25 mm × 0.25 µm film thickness). Initially, the column oven temperature was held at 45 °C, then increased by 5 °C/min to 250 °C and held for 2 min, then increased to 280 °C by 10 °C/min. The injector and MS transfer line temperatures were kept at 250 °C. Helium was used as a carrier gas at a constant flow rate of 1 mL/min. The solvent delay was 2 min and diluted samples of 1 µL were injected automatically using an Autosampler AS1310 coupled with the GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over a range of m/z of 40–600 in full scan mode. The ion source was set at 200 °C. Identification of the constituents was performed on the basis of their retention times and by comparing the mass spectra with those found in the library search (NIST and Wiley) [39]. Type threshold values contained in Xcalibur 3.0 data system of GC/MS were used as match factors and to confirm that all mass spectra are appended to the library with measuring the Standard Index (SI) and Reverse Standard Index (RSI), where the value ≥650 is acceptable to confirm the compounds [40].

2.4. Antibacterial Activity

Antibacterial evaluation of the EOs was assayed against three phytopathogenic bacteria, *Agrobacterium tumefaciens*, *Dickeya solani*, and *Erwinia amylovora* (Microbiology Laboratory, Agricultural Botany Department, Faculty of Agriculture (Saba Basha), Alexandria University, Egypt). The antibacterial evaluation test of the studied four EOs was performed by measuring the inhibition zones (IZs) in millimeters around the loaded filter papers with different amounts of oils (5, 10, 15, 20, and 25 µL) using disc diffusion method [40,41]. Sterile filter paper discs (Whatman filter paper no. 1) with a diameter of 4 mm loaded with different amounts of the studied EOs were placed on the surface of prepared agar plates. All the plates were incubated in incubator at 30 °C for 24 h. Negative control discs were left without any EO. All of the tests were performed in triplicate and the values of the IZs (the clear zones with no bacterial growth around the loaded discs) were reported including the diameter of the disc.

2.5. Statistical Analysis

Values of the bacteria’s inhibition zones were statistically analyzed with analysis of variance (ANOVA) in completely randomized design with two factors (oil type and oil amount) using a computer program, Statistical Analysis System [42], and compared with those of the control. Means were compared with L.S.D. test at \( p < 0.05 \) levels.
3. Results

3.1. Chemical Composition of the EOs

Table 2 presents the chemical composition of EOs from *C. aurantium* green leaves/twigs. The main compounds were 4-terpineol (22.59%), D-limonene (16.67%), 4-carvomenthenol (12.84%), linalool (7.82%), methyl methanthranilate (4.41%), *cis*-4-thujanol (3.72%), *γ*-terpinene (3.58%), tetraneurin-α-diol (2.61%), 6,9,12,15-docosatetraenoic acid methyl ester (2.48%), and linalyl acetate (2.28%).

| Compound                  | Relative Quantity (%) | Molecular Formula | Molecular Weight (g/mol) | SI 1 | RSI 2 |
|---------------------------|-----------------------|-------------------|--------------------------|------|-------|
| Myrcene                   | 0.30                  | C10H16             | 136                      | 803  | 833   |
| β-Pinene                  | 1.21                  | C10H16             | 136                      | 804  | 862   |
| α-Limonene                | 16.67                 | C10H16             | 136                      | 934  | 936   |
| 2-Carene epoxide          | 0.45                  | C11H24             | 156                      | 863  | 920   |
| Undecane                  | 0.92                  | C11H16             | 136                      | 927  | 938   |
| *γ*-Terpinene             | 3.58                  | C10H16             | 136                      |      |       |
| *cis*-4-Thujanol           | 3.72                  | C10H18             | 154                      | 936  | 947   |
| Octadecyl vinyl ether     | 0.76                  | C20H36             | 296                      | 760  | 766   |
| 4-Terpineol               | 22.59                 | C10H18             | 154                      | 961  | 966   |
| Dodecane                  | 1.59                  | C12H26             | 170                      | 883  | 883   |
| *cis*-para-2-Menthen-1-ol | 0.71                  | C10H18             | 154                      | 847  | 886   |
| *trans*,*trans*-5-Caranol | 0.52                  | C10H18             | 154                      | 772  | 841   |
| 2,6,10-Trimethyltetradecane| 0.56                 | C17H36             | 240                      | 768  | 795   |
| 4-Carvomenthenol          | 12.84                 | C10H18             | 154                      | 932  | 943   |
| Linalool                  | 7.82                  | C20H18             | 154                      | 839  | 861   |
| 5,9-Dimethyl-4,8-decadienal| 0.42              | C12H2O             | 180                      | 770  | 805   |
| Linalyl acetate           | 2.28                  | C13H20O2           | 196                      | 825  | 888   |
| *α*-Terpineol             | 0.96                  | C10H18             | 154                      | 762  | 790   |
| Vitamin A aldehyde (Retinal)| 0.32                | C20H32O            | 284                      | 704  | 807   |
| Ascaridol                 | 0.97                  | C10H16O2           | 168                      | 765  | 850   |
| 4,7-Octadecadienoic acid methyl ester | 0.48 | C10H32O2 | 290 | 691 | 712 |
| Arachidonic acid methyl ester | 0.54       | C20H32O3 | 318 | 740 | 777 |
| Thymol                    | 0.90                  | C10H14O            | 150                      | 774  | 864   |
| 6,9,12-Octadecatrienoic acid methyl ester | 0.53 | C10H32O2 | 292 | 719 | 764 |
| 2-(7-Heptadecen-2-yl) tetrahydro-2H-pyran (Z)-Pseudosolasodine diacetate | 0.83 | C31H46NO4 | 499 | 680 | 717 |
| Methyl methanthranilate   | 4.41                  | C9H14NO2           | 165                      | 819  | 929   |
| 3′,4′,7-Trimethylquercetin| 0.41                  | C15H16O7           | 344                      | 661  | 690   |
| 2-[4-Methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde | 1.38 | C23H32O | 324 | 717 | 761 |
| Ethyl iso-allocholate      | 0.61                  | C20H44O3           | 436                      | 717  | 744   |
| Oleic acid                | 0.87                  | C18H34O2           | 282                      | 685  | 754   |
| 6,9,12,15-Docosatetraenoic acid methyl ester | 2.48 | C23H38O2 | 346 | 713 | 797 |
| Tetraneurin-α-diol        | 2.61                  | C15H20O3           | 280                      | 697  | 786   |

Table 3 shows the chemical composition of EOs from *C. aurantium* small green branches. The main compounds in small branches were D-limonene (71.57%), dodecane (4.80%), oleic acid (2.72%), *trans*-palmitoleic acid (2.62%), undecane (2.28%), 1-nonadecanol (2.11%), *γ*-terpinene (1.97%), 4-terpineol (2.13%), and *α*-terpineol (1.04%).
Table 3. Chemical composition of essential oil from *Citrus aurantium* small branches.

| Compound                  | Relative Quantity (%) | Molecular Formula | Molecular Weight (g/mol) | SI ¹ | RSI ² |
|---------------------------|-----------------------|-------------------|--------------------------|------|-------|
| α-Pinene                  | 0.52                  | C₁₀H₁₆             | 136                      | 873  | 934   |
| Decane                    | 0.72                  | C₁₀H₂₂             | 142                      | 859  | 937   |
| Myrcene                   | 1.08                  | C₁₀H₁₆             | 136                      | 819  | 836   |
| 2-Methyldecan-1-ol        | 0.46                  | C₁₃H₂₈O            | 200                      | 788  | 835   |
| n-Limonene                | 71.57                 | C₁₀H₁₆             | 136                      | 940  | 941   |
| (E)-2,3-Epoxycarane       | 0.49                  | C₁₀H₁₆O           | 152                      | 759  | 817   |
| Undecane                  | 2.28                  | C₁₁H₂₄             | 156                      | 928  | 950   |
| γ-Terpinene               | 1.97                  | C₁₀H₁₆             | 136                      | 878  | 910   |
| Myristyl alcohol          | 0.57                  | C₁₄H₃₀O           | 214                      | 774  | 777   |
| 1-Nonadecanol             | 2.11                  | C₁₉H₄₀O           | 284                      | 766  | 775   |
| 4-Terpineol               | 2.13                  | C₁₀H₁₈O           | 154                      | 897  | 942   |
| Dodecane                  | 4.80                  | C₁₂H₂₆             | 170                      | 919  | 934   |
| Tetradecane               | 0.84                  | C₁₄H₃₀             | 198                      | 780  | 788   |
| α-Terpinol                | 1.04                  | C₁₀H₁₈O           | 154                      | 832  | 880   |
| 3,6-Octadecadienoic acid  | 0.49                  | C₁₉H₃₄O₂          | 294                      | 729  | 777   |
| methyl ester              |                       |                   |                          |      |       |
| Octahydro-1,2,4-metheno-1H-| 0.46                  | C₁₀H₁₂O₂           | 164                      | 712  | 778   |
| cyclobuta[c]pentalen-3,5-diol|                   |                   |                          |      |       |
| cis-Z-α-Bisabolene epoxide| 0.96                  | C₁₅H₂₄O           | 220                      | 735  | 759   |
| Oleic acid                | 2.72                  | C₁₆H₃₄O₂           | 282                      | 762  | 781   |
| Arachidonic acid methyl   | 0.82                  | C₂₁H₴₄O₂           | 318                      | 753  | 815   |
| ester                     |                       |                   |                          |      |       |
| (E)-Acrylic acid,         | 0.66                  | C₁₂H₁₆O₄           | 224                      | 604  | 688   |
| 3-(3-methoxycarbonyl-1-cyclohexen-4-yl)-methylester |   |                   |                          |      |       |
| trans-Palmitoleic acid    | 2.62                  | C₁₆H₃₀O₂           | 254                      | 760  | 807   |
| Ethyl iso-allocholate     | 0.66                  | C₂₆H₄₄O₅           | 436                      | 743  | 772   |

¹ SI: Standard Index. ² RSI: Reverse Standard Index.

The chemical constituents of *C. aurantium* branch bark is shown in Table 4. The main components were D-limonene (54.61%), γ-terpinene (6.68%), dodecane (5.73%), dimethyl anthranilate (3.13%), undecane (3.00%), tetradecyloxirane (2.08%), ethyl iso-allocholate (1.96%), 4-terpineol (1.59%), myrcene (1.53%), and 1,3-diolein (1.52%).

Table 4. Chemical composition of essential oil from *Citrus aurantium* branch bark.

| Compound                  | Relative Quantity (%) | Molecular Formula | Molecular Weight (g/mol) | SI ² | RSI ¹ |
|---------------------------|-----------------------|-------------------|--------------------------|------|-------|
| α-Pinene                  | 1.28                  | C₁₀H₁₆             | 136                      | 884  | 938   |
| Decane                    | 1.27                  | C₁₀H₂₂             | 142                      | 817  | 929   |
| Myrcene                   | 1.53                  | C₁₀H₁₆             | 136                      | 812  | 841   |
| β-Pinene                  | 1.38                  | C₁₀H₁₆             | 136                      | 855  | 899   |
| 2,7-Dimethyl-2,6-octadien-1-ol| 0.45                  | C₁₀H₁₈O           | 154                      | 703  | 740   |
| 1-Decene                  | 0.52                  | C₁₀H₂₀             | 140                      | 765  | 786   |
| 1-Tetradecanol            | 0.66                  | C₁₄H₃₀O           | 214                      | 770  | 776   |
| n-Limonene                | 54.61                 | C₁₀H₁₆             | 136                      | 938  | 940   |
| (E)-2,3-Epoxycarane       | 0.96                  | C₁₀H₁₆O           | 152                      | 774  | 829   |
| Undecane                  | 3.00                  | C₁₁H₂₄             | 156                      | 894  | 930   |
| γ-Terpinene               | 6.68                  | C₁₀H₁₆             | 136                      | 908  | 945   |
| cis-p-2-Menthen-1-ol      | 0.41                  | C₁₀H₁₈O           | 154                      | 754  | 822   |
| Hexahydrofarnesol         | 1.2                   | C₁₅H₃₂O           | 228                      | 750  | 740   |
| Tetradecyloxirane         | 2.08                  | C₁₆H₃₂O           | 240                      | 743  | 809   |
Table 4. Cont.

| Compound                        | Relative Quantity (%) | Molecular Formula | Molecular Weight (g/mol) | SI | RSI |
|---------------------------------|-----------------------|-------------------|--------------------------|----|-----|
| 4-Terpineol                     | 1.59                  | C_{10}H_{18}O     | 154                      |    |     |
| Dodecane                        | 5.73                  | C_{12}H_{26}      | 170                      | 893| 923 |
| 2,6,10-Trimethyltetradecane      | 1.17                  | C_{17}H_{36}      | 240                      | 754| 782 |
| 4-Carvomenthol                  | 1.20                  | C_{10}H_{18}O     | 154                      | 782| 800 |
| α-Terpineol                     | 1.15                  | C_{10}H_{18}O     | 154                      | 825| 884 |
| Methyl hexadecanoate            | 0.41                  | C_{17}H_{30}O     | 266                      | 716| 723 |
| trans-(Z)-α-Bisabolene epoxide  | 0.61                  | C_{15}H_{24}O     | 220                      | 729| 801 |
| 4,7-Octadecadienoic acid, methyl ester | 0.61          | C_{16}H_{30}O     | 290                      | 707| 730 |

Table 4 shows the chemical compounds identified in C. aurantium branch wood. The main compounds in the EO were D-limonene (38.13%), dimethyl anthranilate (8.13%), (-)-β-fenchol (6.83%), dodecane (5.31%), 4-carvomenthol (4.21%), γ-terpinene (3.62%), cis-4-thujanol (3.49%), thymol (3.30%), valencene (3.30%), linalool (2.94%), 6,7-dihydrogeraniol (2.15%), and undecane (2.13%).

Table 5. Chemical composition of essential oil from Citrus aurantium branch wood.

| Compound                        | Relative Quantity (%) | Molecular Formula | Molecular Weight (g/mol) | SI | RSI |
|---------------------------------|-----------------------|-------------------|--------------------------|----|-----|
| α-Pinene                        | 1.50                  | C_{10}H_{16}      | 136                      | 941| 948 |
| Decane                          | 0.65                  | C_{10}H_{22}      | 142                      | 880| 939 |
| Myrcene                         | 0.96                  | C_{10}H_{16}      | 136                      | 837| 906 |
| β-Pinene                        | 1.54                  | C_{10}H_{16}      | 136                      | 909| 939 |
| D-Limonene                      | 38.13                 | C_{10}H_{16}      | 136                      | 940| 941 |
| p-Cymene                        | 0.72                  | C_{10}H_{14}      | 134                      | 805| 823 |
| Undecane                        | 2.13                  | C_{11}H_{24}      | 156                      | 934| 951 |
| γ-Terpinene                     | 3.62                  | C_{10}H_{16}      | 136                      | 901| 935 |
| 4-Terpineol                     | 0.95                  | C_{10}H_{18}O     | 154                      | 866| 906 |
| 1-Dodecanol                     | 0.54                  | C_{12}H_{26}O     | 186                      | 769| 798 |
| 1-Eicosanol                     | 1.69                  | C_{20}H_{42}O     | 298                      | 769| 776 |
| Linalool                        | 2.94                  | C_{10}H_{18}O     | 154                      | 873| 898 |
| cis-4-Thujanol                   | 3.49                  | C_{10}H_{18}O     | 154                      | 933| 945 |
| Dodecane                        | 5.31                  | C_{12}H_{26}      | 170                      | 926| 939 |
| 7-Methyl pentadecane            | 1.12                  | C_{16}H_{34}      | 226                      | 850| 885 |
| 4-Carvomenthol                  | 4.21                  | C_{10}H_{18}O     | 154                      | 898| 907 |
| Capraldehyde                    | 0.93                  | C_{10}H_{20}O     | 156                      | 823| 885 |
| (-)-β-Fenchol                   | 6.83                  | C_{10}H_{18}O     | 154                      | 932| 937 |
| 6,7-Dihydrogeraniol             | 2.15                  | C_{10}H_{20}O     | 156                      | 886| 897 |
| β-Citrylidenethanol             | 0.45                  | C_{12}H_{20}O     | 180                      | 730| 742 |
| trans-Carveol                   | 0.83                  | C_{10}H_{18}O     | 152                      | 820| 864 |
| (Z)-Citral                      | 1.42                  | C_{10}H_{16}O     | 152                      | 782| 830 |
The GC–MS chromatograms of the identified compounds of EOs from the studied different parts of C. aurantium are shown in Figure 1.

### Table 5. Cont.

| Compound                  | Relative Quantity (%) | Molecular Formula | Molecular Weight (g/mol) | SI ¹ | RSI ² |
|---------------------------|-----------------------|-------------------|--------------------------|------|------|
| 6-Methyltetraline         | 0.57                  | C₁₁H₁₄             | 146                      | 777  | 841  |
| Dihydro cuminyl alcohol   | 0.91                  | C₁₀H₁₆O            | 152                      | 805  | 854  |
| Thymol                    | 3.30                  | C₁₀H₁₄O            | 150                      | 904  | 917  |
| Farnesol                  | 1.05                  | C₁₅H₂₆O            | 222                      | 801  | 813  |
| Nerolidyl acetate         | 0.66                  | C₁₇H₂₆O₂           | 264                      | 805  | 825  |
| Valencene                 | 3.30                  | C₁₅H₂₄             | 204                      | 931  | 958  |
| Dimethyl anthranilate     | 8.13                  | C₉H₁₁NO₂           | 165                      | 909  | 940  |

¹ SI: Standard Index. ² RSI: Reverse Standard Index.
3.2. Antibacterial Activity of the EOs

From the main effects of the extracted oils from different parts of *C. aurantium* (Figure 2a), oil from leaves/twigs showed the highest activity against all the studied three phytopathogenic bacteria. The main effects of oil amount from all the studied plant parts (Figure 2b) showed that increasing the amount of oil (µL) also increased the antibacterial activity, as measured by the inhibition zone (IZ).

Table 6 presents the antibacterial activity of the studied EOs from different parts of *C. aurantium*. The highest activity against the growth of *A. tumefaciens* was observed by the application of EO from branches at 25 µL (IZ value of 17.66 mm), followed by oil from leaves/twigs at 20 and 25 µL with IZ value of 15.66 mm. On the other hand, EOs from bark and branch wood did not show any activity against *A. tumefaciens*. At 25 µL of leaves/twigs EO, the highest activity (17.33 mm) against *D. solani* was reported, followed by the application of branch EO at 25 µL (16.66 mm) and 20 µL (16.66 mm).

For the antibacterial activity of EOs against the growth of *E. amylovora* at oil amount of 20 and 25 µL from leaves/twigs, the highest IZ value was observed (17.33 mm), followed by branch EO at 25 µL with IZ value of 15.33 mm. Also, the EO from leaves/twigs at 10 and 15 µL showed good activity against *E. amylovora* with IZ value of 15.00 mm.
against *A. tumefaciens*. At 25 µL of leaves/twigs EO, the highest activity (17.33 mm) against *D. solani* was reported, followed by the application of branch EO at 25 µL (16.66 mm) and 20 µL (16.66 mm).

For the antibacterial activity of EOs against the growth of *E. amylovora* at oil amount of 20 and 25 µL from leaves/twigs, the highest IZ value was observed (17.33 mm), followed by branch EO at 25 µL with IZ value of 15.33 mm. Also, the EO from leaves/twigs at 10 and 15 µL showed good activity against *E. amylovora* with IZ value of 15.00 mm.

Figure 2. The main effects of oils from different parts of *C. aurantium* (a) and their amounts (b) on the growth of *A. tumefaciens*, *D. solani*, and *E. amylovora*. 

![Graph showing inhibition zones for *A. tumefaciens*, *D. solani*, and *E. amylovora* against extracts from different parts of *C. aurantium* and different oil amounts.](image-url)
Table 6. Antibacterial activity of essential oils from C. aurantium against three phytopathogenic bacteria.

| Extracted Oil | Oil Amount (µL) | Inhibition Zone Values (mm) | A. tumefaciens | D. solani | E. amylovora |
|---------------|----------------|-----------------------------|----------------|-----------|--------------|
| Leaves/twigs  | 0              | 0.00                        | 0.00           | 0.00      | 0.00         |
|               | 5              | 0.00                        | 9.33 ± 0.57    | 12.66 ± 0.57 |
|               | 10             | 10.00 ± 0.00                | 14.66 ± 0.57   | 15.00 ± 0.00 |
|               | 15             | 11.66 ± 0.57                | 15.00 ± 0.00   | 15.00 ± 0.00 |
|               | 20             | 15.66 ± 0.57                | 16.66 ± 0.57   | 17.33 ± 0.57 |
|               | 25             | 15.66 ± 0.57                | 17.33 ± 0.57   | 17.33 ± 0.57 |
| Branches      | 0              | 0.00                        | 0.00           | 0.00      | 0.00         |
|               | 5              | 0.00                        | 11.33 ± 0.57   | 12.00 ± 0.00 |
|               | 10             | 6.00 ± 0.00                 | 11.33 ± 0.57   | 12.00 ± 0.00 |
|               | 15             | 10.00 ± 0.00                | 14.33 ± 0.57   | 12.33 ± 0.57 |
|               | 20             | 10.00 ± 0.00                | 16.66 ± 1.52   | 14.66 ± 0.57 |
|               | 25             | 17.66 ± 0.57                | 16.66 ± 0.57   | 15.33 ± 0.57 |
| Branch bark   | 0              | 0.00                        | 0.00           | 0.00      | 0.00         |
|               | 5              | 0.00                        | 0.00           | 6.00 ± 0.00 |
|               | 10             | 0.00                        | 0.00           | 10.00 ± 0.00 |
|               | 15             | 0.00                        | 2.00 ± 3.46    | 10.00 ± 0.00 |
|               | 20             | 0.00                        | 7.66 ± 0.57    | 11.66 ± 0.57 |
|               | 25             | 0.00                        | 9.66 ± 0.57    | 12.33 ± 0.57 |
| Branch wood   | 0              | 0.00                        | 0.00           | 0.00      | 0.00         |
|               | 5              | 0.00                        | 0.00           | 6.00 ± 0.00 |
|               | 10             | 0.00                        | 10.00 ± 0.00   | 6.33 ± 0.57 |
|               | 15             | 0.00                        | 10.66 ± 0.57   | 10.00 ± 0.00 |
|               | 20             | 0.00                        | 10.66 ± 0.57   | 11.33 ± 0.57 |
|               | 25             | 0.00                        | 13.66 ± 0.57   | 12.00 ± 0.00 |

p-value < 0.0001 < 0.0001 < 0.0001

4. Discussion

The results of the present work showed the variation in the chemical composition of the EOs from different parts of C. aurantium. Most previous studies have focused on the identification of chemical composition of EOs from the peels, pericarp, blossoms, and leaves, and no core results have been reported from branches, wood, or bark. Additionally, the trials of antimicrobial activities of the EOs were measured against human pathogenic bacteria and plant pathogenic fungi, with no results about the activity against plant bacterial pathogens.

4-terpineol (22.59%) and D-limonene (16.67%) were the most predominate components abundant in green leaves/twigs of C. aurantium, while D-limonene with percentages of 71.57%, 54.61%, and 38.13% was found in small green branches, branch bark, and branch wood, respectively. Results from Wolffenbuttel et al. [29] showed that limonene (39.5–92.7%) and linalool (14.2–24.8%) are the main components of the pericarp and leaves, respectively, of citrus oils obtained by steam distillation, hydrodistillation, or cold press extraction. Linalyl acetate, linalool, a-terpineol, geranyl acetate, geraniol, and geranial as oxygenated monoterpene hydrocarbons were primarily identified in petitgrain oil of C. aurantium var. amara [12], whereas limonene was present only at a concentration of 1.4%.
Terpinen-4-ol, α-pinene, β-pinene, 1,8-cyneol, linalool, and 4-terpineol and their mixture have been shown to have potent antifungal activity [12,43,44]. The most abundant compounds in Tunisian oil were linalool with lower amounts of linalyl acetate and α-terpinol [45]. Algerian C. aurantium leaf EO showed linalool, γ-terpinene, and α-terpineol with percentages of 18.6, 6.9, and 15.1%, respectively, while in peel EO were linalool, cis-linalool oxide, trans-carveol, endo-fenchyl acetate, and carvone with percentages of 12, 8.1, 11.9, 5.5, and 5.8%, respectively [46]. Previously, α-terpineol from *Cinnamomum longepaniculatum* decreased cell size and irregular cell shape, cell wall, and membrane of *E. coli* [47]. α-terpineol, terpinen-4-ol, terpinolene, and α-terpineol had strong antibacterial activities against *Propionibacterium acnes* and *Staphylococcus aureus* [48].

Linalool acetate was present in Sicilian petitgrain oil with a lower amount of linalool [49]. Linalyl acetate and linalool were the main components in petitgrain oil from Turkey [50]. EOs of the peels, flowers, and leaves from *C. aurantium*, collected from northern Greece, exhibited the primary compounds linalool (29.14%), β-pinene (19.08%), *trans*-β-ocimene (6.06%), and *trans*-farnesol (5.14%) [51]. The EOs from blossoms of *C. aurantium* growing in the Darab region in Fars Province, Iran, showed that geraniol, α-terpineol, linalool, and benzene acetaldehyde were the main compounds [52]. Myrcene was found in low percentage of the present work and previously it was reported that myrcene, which found in the EO, is known to possess cytotoxic activity [53,54]. DI-limonene with 94.81% is the main compound identified in peel EO from C. aurantium with promising larvicidal against Anopheles stephensi [9]. Limonene, (E)-nerolidol, α-terpineol, α-terpinyl acetate, and (E,E)-farnesol were the main compounds in the flower EO of *C. aurantium* with good antibacterial activity against *Pseudomonas aeruginosa* [10]. α-terpineol and terpinene-4-ol, found in the leaf EO from *C. hystrix*, were more active against *Acinetobacter baumannii*, *Streptococcus* spp., and *Haemophilus influenzae* than crude oil, while limonene, the most abundant component of *C. hystrix* oil, had lower antibacterial activity [55].

Zest EO had limonene (85.22%), β-myrcene (4.3%), and α-pinene (1.29%) as the main components, and the EO showed higher antioxidant activity than did limonene alone with a potential for antibacterial activity against *Staphylococcus aureus*, *Salmonella* sp., *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Escherichia coli* [13]. Among 34 kinds of citrus EOs, four EOs from *C. aurantium* zest presented good antioxidant activities, as measured by a DPPH assay [16]. Strong fungicidal activity was exhibited by limonene and (E)-nerolidol present in the EO of the flowers of *C. aurantium* L. var. amara [56].

Considering that limonene is the major compound of the EO of *Citrus*, this compound has good antioxidant properties [57]. Additionally, other compounds, such as linalool and bornol, have antitumor effects; sabine and pinene have anti-inflammatory activity; and citral exhibits analgesic functions [58–62].

Although cis-β-terpineol, D-limonene, 4-carvomenthol, and linalool were the main compounds in petitgrain EO in the present study, the compounds of linalyl acetate, linalool, α-terpineol, and geranyl acetate [12,18,63] were the main compounds in petitgrain EO, which exhibited good antibacterial and antifungal activity, especially against *Bacillus subtilis*, *Aspergillus niger*, and *Penicillium expansum*, whereas the weakest fungicidal effects were observed for *Candida krusei* [12]. A mixture of terpenoid containing terpine-4-ol and linalool exhibited high antifungal activity against *Trichophyton mentagrophytes*, *T. rubrum*, *Microsporum gypseum*, *A. niger*, and *A. flavus* [43].

Limonene, linalool, citronellal, and citronellol were the main constituents of EO from *C. aurantifolia* leaves and fruit peels and exhibited promising antibacterial activity against oral pathogenic bacteria *Streptococcus mutans* and *Lactobacillus casei* [64].

Leaves EO of *C. aurantium* grown in Shiraz (south of Iran) showed the presence of limonene, linalool, and *trans*-β-ocimene as major components and exhibited strong antioxidant activity [65]. EO obtained by cold pressing of *C. aurantium* fruits with high percentage of limonene (77.90%) and minor percentages of β-pinene (3.40%) and myrcene (1.81%) was inactive against *Escherichia coli* and *Pseudomonas*, while moderately active against *Staphylococcus aureus* [66]. Limonene from linalool-rich essential oil inhibits *S. aureus* [67].

The variations in the chemical composition of the EOs could be explained by various extraction processes and plant parts used. Furthermore, they are affected by various soils and climatic characteristics.
of the regions where the *C. aurantium* trees grow [36,45,68–71]. For example, the ranges of linalool acetate, linalool, farnesol, nerolidol, and geranyl acetate at 12.2–28.9%, 22.9–54%, 0.2–10.4%, 0.4–21.4%, and 0.97–9.3%, respectively, in *C. aurantium* blossom EO were observed by seven different methods of oil extraction [71].

5. Conclusions

In the present study, variations in essential oils composition from different parts of *C. aurantium* were reported. 4-terpineol, followed by D-limonene, were the main constituents in EO from the leaves/twigs, while D-limonene was the main constituent in small green branches, the branch wood, and the branch bark. EOs from leaves and small branches promised to be potential antibacterial activates against *Agrobacterium tumefaciens*, *Dickeya solani*, and *Erwinia amylovora*. The EOs obtained from different parts of *C. aurantium* displayed bioactive compounds, which have the potential for application as biopreservative agents, antioxidants, antimicrobial compounds, cosmetics, and pharmaceuticals.

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