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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Keywords: clevenger, Citrus aurantium, phytochemical, essential oils, antibacterial activity, hydrodistillation, GC–MS

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-carvomenthenol (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%), ?-terpinene (6.68%), dodecane (5.73%), and dimethyl anthranilate (3.13%), and in branch wood were D-limonene (38.13%), dimethyl anthranilate (8.13%), (-)-?-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.

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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-carvomenthenol (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-terpineol, 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 nerol 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 hydrodistillated for 3 h in a Clevenger-type apparatus [38]. The distillates of the EOs were dried over anhydrous Na_2SO_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. Statsitcal 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%).

Table 2. Chemical composition of essential oils from *Citrus aurantium* green leaves and twigs.

Compound	Relative Quantity (%)	Molecular Formula	Molecular Weight (g/mol)	SI [1]	RSI [2]
Myrcene	0.30	$C_{10}H_{16}$	136	803	833
β-Pinene	1.21	$C_{10}H_{16}$	136	804	862
D-Limonene	16.67	$C_{10}H_{16}$	136	934	936
2-Carene epoxide	0.45	$C_{10}H_{16}O$	152	793	842
Undecane	0.92	$C_{11}H_{24}$	156	863	920
γ-Terpinene	3.58	$C_{10}H_{16}$	136	927	938
cis-4-Thujanol	3.72	$C_{10}H_{18}O$	154	936	947
Octadecyl vinyl ether	0.76	$C_{20}H_{40}O$	296	760	766
4-Terpineol	22.59	$C_{10}H_{18}O$	154	961	966
Dodecane	1.59	$C_{12}H_{26}$	170	883	883
cis-para-2-Menthen-1-ol	0.71	$C_{10}H_{18}O$	154	847	886
trans,*trans*-(+)-5-Caranol	0.52	$C_{10}H_{18}O$	154	772	841
2,6,10-Trimethyltetradecane	0.56	$C_{17}H_{36}$	240	768	795
4-Carvomenthenol	12.84	$C_{10}H_{18}O$	154	932	943
Linalool	7.82	$C_{10}H_{18}O$	154	839	861
5,9-Dimethyl-4,8-decadienal	0.42	$C_{12}H_{20}O$	180	770	805
Linalyl acetate	2.28	$C_{12}H_{20}O_2$	196	825	888
α-Terpineol	0.96	$C_{10}H_{18}O$	154	762	790
Vitamin A aldehyde (Retinal)	0.32	$C_{20}H_{28}O$	284	704	807
Ascaridol	0.97	$C_{10}H_{16}O_2$	168	765	850
4,7-Octadecadienoic acid methyl ester	0.48	$C_{19}H_{30}O_2$	290	691	712
Arachidonic acid methyl ester	0.54	$C_{21}H_{34}O_2$	318	740	777
Thymol	0.90	$C_{10}H_{14}O$	150	774	864
6,9,12-Octadecatrienoic acid methyl ester	0.53	$C_{19}H_{32}O_2$	292	719	764
2-(7-Heptadecynyloxy) tetrahydro-2H-pyran	0.83	$C_{22}H_{40}O_2$	336	714	749
(Z)-Pseudosolasodine diacetate	0.94	$C_{31}H_{49}NO_4$	499	680	717
Methyl methanthranilate	4.41	$C_9H_{11}NO_2$	165	819	929
3′,4′,7-Trimethylquercetin	0.41	$C_{18}H_{16}O_7$	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	$C_{23}H_{32}O$	324	717	761
Ethyl iso-allocholate	0.61	$C_{26}H_{44}O_5$	436	717	744
Oleic acid	0.87	$C_{18}H_{34}O_2$	282	685	754
6,9,12,15-Docosatetraenoic acid methyl ester	2.48	$C_{23}H_{38}O_2$	346	713	797
Tetraneurin-α-diol	2.61	$C_{15}H_{20}O_5$	280	697	786

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

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	$C_{10}H_{16}$	136	873	934
Decane	0.72	$C_{10}H_{22}$	142	859	937
Myrcene	1.08	$C_{10}H_{16}$	136	819	836
2-Methyldodecan-1-ol	0.46	$C_{13}H_{28}O$	200	788	835
D-Limonene	71.57	$C_{10}H_{16}$	136	940	941
(E)- 2,3-Epoxycarane	0.49	$C_{10}H_{16}O$	152	759	817
Undecane	2.28	$C_{11}H_{24}$	156	928	950
γ-Terpinene	1.97	$C_{10}H_{16}$	136	878	910
Myristyl alcohol	0.57	$C_{14}H_{30}O$	214	774	777
1-Nonadecanol	2.11	$C_{19}H_{40}O$	284	766	775
4-Terpineol	2.13	$C_{10}H1_{8O}$	154	897	942
Dodecane	4.80	$C_{12}H_{26}$	170	919	934
Tetradecane	0.84	$C_{14}H_{30}$	198	780	788
α-Terpineol	1.04	$C_{10}H_{18}O$	154	832	880
3,6-Octadecadienoic acid methyl ester	0.49	$C_{19}H_{34}O_2$	294	729	777
Octahydro- 1,2,4-metheno-1H-cyclobuta[cd]pentalene-3,5-diol	0.46	$C_{10}H_{12}O_2$	164	712	778
cis-Z-α-Bisabolene epoxide	0.96	$C_{15}H_{24}O$	220	735	759
Oleic acid	2.72	$C_{18}H_{34}O_2$	282	762	781
Arachidonic acid methyl ester	0.82	$C_{21}H_{34}O_2$	318	753	815
(*E*)-Acrylic acid, 3-(3-methoxycarbonyl-1-cyclohexen-4-yl)-methylester	0.66	$C_{12}H_{16}O_4$	224	604	688
trans-Palmitoleic acid	2.62	$C_{16}H_{30}O_2$	254	760	807
Ethyl iso-allocholate	0.66	$C_{26}H_{44}O_5$	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	$C_{10}H_{16}$	136	884	938
Decane	1.27	$C_{10}H_{22}$	142	817	929
Myrcene	1.53	$C_{10}H_{16}$	136	812	841
β-Pinene	1.38	$C_{10}H_{16}$	136	855	899
2,7-Dimethyl-2,6-octadien-1-ol	0.45	$C_{10}H_{18}O$	154	703	740
1-Decene	0.52	$C_{10}H_{20}$	140	765	786
1-Tetradecanol	0.66	$C_{14}H_{30}O$	214	770	776
D-Limonene	54.61	$C_{10}H_{16}$	136	938	940
(E)- 2,3-Epoxycarane	0.96	$C_{10}H_{16}O$	152	774	829
Undecane	3.00	$C_{11}H_{24}$	156	894	930
γ-Terpinene	6.68	$C_{10}H_{16}$	136	908	945
cis-*p*-2-Menthen-1-ol	0.41	$C_{10}H_{18}O$	154	754	822
Hexahydrofarnesol	1.2	$C_{15}H_{32}O$	228	750	740
Tetradecyloxirane	2.08	$C_{16}H_{32}O$	240	743	809

Table 4. *Cont.*

Compound	Relative Quantity (%)	Molecular Formula	Molecular Weight (g/mol)	SI [2]	RSI [1]
4-Terpineol	1.59	$C_{10}H_{18}O$	154	850	920
Dodecane	5.73	$C_{12}H_{26}$	170	893	923
2,6,10-Trimethyltetradecane	1.17	$C_{17}H_{36}$	240	754	782
4-Carvomenthenol	1.20	$C_{10}H_{18}O$	154	782	800
α-Terpineol	1.15	$C_{10}H_{18}O$	154	825	884
Methyl hexadecadienoate	0.41	$C_{17}H_{30}O_2$	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	$C_{19}H_{30}O_2$	290	707	730
2-[4-Methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde	0.48	$C_{23}H_{32}O$	324	703	714
Oleic acid	1.33	$C_{18}H_{34}O_2$	282	780	804
9-Hexadecenoic acid	1.16	$C_{16}H_{30}O_2$	254	776	810
Dimethyl anthranilate	3.13	$C_9H_{11}NO_2$	165	669	893
Methyl hexadecadienoate	0.92	$C_{17}H_{30}O_2$	266	764	801
1,3-Diolein	1.52	$C_{39}H_{72}O_5$	620	753	780
Ethyl iso-allocholate	1.96	$C_{26}H_{44}O_5$	436	744	767

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

Table 5 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-carvomenthenol (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 [1]	RSI [2]
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-Carvomenthenol	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-Citrylideneethanol	0.45	$C_{12}H_{20}O$	180	730	742
trans-Carveol	0.83	$C_{10}H_{16}O$	152	820	864
(Z)-Citral	1.42	$C_{10}H_{16}O$	152	782	830

Table 5. *Cont.*

Compound	Relative Quantity (%)	Molecular Formula	Molecular Weight (g/mol)	SI [1]	RSI [2]
6-Methyltetraline	0.57	$C_{11}H_{14}$	146	777	841
Dihydro cuminyl alcohol	0.91	$C_{10}H_{16}O$	152	805	854
Thymol	3.30	$C_{10}H_{14}O$	150	904	917
Farnesol	1.05	$C_{15}H_{26}O$	222	801	813
Nerolidyl acetate	0.66	$C_{17}H_{28}O_2$	264	805	825
Valencene	3.30	$C_{15}H_{24}$	204	931	958
Dimethyl anthranilate	8.13	$C_9H_{11}NO_2$	165	909	940

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

The GC–MS chromatograms of the identified compounds of EOs from the studied different parts of *C. aurantium* are shown in Figure 1.

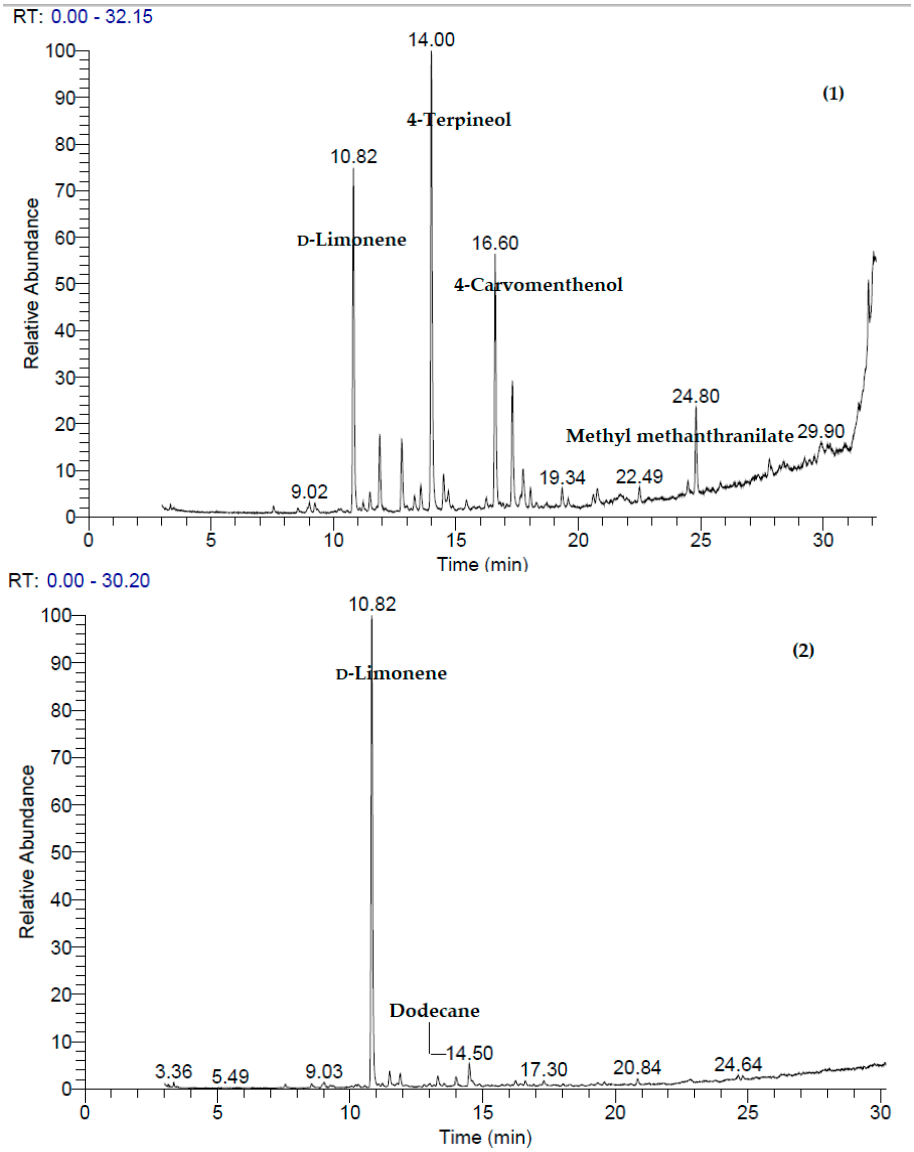

Figure 1. *Cont.*

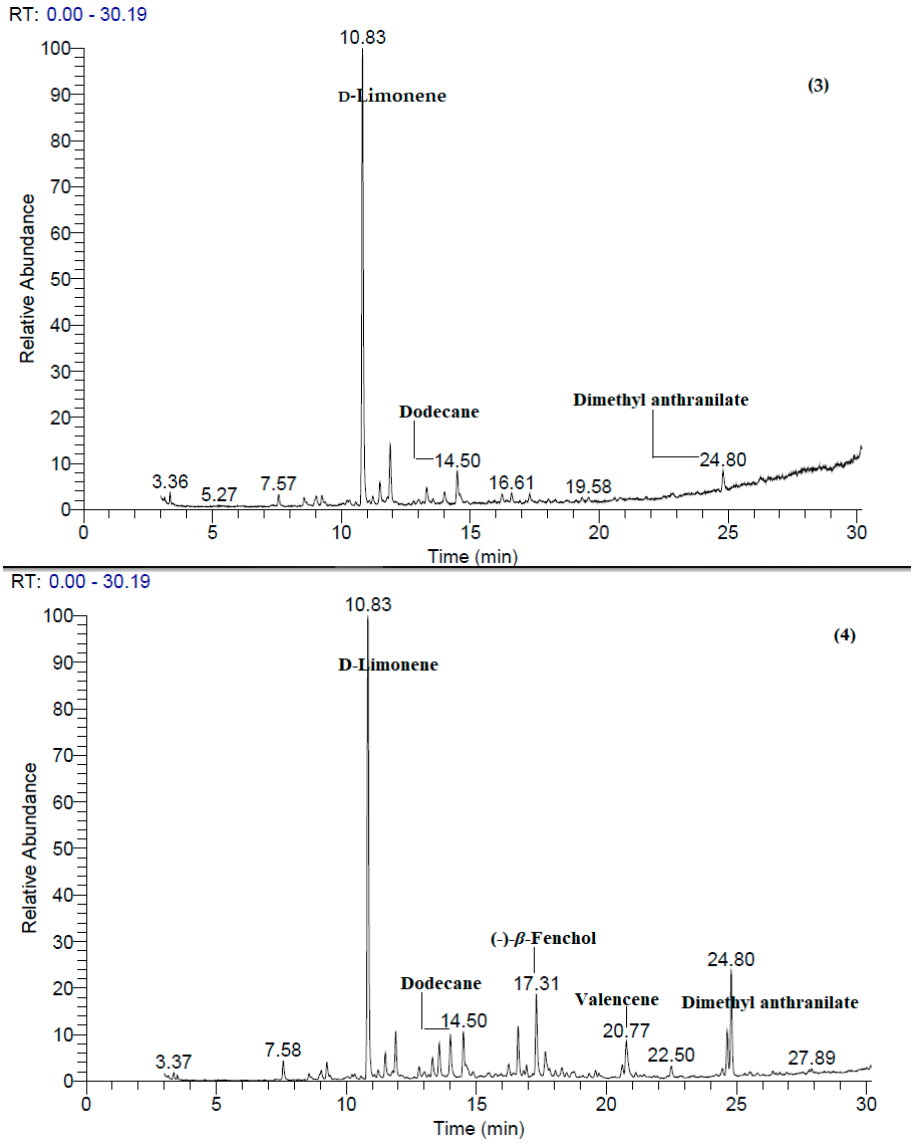

Figure 1. GC–MS chromatogram showing the chemical analysis of essential oils from leaves/twigs (**1**), small branches (**2**), bark of branches (**3**), and wood of branches (**4**).

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.

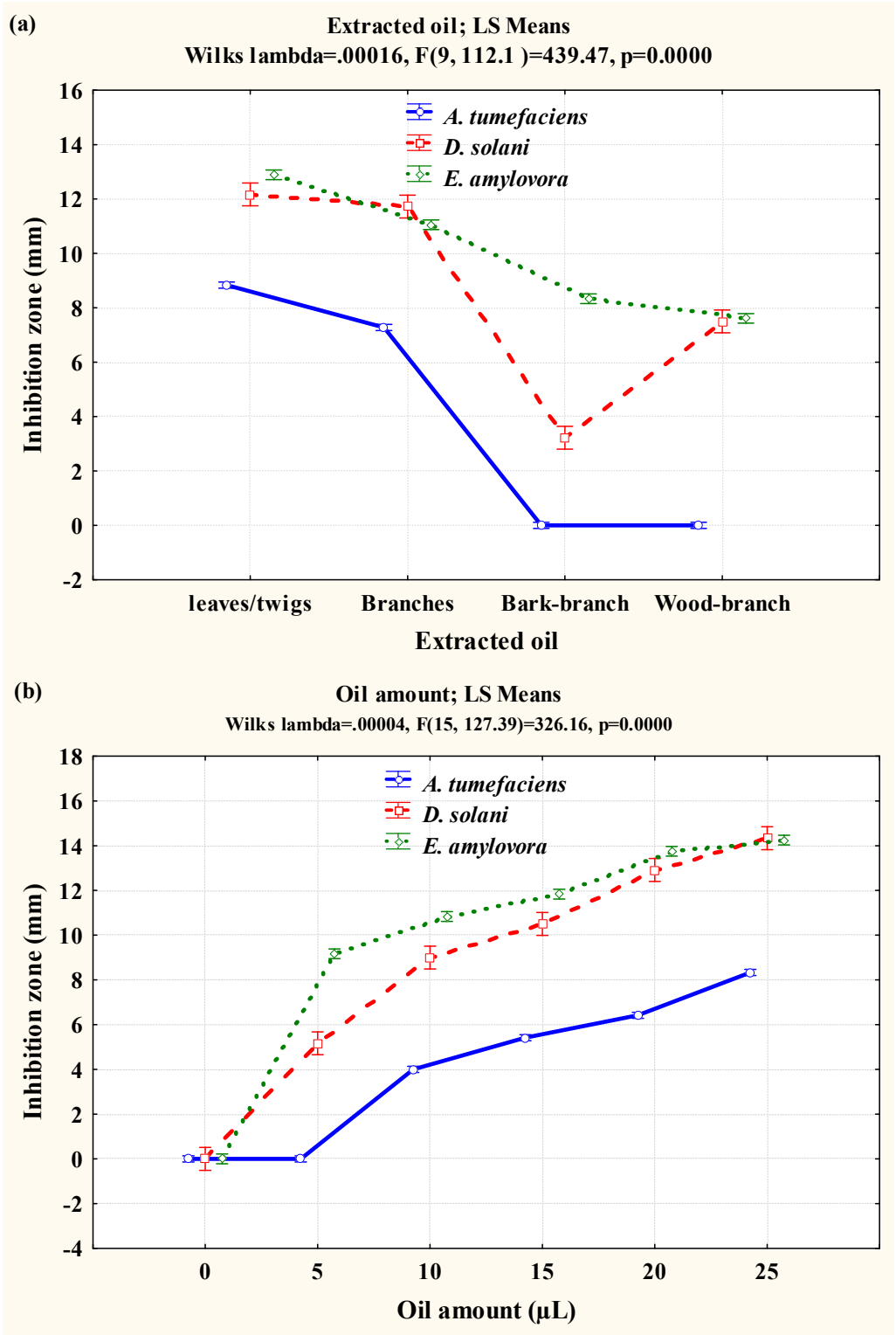

Figure 2. The main effects of oils from different parts of *C. aurantium* (**a**) and their amounts (**b**) on the growth of *A. tumefaciens*, *D. solani*, and *E. amylovora*.

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
	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
	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
	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
	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, α-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-terpineol [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-terpinene, terpinen-4-ol, terpinolene, and α-terpineol had strong antibacterial activities against *Propionibacterium acnes* and *Staphylococcus aureus* [48].

Linalyl 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]. Dl-limonene with 94.81% is the main compound identified in peel EO from *C. aurantium* with promising larvicide 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 borneol, have antitumor effects; sabinene and pinene have anti-inflammatory activity; and citral exhibits analgesic functions [58–62].

Although *cis*-β-terpineol, D-limonene, 4-carvomenthenol, 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 terpinen-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 *Stapylococcus 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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