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Chemical Composition and Antimicrobial Activity of *Fraxinus excelsior* L. Seeds Essential Oil

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Abstract

Background: *Fraxinus excelsior* L. has been traditionally used as a diuretic, carminative and gallstone crusher. The antimicrobial activity of *Fraxinus excelsior* L. leaves and bark extract has been confirmed against bacteria and fungi.
Objectives: The aim of this study was to evaluate the chemical composition and antimicrobial activity of *Fraxinus excelsior* L. seed essential oil.
Methods: Chemical composition of *F. excelsior* was analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS), and its antimicrobial activity was evaluated by disc diffusion and micro broth dilution assays.
Results: Overall, 53 components were identified in the essential oil, which constitute 99.98% of total oil composition. Carotol (16.25%), α-cadinol (13.33%), δ-cadinene (12.4%), bicyclogermacrene (10.34%), α-muurolol (9.69%), and E-caryophyllene (5.9%) were the main components of the essential oil. The essential oil showed the best activity against the standard strain of *Staphylococcus aureus*, while *Pseudomonas aeruginosa* and *Candida albicans* had less sensitivity to it. The mean minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of the essential oil against clinical isolates of *S. aureus* were 0.162 ± 0.024 and 0.31 ± 0.067 μL/mL, respectively.
Conclusions: Therefore, the essential oil can be a suitable candidate for further studies against staphylococcal infections.

Keywords: *Fraxinus excelsior*, Seed, Essential Oil, *Staphylococcus aureus*, Carotol

1. Background

Fraxinus excelsior L., a member of Oleaceae family, has been traditionally used as a gallstone crusher and carminative (1). FraxiPure™ is a safe natural extract obtained from *F. excelsior* that was shown to reduce glycaemia in animal models and human clinical trials (2). Hippocrates used *F. excelsior* leaves and bark as diuretics and for the treatment of rheumatoid, fever, wound, diarrhea, and dysentery (3). Coumarins (e.g., esculin and fraxin), secoiridoids, phenylethanoid glycosides (i.e., verbascoside, salidroside, calceolarioside A, B, lugrandoside, isolugrandoside, and isoacteoside), lignans, flavonoids, phenolic compounds (e.g., p-hydroxybenzoic acid, protocatechuic acid, vanillic acid, synergic acid, 2, 4-dihydroxybenzoic acid, and gallic acid), sterols, and triterpenes were the isolated chemical components of *F. excelsior* (3).

The ethyl ether fraction of *F. excelsior* bark ethanolic extract has inhibitory effects against *Bacillus subtilis* (4). *F. excelsior* leaves extract has antifungal activity against *Gloeosporum limetticola* and *Alternaria tennis* (5). *F. excelsior* leaves aqueous extract has been found to suppress the growth of *Candida albicans*, while its bark aqueous extract showed antimicrobial activity against *Staphylococcus aureus* and *Proteus mirabilis*. The antibacterial (6), anti-inflammatory (7), anti-oxidant (6), diuretic (3), antihypertensive (8), analgesic, antipyretic (9), and hypoglycemic activity (10-12) of *F. excelsior* extract have also been confirmed. Although the antimicrobial activity of *F. excelsior* leaves and bark extracts were confirmed against bacteria and fungi, there is a scarcity of studies evaluating the chemical composition and antimicrobial activity of *F. excelsior* seed essential oil.

2. Objectives

The aim of this study was to evaluate the chemical composition and antimicrobial activity of *F. excelsior* seed essential oil.

3. Methods

3.1. Plant Materials, Essential Oil Extraction and Analysis

F. excelsior seeds were collected from the research garden of BarijEssence Pharmaceutical Company, Kashan,

Iran. The plant materials were identified and authenticated. Then, 100 g of seed was hydrodistilled by water in Clevenger type apparatus for 4 h. The essential oil was gathered and subjected to identification by gas chromatography (GC) and gas chromatography–mass spectrometry (GC-MS) analyses. The GC and GC-MS analyses were performed by Shahid Beheshti Medicinal Plant Research Center. The GC analysis was carried out on Trace MS (Thermo Quest- Finnigan, China) using capillary column of DB-5 (30 m $\times$ 0.25 mm; film thickness, 0.25 μm), the oven temperature program was initiated at 60°C for 1 min, then increased up to 250°C at a rate of 3°C/min, and held isothermal for 10 min.

The injector and detector temperatures were fixed at 230°C and 250°C, respectively. GC-MS analysis was performed using Trace MS (Thermo Quest-Finnigan) equipped with 5973 network mass selective detector system. The carrier gas was helium used at a flow rate of 1.1 mL/min with a split ratio of column sample injection equal to 1/100. Oven temperature program was set the same as GC mentioned above. The results were interpreted and reported according to comparison with retention indices (RI) relative to homologous series of n-alkanes and using libraries of Wiley 275.L and Wiley 7n.1 and through comparison of the fragmentation pattern of the mass spectra with data published in the literature (13).

3.2. Microbial Strains

The microbial strains used in this study included *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, and *Candida albicans* ATCC 10231. Eleven clinical strains of *S. aureus* were used for further evaluations. These strains were isolated from patients with staphylococcal infections. The microbial strains were cultured on Soybean Casein Digest Agar and incubated at 37°C in aerobic conditions for 24 - 72 h. Sabouraud Dextrose Agar was used for *Candida albicans*. One or two colonies of each strain were suspended in sterilized buffer with pH 7.2 and its turbidity was adjusted to 0.5 McFarland by spectrophotometric method (1×10^8 CFU/mL for bacteria and 1×10^6 CFU/mL for *C. albicans*).

3.3. Antimicrobial Evaluations of Essential Oil

The antimicrobial activity screening of *F. excelsior* essential oil against microbial strains was screened by two different methods including disc diffusion method and micro-broth dilution assay.

In the disc diffusion method, the adjusted microbial strains to 0.5 McFarland were cultured on Muller Hinton Agar or Sabouraud Dextrose Agar by swab, and the discs containing 2.5, 5, and 7.5 μL of essential oil dissolved in dimethyl sulfoxide were put on culture media. The plates were incubated at 37°C in aerobic conditions for 24 h. After incubation, the inhibition zone diameters were measured in millimeter and reported. All the experiments were performed in triplicate and reported as mean $\pm$ standard deviation (SD). Vancomycin (30 μg/disc), gentamicin (10 μg/disc), and amphotericin B (10 μg/disc) (Rosco Diagnostica) were used as positive controls.

In micro-broth dilution assay, the microbial suspensions were diluted in 1/200. The colony-forming units (CFUs) were 1×10^6 and 1×10^4 CFU/mL for bacteria and yeasts, respectively. The essential oil was dissolved in dimethyl sulfoxide and diluted in Muller Hinton broth or RPMI 1640 (Roswell Park Memorial Institute medium) within the range of 0.03 - 16 μL/mL of essential oil. Then, 100 μL of each dilution was mixed with 100 μL of diluted microbial strains in 96-well microtiter plates. The plates were incubated in the above conditions. The lowest concentration of essential oil that inhibits the microbial strains in wells is the minimum inhibitory concentration (MIC) and the lowest concentration of essential oil that inhibits the growth of microbial strains on solid media is minimal lethal concentration (MLC) (14).

4. Results

4.1. Chemical Composition of F. excelsior Seed Essential Oil

GC-MS analysis of *F. excelsior* seed essential oil showed the presence of 53 components, which account for 99.98% of total oil composition. Carotol (16.25%), α-cadinol (13.33%), δ-cadinene (12.4%), bicyclogermacrene (10.34%), α-muurolol (9.69%), E-caryophyllene (5.9%), and β-elemene (3.75%) were the main components of essential oil, followed by β-cadinene (3.18%), δ-cadinol (2.99%), and spathulenol (2.54%), respectively (Table 1).

4.2. The Antimicrobial Activity of F. excelsior Seed Essential Oil

The results of the first antimicrobial screening showed that among Gram-positive and Gram-negative bacteria and yeasts, the most sensitive microorganism was the Gram-positive bacterium of *S. aureus* with inhibition zone diameters of 9.7 - 11 mm in the presence of 2.5 - 7.5 μL/disc *F. excelsior* essential oil and the MIC and MLC values of 0.06 and 0.108 μL/mL, respectively (Table 2).

Due to acceptable antibacterial activity of *F. excelsior* essential oil against *S. aureus*, the antibacterial activity of *F. excelsior* essential oil was evaluated against clinical isolates of *S. aureus*. The results of screening showed that the inhibition zone diameter of essential oil increased dose-dependently and this inhibition zone diameter was lower than that of vancomycin. The mean inhibition zone diameter for 7.5 μL of essential oil against clinical isolates of *S.*

Table 1. The Chemical Composition of *Fraxinus excelsior* L. Seed

Row	Compounds	Retention Index	Percent
1	α-pinene	933	0.07
2	Sabinene	973	0.07
3	β-pinene	978	0.23
4	P-cymene	1025	0.16
5	Limonene	1029	0.96
6	1, 8-cineole	1032	0.11
7	Linalool	1100	0.14
8	Menthol	1175	0.02
9	Terpinen-4-ol	1180	0.06
10	α-terpineol	1194	0.06
11	β-citronellol	1231	0.06
12	Z-citral	1242	0.04
13	Geraniol	1257	0.22
14	E-citral	1272	0.06
15	Bicycloelemene	1340	0.49
16	α-cubebene	1353	0.12
17	Neryl acetate	1366	0.01
18	Cyclosativen	1369	0.03
19	α-copaene	1379	0.27
20	Geranyl acetate	1385	0.02
21	β-elemene	1397	3.75
22	Junipene	1400	0.03
23	α-gurjunene	1414	0.74
24	E-caryophyllene	1426	5.91
25	Calarene	1433	0.12
26	Trans-α-bergamotene	1439	0.21
27	Aromadendrene	1443	0.18
28	α-humulene	1459	1.19
29	Alloaromadendrene	1466	1.01
30	γ-muurolene	1482	1.19
31	Germacrene D	1486	1.18
32	Bicyclogermacrene	1506	10.34
33	α-muurolene	1506	1.92
34	β-bisabolene	1513	0.39
35	β-cadinene	1521	3.18
36	D-nerolidol	1526	1.32
37	δ-cadinene	1533	12.41
38	β-elemol	1555	2.15
39	β-calacorene	1569	0.22
40	Palustrol	1574	0.25
41	δ-cadinol	1584	2.99
42	Spathulenol	1586	2.54
43	Globulol	1591	1.11
44	Viridiflorol	1598	0.42
45	Ledol	1610	0.65
46	1, 10-di-epi-cubenol	1621	0.27
47	1, epi-cubenol	1634	0.65
48	γ-eudesmol	1638	0.36
49	α-muurolol	1651	9.69
50	α-cadinol	1667	13.33
51	Carotol	1705	16.25
52	(2Z, 6E)-farnesol	1727	0.47
53	Oplopanone	1746	0.36

aureus was 9.3 $\pm$ 0.19 mm versus 19.6 $\pm$ 0.19 for 30 μg of vancomycin.

MIC values of essential oil against clinical isolates of *S. aureus* were within the range of 0.05 - 0.21 μL/mL with the mean of 0.162 $\pm$ 0.024 μL/mL. These values were 0.25 - 1 and 0.58 $\pm$ 0.27 μg/mL for vancomycin. The MBC values of essential oil and its mean were 0.125 - 1 and 0.31 $\pm$ 0.067 μL/mL, respectively. The corresponding values for vancomycin were 0.5 - 2 and 1.04 $\pm$ 0.49 μg/mL (Table 3).

5. Discussion

F. excelsior seed, leaves, and bark extract have been traditionally used for the treatment of different ailments in various parts of the world. In this study, we extracted *F. excelsior* seed essential oil for the first time. The inhibition zone diameter of *F. excelsior* seed essential oil against clinical isolates of *S. aureus* was not correlated with the results of micro broth dilution assay. This phenomenon is related to the molecular weight of essential oil components, solubility in agar media, and vaporization of essential oil; therefore, the disc diffusion method was not a suitable assay for evaluating the efficacy of the essential oil.

Carotol, a squiterpenoid compound, is the main component of carrot seed oil (15), carotol, and caryophyllene as the main components of carrot seed oil inhibit the radial growth of *Alternaria alternata* by 65% (16), *Staphylococcus aureus*, and *Bacillus subtilis* (17). Therefore, the antimicrobial activity of *F. excelsior* seed essential oil can be related to its contents such as carotol and caryophyllene. The antimicrobial activity of caryophyllene was confirmed against *S. aureus*, *S. typhimurium*, *E. coli*, *Enterococcus faecalis*, *Aspergillus niger*, *Fusarium solari*, *Aspergillus fumigatus*, and *Aspergillus parasiticum* (18).

S. aureus as an important pathogen in nosocomial infections showed sensitivity to *F. excelsior* seed essential oil; therefore, it can be concluded that the essential oil can be a suitable choice for further studies to combat *S. aureus* infections.

Footnotes

Authors' Contribution: Elaheh Mahdizadeh and Rezvan Heidary Tabar performed the examinations; Mohaddese Mahboubi supervised the study and wrote the manuscript.

Conflict of Interests: There is no conflict of interest.

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Table 2. The Antimicrobial Activity of *F. excelsior* Seed Essential Oil

	Disc Diffusion Method[a]				Broth Dilution	
	Essential Oil (μL/disc)			Antibiotics (μg/disc)	Essential Oil (μL/mL)	
	2.5	5	7.5		MIC	MLC
S. aureus	9.67 ± 0.49	9.82 ± 0.66	10.7 ± 0.46	21.6 ± 0.6[A]	0.06	0.108
P. aeruginosa	-	-	-	19 ± 0.0[B]	8	8
C. albicans	-	-	-	21 ± 0.0[C]	7	7

[a] In disc diffusin method: A, vancomycin 30; B, gentamicin 10; C, amphotericin B 10.

Table 3. The Antimicrobial Activity of *F. excelsior* Essential Oil Against Clinical Strains of *S. aureus*

	Disc Diffusion Method				Microbroth Dilution			
	Essential Oil (μL/disc)			Van (μg/disc)	Essential Oil (μL/mL)		Van (μg/mL)	
	2.5	5	7.5	30	MIC	MBC	MIC	MBC
SA-27	6.8 ± 0.0	8.5 ± 0.4	9.41 ± 0.8	19.3 ± 0.03	0.125 ± 0.0	0.125 ± 0.0	0.5	1
SA-4	7.26 ± 0.8	8.6 ± 0.3	9.3 ± 0.46	19.1 ± 0	0.125 ± 0.0	0.25 ± 0.0	1	2
SA-A	7.63 ± 0.7	7.75 ± 0.9	9.1 ± 0.5	18.6 ± 0.1	0.25 ± 0.0	8.0 ± 0.0	0.5	1
SA-3	6.8 ± 0.0	10.2 ± 0.4	10.6 ± 0.4	20.2 ± 0.3	0.06 ± 0.0	0.125 ± 0.0	0.25	0.5
SA-K	6.8 ± 0.0	7.6 ± 0.7	8.52 ± 0.14	16.5 ± 0.01	0.125 ± 0.0	0.25 ± 0.0	0.5	0.5
SA-6	6.8 ± 0.0	8.6 ± 0.6	8.97 ± 0.5	21.2 ± 0.14	0.05 ± 0.0	1.0 ± 0.0	1	2
SA-25	6.8 ± 0.0	7.8 ± 0.9	9.02 ± 0.1	20.8 ± 0.09	0.125 ± 0.0	0.125 ± 0.0	0.25	1
SA-33	6.8 ± 0.0	9.4 ± 0.3	9.93 ± 0.4	19.8 ± 0.28	0.06 ± 0.0	0.125 ± 0.1	0.5	1
SA-32	8.3 ± 0.2	8.7 ± 0.3	8.92 ± 0.3	19.2 ± 0.42	0.125 ± 0.0	0.33 ± 0.14	0.5	1
SA-34	6.8 ± 0.0	9.1 ± 0.23	9.67 ± 0.4	21.0 ± 0.6	0.082 ± 0.04	0.33 ± 0.14	0.5	1
SA-26	6.8 ± 0.0	7.8 ± 0.87	8.98 ± 0.3	20.4 ± 0.37	0.21 ± 0.07	0.33 ± 0.14	1	1
Means	7.1 ± 0.3	8.6 ± 0.27	9.3 ± 0.19	19.6 ± 0.19	0.162 ± 0.02	0.31 ± 0.06	0.58 ± 0.27	1.04 ± 0.49

Abbreviations: MBC, minimal bactericidal concentration; MIC, minimal inhibitory concentration; SA, *S. aureus*.

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