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GC/MS analysis of the oils and lipid constituents and evaluation of different extracts from *Salvia fruticosa* Mill. seed

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Abstract

The main goal of this work is to investigate *Salvia fruticosa* Mill. seed oils (essential and fixed) and lipid constituents (unsaponifiable matter and fatty acids) in addition to evaluating the antimicrobial activity of different seed extracts of various concentrations using Disc-diffusion under the Kirby-Bauer method against several selected strains of bacteria and fungi. The volatile oil of the seeds was obtained using the hydrodistillation method, while the fixed oil was extracted with n-hexane to give a yellowish oil. Both of the oils were analyzed using GC/MS. The identified compounds constitute 98.35% and 89.85%, respectively. The results prove that the volatile oil contains thirty (30) compounds with a high percentage of diterpenes (28.16%), of which sclareoloxide is the most abundant (14.47%), sesquiterpenes (27.91%) with □- cadinene as the main constituent (16.9%), ketones (12.33%), esters and phthalates (10.5%) and monoterpene hydrocarbon constituents (3.89%), . The fixed oil consists of nineteen (19) compounds of which linolenic acid (14.68%) represents the main compound. The main classes are acids (38.46%) with linoleic acid as the major acid (14.68%), esters (12.82%) of which methyl 10-octadecenoate forms 5.09%, hydrocarbons are represented by 7.51% with heptacosane as the main hydrocarbon (4.9%) and diterpenes are present only as phytol (6.01%). It is also noticed that the phthalates are present in the fixed oil in a higher percentage (12.83%) than that in the volatile oil (9.21%).

The results of antimicrobial activity show that the unsaponifiable fraction of the seeds have variable antimicrobial activities against bacteria and fungi, with the highest activity being against *Sarcina spp.* and *Staphylococcus aureus*, while the volatile oil, the fixed oil as well as the saponifiable fraction had no effect at all concentrations against all of the tested strains used in study.

Keywords: *Salvia fruticosa* Mill., Labiateae , volatile oil, fixed oil, lipid constituents, and antimicrobial activity.

Introduction

Plants have been a rich source of the most important, low cost and easily available natural products which have played a vital role in pharmacy to synthesis and isolate new herbal and useful drugs. A few families are: which have proven to be efficient in providing precious essential oils and are a rich source of innumerable bioactive compounds. [1-2]

The is one of the most diverse and widespread plant families in terms of ethno medicine and its medicinal value is based on the volatile oils [3]. Many species of the family are aromatic, due to the presence of volatile oils in their external glandular structures [4]. These oils are important in cosmetic industries, flavoring, fragrance perfumery and pharmaceutical preparations [5]. The genus *Salvia*, belonging to Lamiaceae, comprises about 900 species that grow in South-West Asia, South Africa and America and the Oriental Mediterranean [6]. In Libya, the genus *Salvia* is represented by ten species, three of which are cultivated [7]. Close attention has been paid to the *Salvia* species due to the wide range of its biological such as antibacterial, cytostatic [8], antiparasites [9], antiviral , antitrypanosomal [10-11], anticancer [12-13] and antioxidant activities [14]. Moreover, they are frequently used in traditional medicine to treat diarrhea and gonorrhea, have antiseptic and antispasmodic effects and are used for the treatment of

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eye diseases [15]. It was found that *Salvia* species contain many chemical constituents, such as volatile oils, lipids, flavonoids phenolics, etc. [16].

The essential oil of the *S. hypoleuca* root's main components were identified as hexadecanoic acid (27.4%), β-caryophyllene (22.0%), germacrene D (15.1%) and viridiflorol (14.9%) [17-18].

Forty-five compounds were identified from *S. fruticosa* using GC/MS, including 1,8-cineole (49.34%), camphor (7.53%), β-pinene (7.38%), myrcene (7.38%), α-pinene (5.15%), β-caryophyllene (4.13%) and α-terpineol (3.25) [19]. Also, in another study, it was found that the main component of the essential oil of *S. fruticosa* was trans-thujone (54.2%) and of the essential oil *S. ringens* was α-pinene (28.1%) [20].

Eighty-two constituents were identified by GC and GC/MS analyses in the oil of *S. sclarea* aerial parts with oxygenated monoterpenes (74%), monoterpene hydrocarbons (8.8%), sesquiterpene hydrocarbons (6.6%), oxygenated sesquiterpenes (5.8%), and oxygenated diterpenes (1.1%). The main components are linalool (42.3%), α-terpineol (13.4%), geraniol (6.3%), its acetate derivative (5.4%), and myrcene (3.3%) [21].

The chemical composition of the essential oil from *Salvia acetabulosa* was characterized by its high content of hydrocarbons and oxygenated monoterpenes. The major chemical compounds ~~identified~~ were ocimene (25.22%), camphor (23.17%), 1,8 cineol (22.06%), isoborneol (11.59%) and isoborneol acetate (7.44%), respectively [22]. Savelev *et al* in 2003 also reported that 1,8-cineole, camphor, borneol caryophyllene oxide, α, β -pinene, bornyl acetate, α-, β-thujone, and linalool are the main compounds in *S. lavandulifolia* [23].

Many triterpenoids have been isolated from aerial parts of different *Salvia* sp. by different authors. These are 3α,24dihydroxy-olean-12-en-28-oicacid, 3α,24-dihydroxy-olean-12-en-28,30-dioic acid, 2-α-hydroxy-3-b-methoxyurs-12-en-28-oic acid, 3α-hydroxy-2α-methoxyurs-12-en-28-oic acid, 3-oxo-11α,19β,20,22β-tetrahydroxy-lupane, 3β,11α,19β,20,22β-pentahydroxy-lupane, 2α,3α-dihydroxy-urs-12-en-28-oic acid,2α,3α,24-trihydroxy-urs-12-en-28-oic acid, 2-acetoxylupeol, lupine-2,3-diol, 11α-methoxyurs-12-ene-1β,3β,15α-triol,urs-12-ene-1β,3β,11α,15α-tetraol, 11α-methoxyurs-12-ene-1β,3β-diol, 1β,3β,15α-trihydroxy-11α-methoxyurs-12-en-28-al, 1β,3β,15α-trihydroxyurs-12-en-28-al, urs-12-ene-1β,3β,15α,28-tetraol , 11α-methoxyurs-12-ene-1β,3β,28-triol, 13β,28-epoxyurs-12-ene-1β,3β-diol, urs-12-ene-3β,7β,15α,28-tetraol and olean-12-ene-3β,7β,15α,28-tetraol with three sterols (β-sitosterol, stigmasterol and campasterol) [24-30].

However, to the best of our knowledge, there are few reports on the chemical composition of *S. fruticosa* growing in Libya, so this study aimed to isolate some of the chemical constituents and antimicrobial activity of *S. fruticosa* seed extracts.

Material and methods

Plant Material

The plant was collected in April 2015 from Sirt city (the area around Sirt university) and identified by Prof. Mohammed Eldarawy, Prof. of Botany and Ecology, Biology Department, Faculty of Sciences, Benghazi University, Libya, while the seeds were collected in June 2015, air dried and ground to a fine powder.

1- Preparation of the volatile oil of *S. fruticosa* by Hydrodistillation:

About 150 g of the powdered seeds of *S. fruticosa* was subjected to hydrodistillation in an all-glass apparatus (Clevenger) for about three hours according to the Gunther method [31]. The trapped oil in the side arm was removed after complete distillation and dried over anhydrous sodium sulphate to create a pale-yellow oil with a characteristic odor (0.15 % ; V/W).

2- Extraction of fixed oil seed:

About 100 g of dried powdered seeds were extracted with 400 ml of n-hexane till exhaustion. The combined extracts were evaporated till free from solvent under reduced pressure at 35 ˚C to give a pale-yellow oil (20 g).

The chemical constituents and percentage of each component in-volatile and fixed oils were determined using GC/MS under the following conditions: **Gas chromatography:** Instrument: TRASC GC, Splitless Mode. Column: BD-5 capillary column (30 m, 0.25 mm internal diameter, 0.25 µm film). Temperature program: Injector 50˚C, Initial Temp. 38˚C, Rate, 2˚C / min, to 200˚C, Final Temp. 200˚C for 5 min. Flow gas Helium at 10 ml/min. **Mass spectroscopy:** Instrument: TRACE DSQ. Full scan 50-450, positive ion, Ion source 200˚C, mass transfer line 200 ˚C. Library: NIST. The mass spectra were measured in EI scan Mode at (70 ev) from a 50-450 mass unit, as summarized in table1.

3- Extraction of lipid constituents:

About 960 g of the dried, powdered *S. fruticosa* seeds were extracted with n- hexane in a soxhlet apparatus. The combined n- hexane extract was passed through fuller's earth to remove the colored pigments, filtered, dried over anhydrous sodium sulfate and evaporated in *vacuo* at 40 ^{0}C till dryness to create a pale-yellow residue (135 g).

3.1 Saponification of hexane extract: The n-hexane extract (10 g) was saponified by refluxing with 100 ml N/2 alcoholic KOH. The alcoholic solution was concentrated to about 20 ml and diluted with cold distilled water. The unsaponifilable constituents were extracted by partition with successive portions of

diethyl ether (3×100 ml). The combined ether extract was washed with distilled water, dehydrated over anhydrous sodium sulfate and evaporated in *vacuo* till dryness to give a yellowish-brown semi-solid residue of unsaponifiable matter (1.99 g). The unsap. fraction was subjected to GLC analysis under the following conditions and the obtained results are shown in table 2.

Table (1):GC/MS data of fixed and volatile oils of *S. fruticosa* seeds

Peak	R_t	%		Mass data			Compounds
		V oil	Fixed oil	Mol. For	MW	B.P	
1	5.47	1.79	-	$C_3H_4O_3$	88	43	Pyruvic acid
2	5.7	0.84	-	$C_3H_6O_3$	90	43	L-(+)-Lactic acid
3	8.08	-	4.01	$C_{10}H_{16}$	136	93	□-carene
4	8.14	1.20	-	$C_9H_{14}O$	138	138	Camphenilone
5	8.18	2.16	-	$C_8H_{16}O_2$	١٤٤	٤٣	2-acetoxy hexane
6	11.50	-	3.54	$C_6H_4Cl_2$	146	146	1,4-dichlorobenzene
7	17.11	1.29	-	$C_8H_{14}O_2$	142	43	2E-hexenyl acetate
8	15.73	1.58	-	$C_{10}H_{20}O$	١56	٥٧	n-Decanal
9	18.62	0.78	-	$C_{10}H_{18}O_2$	١٧٠	٨١	Trans-mentholactone
10	17.65	1.48	-	$C_{11}H_{18}O$	١٧٠	٤١	2,4undecadienal
11	16.46	1.78	-	$C_{13}H_{18}O$	190	69	á-Damascenone
12	21.34	1.35	-	$C_{14}H_{24}$	192	17٧	1,3,5,7-Tetramethyl-adamantane
13	21.45	2.16	-	$C_{13}H_{22}O$	194	105	(E)-Geranylacetone
14	21.61	1.23	-	$C_{13}H_{22}O$	194	111	5,5,8a-Trimethyldecalin-1-one
15	21.74	0.65	-	$C_{12}H_{22}O_2$	١98	٥٥	9-dodecenoic acid
16	22.02	-	3.74	$C_{12}H_{24}O_2$	200	73	Lauric acid
17	22.62	1.67	-	$C_{15}H_{24}$	٢٠٤	٢٠٤	□-gurjunene
18	22.80	2.69	-	$C_{15}H_{24}$	204	105	□-copaene
19	23.35	16.9	-	$C_{15}H_{24}$	٢٠4	161	β- Cadinene
20	24.98	2.39	-	$C_{15}H_{22}$	202	159	trans-calamenene
21	25.86	4.35	-	$C_{15}H_{24}$	204	161	Bicyclo sesquiphellandrene
22	26.21	2.21	-	$C_{15}H_{20}O$	216	91	à-hexyl Cinnamaldehyde
23	26.68	0.81	-	$C_{15}H_{20}O$	220	95	Longifolenaldehyde
24	29.05	-	2.00	$C_{14}H_{28}O_2$	228	73	Myristic acid
25	25.28	-	0.76	$C_{16}H_{31}N$	237	43	Palmitonitrile
26	31.09	5.27	9.2	$C_{16}H_{32}O_2$	256	43	palmitic acid
27	31.71	14.5	-	$C_{18}H_{30}O$	262	43	Sclareoloxide(Cis-A/B)
28	32.26	3.28	-	$C_{18}H_{30}O$	262	69	Farnesyl acetone A
29	33.03	-	4.42	$C_{18}H_{36}O$	268	43	Hexahydrofarnesyl acetone
30	33.43	1.80	4.77	$C_{17}H_{34}O_2$	270	74	Methyl palmitate
31	35.12	-	8.77	$C_{16}H_{22}O_4$	278	149	Isobutyl phthalate
32	35.31	9.21	-	$C_{16}H_{22}O_4$	278	149	Butyl phthalate
33	36.89	-	14.68	$C_{18}H_{32}O_2$	280	67	Linoleic acid
34	37.56	-	8.84	$C_{18}H_{34}O_2$	282	55	Oleic Acid
35	39.89	9.11	-	$C_{20}H_{34}O$	290	69	Geranyl linalool
36	41.2	2.89	-	$C_{20}H_{34}O$	290	69	trans-Geranylgeraniol
37	42.06	-	2.86	$C_{19}H_{34}O_2$	294	67	Methyl linolelaidate
38	42.80	-	5.09	$C_{19}H_{36}O_2$	296	67	Methyl 10-octadecenoate
39	43.89	1.69	5.01	$C_{20}H_{40}O$	296	71	Phytol
40	44.99	-	0.78	$C_{19}H_{38}O_4$	330	55	1-Monopalmitin
41	45.12	-	2.38	$C_{22}H_{34}O_2$	362	149	butyl 8-methylnonyl Phthalate
42	46.63	-	4.90	$C_{27}H_{56}$	380	57	n-Heptacosane
43	48.49	-	1.68	$C_{24}H_{38}O_4$	390	149	Diisooctyl phthalate
44	50.31	-	2.61	$C_{32}H_{66}$	450	57	n-Dotriacontane

Instrument: Aglient technologies 6890N Network GC system. Column: Capillary column (ZB-5), (length 30m, i.d. 530µm, Film- thickness 50µm). Temperature program: Oven: initial temp.: 80 °C, rate: 8°C/min., final temp.: 250°C, final time: 50 min. Inlet: 270 °C, (split) = mode, Split ratio = 15:1. Detector:

(FID) 300 °C. Carrier gas: N_2:30 ml/min. H_2 :30 ml/min. Air: 300 ml/min.

Table (2): GLC of the unsap. fraction of *S. fruticosa* seeds

Peak no.	RT (min.)	%	Compounds
1	4.30	2.44	Decane. n-C10
2	5.52	0.49	Undecane, n-C11
3	7.12	2.83	Dodecane, n-C12
4	9.59	0.56	tetradecane , n-C14
5	11.99	3.21	Hexadecane , n-C16
6	13.18	0.49	Heptadecane, n-C17
7	14.43	1.50	Octadecane, n-C18
8	14.95	6.15	Nonadecane, n-C19
9	15.51	1.10	Eicosane, n-C20
10	16.57	0.74	Heneicosane, n-C21
11	18.35	23.62	Tricosane, n-C23
12	20.45	36.30	Tetracosane, n-C24
13	21.86	3.7	Pentacosane, n-C25
14	23.34	4.62	Hexacosane, n-C26
15	25.54	1.48	Heptacosane, n-C27
16	25.98	0.62	Nonacosane, n-C29
17	27.43	1.20	Triacontane, n-C30
18	29.92	4.80	Cholesterol
19	33.70	0.6	stigmasterol
20	35.24	054	□- sitosterol
21	36.74	3.01	□-amyrine

3.2 Extraction of the otal fatty acids: The sap. fraction was extracted from hydroalcoholic soap solution after the saponification was rendered acidic (pH=1) with 5% sulphuric acid (H_2SO_4). The liberated fatty acids were thoroughly extracted several times with diethyl ether. The combined ether extract was washed with distilled water till free from acidity and dehydrated over anhydrous sodium sulfate. The solvent was evaporated in *vacuo* at about 40°C till dryness to give 0.44 g.

3.3 Preparation of the fatty acid methyl esters: About 400 mg of the total fatty acids were dissolved in 75 ml dry methanol containing 4-5 % dry HCl and refluxed in a boiling water bath for four hours. The solvent was concentrated by evaporation till 25 ml and diluted with 100 ml distilled water. The reaction mixture was extracted with successive portions of diethyl ether (3×100 ml). The combined ether extract was washed with distilled water till free from acidity, dried over anhydrous sodium sulfate, filtered and then the solvent was evaporated in *vacuo* at 40 °C to give 0.3 g. The composition and the percentages of the fatty acid methyl esters were determined by GLC analysis using the following conditions and the obtained results are given in table 3.

Instrument: Hewlett Packar DHP-6890 series. Column: Capillary column HP- wax Bonded Polyethlene glycol (length: 60m, Dimeter : 320µm, Film thickness : 0.25µm).Temperature program: 70 °C for 2min, rate 4 °C/min, Final Temp. 200°C, Final time, 30 min. Detector temp.: 275°C (FID). Injector temp.: 250°C. Flow rates: N_2: 30 ml/min.H_2: 30 ml/min. Air: 350 ml/min.

Biological activity study:

Preparation of different extracts for antimicrobial evaluation:

Three concentrations were prepared from each extract (fixed oil, volatile oil, unsap and sap. fractions) as 1, 2 and 3 which are 0.05, 0.1 and 0.15 g / ml, respectively.

Microbiological media: A- The nutrient broth and nutrient agar which were made by Oxide, UK Company were used for bacteria. B- Saturated dextrose produced by the same company was used for yeasts. C- Czapek solution was used for the fungi.

Cultures: Seven bacteria strains were used for the study viz. *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Bacillus subtillis*

ATCC 6623, *Bacillus cereus* 14579, *Pseudomonas aeruginosa* ATCC 10145, *Salmonella Typhi* ATCC 13311 and *Sarcina sp.* (from the chemistry of natural and microbial products, National research center, Cairo, Egypt), two fungi strains *Aspargillus niger* MTCC 1344 and *Asprrgilus flavus* MTCC 871 and one yeast *Candida albicans* ATCC 10231 (Biotech. Center, Tropoli, Libya).

Antimicrobial control : Three antimicrobial agents were used as control as follows : Tetracycline, Mycostatin and Metradinazol.

testing the extracts as antimicrobial [32]: The culture of microbiological media was prepared using the Disc-diffusion-modified-Kirby-Bauer and streaking method, sterilized by autoclaving at 120 ˚C for 20 minutes to become a broth culture of the strain under test and then placed in an empty sterile Petri dish. The melted microbiological media, that must be of a homogenous distribution of the inoculums in the medium, was left to solidify, then placed on the saturated discs (three discs for each extract) and placed in an incubator at 37 ˚C for 24 hours for bacteria, for 48 hours for yeasts while for fungi they were incubated at 30˚C for one week. The inhibition zone was measured for each extract. The obtained results are shown in table 4.

Results and discussion:

The volatile oil of the seeds was obtained by the hydrodistlaation method (0.15% w/v) while the fixed oil was extracted with n-hexane to afford a yellowish oil (20 % w/v). Both oils were analyzed using GC/MS. The compounds were identified by comparing their mass spectra with those given in the literature [33], as shown in table 1. The results showed that the volatile oil contains thirty compounds with □- cadinene as the major component (16.9%) while the fixed oil consists of ninety compounds, including linolenic acid (14.68%) and isobutyl phthalate (17.77%). The identified compounds constitute 98.35% and 89.85%, respectively. It was found that the volatile oil of *S. fruticosa* seeds showed a higher percentage of diterpenes (28.16%) in which sclareoloxide is the most abundant (14.47%), then sesquiterpenes (27.91%), with □- cadinene as the main constituent (16.9%), ketones (12.33%), esters and phthalates (10.5%), monoterpene hydrocarbons ~~constitutes~~ (3.89%) and acids (2.63%). These data are not in line with the results reported by Taârit et al [34], who found that the essential oil of *S. officinalis* seeds showed a higher percentage of monoterpenes (56.59%) than sesquiterpenes (17.32%). Also, the essential oil was characterized by the presence of α-thujone (14.77%), camphor (13.08%), 1,8-cineole (6.66%), α-humulene (3.71%) and Viridiflorol (2.66%).

For the fixed oil of *S. fruticosa* seeds, the main classes are acids (38.46%) with linoleic acid as ~~the m~~ajor acid (14.68%), esters (12.82%) in which methyl 10-octadecenoate forms 5.09%, hydrocarbons are represented by 7.51%, with heptacosane as the main hydrocarbon (4.9%) and diterpenes are present only as phytol (6.01%). As reported in a previous study by Maryam and Jinous in 2015, the Lamiaceae family is characterized by the presence of linoleic and linolenic acids as the main acids in their seed oils which are considered important chemotaxonomic markers and valued in the nutritional medicinal and cosmetic industries [35].

It is also noticed that the phthalates are present in fixed oil in a higher percentage (12.83%) higher than that in volatile oil (9.21%). The presence of phthalates in the oil of the *Salvia* species was confirmed through the study by Nadaf *et al* in 2012, where they analyzed the n-hexane extract from aerial parts of *S. nemorosa* using GC-MS and identified, among other products, bis(2-ethylhexyl)phthalate (8.6%) in addition to palmitic acid (7.2%), bromocyclohexane (5.0%) and 1-hexadecanol (4.7%) [36]; also, Jun-Feng *et al* [37] isolated bis(2-ethylhexyl) phthalate from *S. chinensis*. In addition, Akhgar *et al* investigated the stems and roots of the essential oil of *S. macilenta* and identified dibutyl phthalate in both oils (4.0% and 10.6%, respectively) [38]. Also, Jibao *et al* in 2006 discovered the presence of a large amount of diethylphthalate, methyl-ethylphthalate and dimethyl phthalate in the oil of *S. sclarea* [39].

The n-hexane extract was subjected to saponification to yield the unsaponifiable fraction (~~unsap,~~ table 2) and the saponifiable fraction which represents fatty acids which were identified as methyl esters (FAME) using GLC analysis, as shown in table 3. The results in table 2 prove that the unsap fraction consists of a series of hydrocarbons (91.02%), sterols (5.94%) and triterpene (3.01%). The hydrocarbons start from decane (n-C10) to triacontane (n-C30), with tetracosane (n-C24, 36.3%) and tricosane (n-C23, 23.62%) as the main compounds. The sterol fraction includes cholesterol, stigmasterol and □- sitosterol, while only one triterpene was identified as a-amyrine. Ghena and Amany in 2020 [40] reported that the unsap fraction of *S. hispanica* contains a sterol fraction which is rich in β-sitosterol (6.33), stigmasterol (4.83%), and campesterol (3.77%) while Taghreed in 2012 [41] stated that the unsap fraction of

Salvia bicolor was characterized by a large amount of hydrocarbons, which constituted 48.05% of the fraction, with octacosane as the major one (11.01%), Both α- and β-amyrins were found in *S. amplexicaulis* and *S. apiana* [21], β-amyrin, lupeol, β-sitosterol, and stigmasterol were also detected in *S. aegyptiaca* .

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Table (3): GLC data of FAME of *S. fruticosa* seeds

Peak no.	Rt. (min.)	%	Compounds	Molecular formula
1	12.15	0.64	Myrestic acid	$C_{14}H_{28}O_2$, C14(0)
2	15.12	20.08	Palmitic acid	$C_{16}H_{32}O_2$, C16(0)
3	16.39	1.1	Palmitoleic acid	$C_{16}H_{30}O_2$, C16(1)
4	17.29	4.25	Magaric acid	$C_{17}H_{34}O_2$, C17(0)
5	18.61	6.53	Stearic acid	$C_{18}H_{36}O_2$, C18(0)
6	19.09	36.86	Oleic acid	$C_{18}H_{34}O_2$, C18(1)
7	20.01	20.77	Linoleic acid	$C_{18}H_{32}O_2$, C18(2)
8	21.17	8.30	Linolenic acid	$C_{18}H_{30}O_2$, C18(3)
10	24.57	0.64	Arachidonic acid	$C_{20}H_{32}O_2$, C20(4)
11	29.60	0.77	Cerotic acid	$C_{26}H_{52}O_2$, C26(0)

The data in table 3 is the first study of the seed oil of *S. fruticosa* which revealed the presence of eleven fatty acids, of which four saturated fatty acids (SFA) constitute about 32.27%, with palmitic acid (C16:0) as the main one (20.08%), while the unsaturated fatty acids (USFA) are represented by seven acids (67.03%), which include mono unsaturated fatty acids (MUSFA) (37.96%) with oleic acid (36.86%), only one di-unsaturated fatty acid (DUSFA) (20.77%) linoleic acid, poly unsaturated fatty acids (PUSFA) (8.94%) with linolenic acid (8.3%). Adil *et al* in 2016 [42] investigated the seed oils of six *Salvia* taxa and reported that the linoleic acid amounted to 58.5%, to 69.2% in some of the species and 22.9-44.19% in the other studied species, while oleic acid had similar concentrations in the studied *Salvia* species (16.8–23.1%). In our study, the linoleic acid and oleic acid amounted to 20.77% and 36.86%, respectively. Also, Nejad *et al* in 2007 stated that the major fatty acids were linolenic acid, oleic acid and palmitic acid in different studied *Salvia* species [43]. It was found that the seed oil of *S. sclarea* is rich in linolenic (36.6%), as the main **fatty acid** constituent, followed by oleic acid (19.4%) and linolenic acid (18.1%), while the seed oil of *S. candidissima* was reported to be rich in oleic acid (21.1%), palmitic acid (21%), Linolenic acid (20.9%) and linolenic acid (19.2%) [44-45]. Finally, Motyka *et al* in 2022 reported that the oil of *S. hispanica* seeds contains 30–33% of fatty acids, in which the main fatty acids are unsaturated [46].

The antimicrobial activity of fixed oil and different fractions was studied using the disc diffusion method. The results in table 4 prove that the unsap fraction exhibited different degrees from inhibition against the tested strains. The highest activity was noticed with *St. aureus* and *Sarcina sp.* with the inhibition zone (14 mm and 16 mm respectively) at high concentrations (3), while it exhibited moderate activity against Gram-negative bacteria (both *E coli* and *B. cereus*) and the fungus *A. Flavus* at the same concentration and weak activity against *C. albicans*.

The other tested fractions had no activity against any of the tested strains. These findings disagree with those reported by Milica *et al* in 2022 [47], who found that Gram-negative bacteria were more sensitive to *S. sclarea* essential oil in comparison to Gram-positive bacteria. In addition, Murat *et al* [48] found that the antimicrobial activities of the seed fatty acids of twelve *Salvia* species have variable antimicrobial activities against bacteria, yeasts and dermatophyte and no effect against some bacteria and yeasts. Moreover, Mansureh *et al* in 2020 [49] stated that both the leaves and flowers essential oils of *S. hydrangea* may have bactericidal activity against some bacteria.

Table (4): Antimicrobial activity of different extracts of *S. fruticosa*

Extract	Conc. g/ml	Tested strains									
		G +ve Bacteria			G -ve Bacteria				Fungi		
		St aureus	*B. subtilus*	*Sarcina sp.*	*Ps. aerugi nosa*	*E coli*	*Sal. typhi*	*B. cereus*	*C. albicans*	*A. Flavus*	*A. niger*
Fixed Oil	**1**	R^-	R^-	R^-	R^-	R^-	R^-	R^-	R^-	R^-	R^-
	2	R^-	R^-	R^-	R^-	R^-	R^-	R^-	R^-	R^-	R^-
	3	R^-	R^-	R^-	R^-	R^-	R^-	R^-	R^-	R^-	R^-
volatile Oil	**1**	R^-	R^-	R^-	R^-	R^-	R^-	R^-	R^-	R^-	R^-
	2	R^-	R^-	R^-	R^-	R^-	R^-	R^-	R^-	R^-	R^-
	3	R^-	R^-	R^-	R^-	R^-	R^-	R^-	R^-	R^-	R^-
Sap of seeds	**1**	R^-	R^-	R^-	R^-	R^-	R^-	R^-	R^-	R^-	R^-
	2	R^-	R^-	R^-	R^-	R^-	R^-	R^-	R^-	R^-	R^-
	3	R^-	R^-	R^-	R^-	R^-	R^-	R^-	R^-	R^-	R^-
Unsap of seeds	**1**	$S^{+١٠}$	$S^{+٨}$	$S^{+١٠}$	R^-	$S^{+٦}$	R^-	$S^{+٦}$	R^-	$S^{+٨}$	R^-
	2	$S^{+١٢}$	$S^{+١٠}$	$S^{+١٤}$	R^-	$S^{+٨}$	R^-	$S^{+٨}$	$S^{+٨}$	$S^{+١٠}$	R^-
	3	$S^{+١٤}$	$S^{+١٢}$	$S^{+١٦}$	R^-	$S^{+١٠}$	R^-	$S^{+١٠}$	$S^{+١٠}$	$S^{+١٢}$	R^-

Notes: R = Resistant -ve S = Sensitive +ve

Conclusion:

The main components in the volatile oil of the seed are sclareoloxide (14.47%) and □- cadinene (16.9%), while in the fixed oil they are linolenic acid (14.68%), methyl 10-octadecenoate forms 5.09%, and phytol (6.01%). It is also noticed that the phthalates are present in fixed oil in a higher percentage (12.83%) than that in volatile oil (9.21%). This is the first report on the volatile oil and lipid constituents of *S. fruticosa* seeds. The results of antimicrobial activity prove that the unsap fraction of the seeds have different antimicrobial activities against bacteria and fungi, with the highest activity against *Sarcina sp.* and *St. aureus* so the unsap fraction could be a good substituent for many chemical synthesized antibiotics, especially due to the high awareness concerning the toxicity, ineffectiveness, antibiotic resistance and adverse effects caused by the widespread use of synthetic drugs.

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