BIOCHEMICAL COMPOSITION, AND IN VITRO ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF *SALVIA FRUTICOSA*, AN ETHNOMEDICINAL PLANT

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Abstract. *Salvia fruticosa* (Anatolian sage) is a medicinal plant with a natural distribution in the Central and Eastern Mediterranean. It has ethnomedicinal uses in traditional medicine. The aim of this study was to investigate the biochemical composition and *in vitro* antimicrobial and antioxidant activities of *S. fruticosa*. The *in vitro* antimicrobial activity of *S. fruticosa* ethanol extract (SFEt) by disk diffusion method against thirty-nine bacterial (including eleven multidrug-resistant strains) and two fungal strains was examined. The determination of antioxidant activity was carried out by the DPPH method. The identification of biochemical composition was by GC-MS. Twenty-three components were identified in SFEt and the main one was D-camphor (20.27%). SFEt has antimicrobial activity against a wide range of microorganisms tested. The highest activity has been demonstrated against *Staphylococcus epidermidis* DSMZ 20044 (ST12) as a Gram-positive bacteria with a 21 mm zone of inhibition and *Proteus vulgaris* (MDR6) as a Gram-negative bacteria with a 13 mm zone of inhibition. Also, the data from *Streptococcus pneumonia* (MDR7) was very striking because a higher activity has been observed than fourteen positive controls. SFEt showed antioxidant activity almost as high as Ascorbic acid. These results have shown that *S. fruticosa* has a high antimicrobial and antioxidant potential.
Keywords: *Salvia fruticosa, Anatolian sage, GC-MS, disk diffusion, DPPH, multidrug-resistant bacteria*

Introduction

Since ancient times, humans have harnessed natural elements such as water, soil, and plants in their environment for therapeutic purposes (Baytop, 1999). Notably, the discovery of plants, such as hollyhock, cornflower, yarrow, groundsel, and purple hyacinth, in 60,000-year-old Neanderthal tombs within the Shanidar Cave in northern Iraq provides some of the earliest evidence of human plant utilization (Lewin, 2000; Heinrich et al., 2004). The body of knowledge surrounding the therapeutic properties of plants has expanded throughout human history and remains relevant in contemporary times (Principe, 1991; Tez, 2017). Historical records dating back to the 3000s BC demonstrate the utilization of plants for therapeutic purposes across various civilizations, including Mesopotamia, ancient China, and Egypt. (Blackwell, 1990;

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Principe, 1991; Tez, 2017). Plants native to Anatolia, such as mandrake, hawthorn, sneezeweed, barley, almond, henbane, wheat, bay, toothpickweed, mustard, poppy, apricot, fir, thuja, myrtle, licorice, saffron, garlic, cedar, cypress, onion, willow, sesame, spurge, boxwood, grapes, and olives, can be found in recipes recorded in Hittite tablets dating back to the 1300s BC (Baytop, 1999).

One of the most ancient and significant sources on Anatolian medicinal plants is the work of Dioscorides, who was born in AD 40 in Anazarbus, near Adana. The work, originally written in Greek, has been translated into Latin as De Materia Medica (Knowledge of Medicine). This renowned work, comprising five books, details the morphological, pharmacological, and toxicological properties of approximately 500 medicinal plants. For nearly 1500 years, De Materia Medica served as the primary reference in the field of medicine and was extensively consulted by experts on the subject. Although the original manuscript of Dioscorides' work has not survived to the present day, copies made from the original can be found in numerous libraries. The oldest of these copies, known as the "Istanbul Codex," is currently housed in Vienna (Büyüknisan, 2011). According to a study prepared by the World Health Organization (WHO) based on pharmacopeias of 91 countries and publications on medicinal plants, the total number of medicinal plants used for treatment is about 20,000. In Turkey, this number is at least about 500 (Ertürk, 2010).

Herbal drugs have been widely recognized for their use in treatments over an extended period and are often employed to address multiple diseases due to their diverse active ingredients and fewer side effects. In contrast, synthetic drugs, while more effective in targeting a single disease, tend to exhibit greater side effects. The primary limitation of herbal drugs is their delayed efficacy, necessitating prolonged use for optimal results (Özata, 2019). Despite advances in drug research and recent developments, bacterial, fungal, and viral infections continue to pose significant threats to public health, particularly in developing countries (Cos et al., 2006). Access to medication in these regions is comparatively limited, and the rise of antibiotic resistance jeopardizes global health (Okeke et al., 2005). Consequently, alternative approaches have been sought to combat infections, with researchers increasingly turning to plants in the pursuit of antimicrobial agents (Cowan, 1999).

The Lamiaceae family exhibits a natural distribution in Turkey, encompassing 49 genera and 629 species. Within the Turkish flora, there are 763 taxa, including subspecies, varieties, and hybrids. Remarkably, 44.2% of the Lamiaceae family is endemic to Turkey with 360 endemic taxa. The most significant genera of Lamiaceae in Turkey comprise *Salvia*, *Sideritis*, *Origanum*, *Mentha*, *Phlomis*, *Thymus*, and *Stachys*. Due to their medical importance, research has primarily focused on *Salvia*, *Origanum*, *Mentha*, and *Thymus* species.

In general, *Salvia* L. species are used in traditional medicine for their antiseptic, antisudorific, carminative, digestive, diuretic, sedative, and wound healing properties. This genus exhibits various biological activities, such as analgesic, antibacterial, antidiabetic, anticancer, antifungal, antioxidant, antiseptic, antispasmodic, antiviral, astringent, cardiovascular, central nervous system depressant, hallucinogenic, insecticidal, and tuberculostatic activities. *Salvia fruticosa* Mill. (Anatolian sage) is one of the Mediterranean species, with its natural distribution areas spanning from Northern Libya, Sicily, and Southern Italy to the southern part of the Balkan Peninsula, and from Western Anatolia to Western Syria (Karık, 2015).

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In Turkey, *Salvia* species are classified based on the primary component of their essential oil. Accordingly, *S. fruticosa* belongs to the 1,8-cineole/camphor group. The essential oil content ranges between 0.9-2.8%, with 35-51% comprising 1.8-cineole and 7-13% consisting of camphor (Başer, 2002).

The rise of multidrug-resistant (MDR) bacteria poses a significant global health challenge, as these pathogens are responsible for difficult-to-treat infections. The improper and overuse of antibiotics, coupled with their ineffectiveness against biofilm-related infections, has contributed to the rapid emergence of MDR bacteria. In response to this threat, researchers are urgently searching for alternative antimicrobial agents and strategies to combat these infections (Catalano et al., 2022).

Although there are numerous recent publications in the literature reporting the antimicrobial activity of *S. fruticosa*, our study is the first to assess its potency against a wide range of microbial strains, including multidrug-resistant (MDR) microorganisms and yeast strains. This unique aspect of our research highlights the novelty of our work and emphasizes its potential contribution to the development of alternative antimicrobial agents. By investigating the antimicrobial properties of *S. fruticosa* against various microbial strains and MDR microorganisms, we aim to expand the current understanding of its potential therapeutic applications and pave the way for further studies in this field. Free radicals are involved in essential physiological processes, but their uncontrolled accumulation can lead to cellular damage and various diseases. Antioxidants, a diverse group of molecules, can counteract oxidative stress by neutralizing free radicals and protecting cellular structures. As a result, there is a growing interest in identifying novel and effective antioxidants from natural sources like plants (Spigel, 2022).

As we delve deeper into the intricacies of bioactivity studies, it becomes increasingly evident that complementing these investigations with biochemical content analyses is essential for a comprehensive understanding of the underlying mechanisms and variations in the active compounds of plants. Ecological, seasonal, and geographical factors can cause fluctuations in the concentration and composition of these bioactive substances. In addition, given that the majority of these bioactive compounds are volatile in nature, gas chromatography-mass spectrometry (GC-MS) analysis has been recognized as a suitable and reliable method to investigate and quantify these compounds (Piccolella et al., 2018).

In this study, we investigated the bioactive properties of *S. fruticosa* ethanol extract SFEt through a comprehensive analysis that included gas chromatography-mass spectrometry (GC-MS) for the identification and quantification of the volatile compounds present in the extract. We assessed the antimicrobial activity of the extract against various pathogens, including multi-drug resistant strains. Additionally, we examined the antioxidant properties of SFEt. By combining these analyses, we hope to contribute to the existing body of knowledge and promote further research on natural products and their applications.

Materials and methods

Plant samples

S. fruticosa samples were collected from near Kalkım Pond, Çanakkale, Türkiye (39°48’20.64”N, 27°11’34.41”E). A total of 50 individual plants were gathered from different points across the distribution area. The appropriate authorization has been obtained for the collection of the plant, and its use has been carried out in accordance with

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the relevant guidelines. The plant was identified by Dr. Mustafa Eray Bozyel from the Çanakkale Onsekiz Mart University with voucher specimen number: FFDEU-ERA1762 which was deposited at the Fauna and Flora Research and Application Center, Dokuz Eylül University, Buca, Izmir, Türkiye. According to IUCN regulations and standards, the plant is of the Least Concern (LC). The aerial parts of the plant were placed in sample bags and transported to our laboratory. The samples were air-dried at room conditions.

Extraction method

Dried *S. fruticosa* aerial part samples were ground to obtain a fine powder, to increase the surface area for extraction. The crude extract was extracted by pure ethanol (Sigma Aldrich, Saint Louis, MO, USA) through shaking at room temperature for 48 h at 160 rpm. After filtering through Whatman No. 1 filter paper, the ethanol in the extract was evaporated at 45°C under vacuum by using a rotary evaporator (Heidolph Hei-Vap Value HL/HB-G1, Heidolph Instruments GmbH & CO. KG, Schwabach, Germany) (Canli et al., 2016). The remnant was weighed and an extract stock was prepared by using a defined volume of ethanol, and 50 µL and 200 µL of the extracted stock were transferred on empty sterile antibiotic disks to load 3.35 and 13.4 mg extracts on disks respectively.

Antimicrobial activity

The disk diffusion test, as previously described in detail by Bozyel et al. (2021) was employed to evaluate the antimicrobial activity of SFEt against 11 standard, 11 multidrug-resistant (MDR), 10 clinical isolated (CI), and 7 food isolated (FI) bacterial strains, as well as 1 standard and 1 clinical isolated (CI) fungal strains. The incubation conditions for microorganisms, excluding fungal strains, were 37°C for 24 h, while *C. albicans* and *C. tropicalis* were incubated at 27°C for 48 h. Inoculum for each microorganism was prepared in 0.9% sterile saline solution, and the turbidity of all inocula was adjusted by comparing with 0.5 McFarland standard. The Petri dishes containing disks, on which the ethanol extract was loaded, incubated according to the suitable time-temperature combinations mentioned above, and the inhibition zones were observed and recorded in millimeters. Empty antibiotic disk and ethanol-loaded disk were used as negative controls. A wide range of antibiotics was used as positive controls, including Gentamicin, Tobramycin, Ciprofloxacin, Cefazolin, Clindamycin, Chloramphenicol, Ceftriaxone, Ampicillin, Cephalothin, Cefuroxime, Vancomycin, Amoxicillin/Clavulanic acid, Trimethoprim/Sulfamethoxazole, Clarithromycin, Aztreonam, Piperacillin/Tazobactam, Ampicillin/Sulbactam, Ceftazidime, Rifampicin, Oxacillin, Piperacillin, Linezolid, Teicoplanin, Amicasin, Polymyxin B, Cefoxitin, Imipenem, Sulbactam/Cefoperazone, Colistin sulfate, Furazolidone, Optochin, Bacitracin, and Cefotaxime.

Antioxidant activity

DPPH• (2,2-diphenyl-1-picryl-hydrazyl) free radical scavenging activity of SFEt and ascorbic acid as a positive control were tested with the method described by Mensor et al. (2001). Decreasing of DPPH• absorbance, induced by the extract was monitored at 517 nm after incubation at 28°C for 30 min with a spectrophotometer (BioTek Microplate Spectrophotometer, Winooski, VT, USA) (Turu et al., 2020).

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GC-MS analysis

The composition of SFEt was determined according to the protocol given in previous studies by GC-MS analysis. For the identification of chemical components, each sample was analyzed by Agilent GC 6890N-Agilent MS 5973 (Agilent Technologies Inc., Santa Clara, CA, USA) equipped with HP5-MS capillary column (30 m × 0.25 mm; coating thickness 0.25 μm). Analytical conditions were an injector temperature of 350°C; carrier gas Helium at 1 mL/min; injection mode: split, split ratio 10:1; volume injected: 1 μL of sample in ethanol extract; oven temperature programmed from 40°C to 350°C at 4°C/min; pressure: 48.2 kPa; and split flow: 9.9 mL/min. The MS scan conditions were a transfer line temperature of 280°C, an interface temperature of 280°C, and an ion source temperature of 230°C. Identification of the components was conducted by matching the retention times against Wiley-Nist MS data libraries, and crosscheck was applied with previously published data (Canlı et al., 2019).

Statistics

All tests were applied as triplicates. One-way analysis of variance (ANOVA), which is a parametric method was performed ($P = 0.05$). Pearson correlation coefficient was determined for any possible correlation between the intensity of antimicrobial activity and concentration. On the other hand, the activity of the positive controls and SFEt were compared by their z-scores. R Studio, version 2022.12.0 was used for statistical analysis (Canli et al., 2020).

Results

Antimicrobial activity of SFEt

The data obtained from the study about the inhibition zone diameters are shown in *Table 1*. According to the results, negative controls show no activity (Canli et al., 2017). Additionally, statistical analysis verified that the differences between the results of three replicates of each extract volume were statistically non-significant ($p > 0.05$). In addition, obtaining a Pearson correlation coefficient of 0.2276 presented a very weak positive correlation between the antimicrobial activity and the volumes of extracts used. Also, thirteen antibiotics in standard, food isolated, and clinical isolated strains (*Table 2*) and thirty-three antibiotics in multidrug-resistant strains were used as positive controls (*Table 3*).

According to *Table 1,* SFEt shows antimicrobial activity against 21 of 41 strains. Seven of them have a high (≥15 mm), eight of them have a moderate (14-10 mm), and six of them have a low susceptibility (9-7 mm) (Canlı et al., 2019). The most susceptible Gram-positive bacterial strain is ST12 with a 21 mm zone of inhibition, and the Gram-negative bacterial strain is MDR6 with a 13 mm zone of inhibition. SFEt showed higher antimicrobial activity than one or more antibiotics against fifteen bacterial strains.

Antioxidant activity of SFEt

The results of the DPPH• free radical scavenging activity of SFEt and ascorbic acid are shown in *Table 4*. According to the results, SFEt showed the highest antioxidant activity at 200 μg/mL concentration. Also, EC_{50} values of SFEt and ascorbic acid were determined as 0.435 and 0.359 μg/mL, respectively.

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Table 1. *Disk diffusion test results of SFEt (inhibition zones diameters in mm)*

Lab. Code	Microorganisms	**50** μL*	**200** μL*
ST1	*Bacillus subtilis* DSMZ 1971	-	14.00 ± 0.00
ST2	*Candida albicans* DSMZ 1386	10.00 ± 0.00	10.00 ± 0.00
ST3	*Enterobacter aerogenes* ATCC 13048	-	8.00 ± 0.00
ST4	*Enterococcus faecalis* ATCC 29212	9.00 ± 0.00	11.00 ± 0.58
ST5	*Escherichia coli* ATCC 25922	-	8.00 ± 0.00
ST6	*Listeria monocytogenes* ATCC 7644	18.00 ± 0.00	17.00 ± 0.00
ST7	*Pseudomonas aeruginosa* DSMZ 50071	8.00 ± 0.00	13.00 ± 0.00
ST8	*Pseudomonas fluorescens* P1	11.00 ± 0.58	10.00 ± 0.00
ST9	*Salmonella enteritidis* ATCC 13076	9.00 ± 0.00	10.00 ± 0.00
ST10	*Salmonella typhimurium* SL 1344	-	-
ST11	*Staphylococcus aureus* ATCC 25923	16.00 ± 0.00	15.00 ± 0.58
ST12	*Staphylococcus epidermidis* DSMZ 20044	19.00 ± 0.00	21.00 ± 0.00
FI1	*Enterococcus durans*	10.00 ± 0.00	15.00 ± 0.00
FI2	*Enterococcus faecium*	19.00 ± 0.00	18.00 ± 0.00
FI3	*Klebsiella pneumoniae*	-	8.00 ± 0.00
FI4	*Listeria innocua*	10.00 ± 0.00	19.00 ± 0.58
FI5	*Salmonella infantis*	-	-
FI6	*Salmonella kentucky*	-	-
FI7	*Escherichia coli*	-	-
CI1	*Staphylococcus aureus*	17.00 ± 0.00	12.00 ± 1.15
CI3	*Staphylococcus hominis*	-	-
CI4	*Staphylococcus haemolyticus*	-	-
CI5	*Staphylococcus lugdunensis*	-	-
CI6	*Shigella boydii*	-	-
CI7	*Acinetobacter baumannii*	-	-
CI8	*Shigella flexneri*	-	-
CI9	*Staphylococcus aureus*	-	-
CI10	*Enterococcus faecalis*	-	-
CI11	*Klebsiella pneumoniae*	-	-
CI12	*Candida tropicalis*	-	-
MDR1	*Escherichia coli*	-	-
MDR2	*Klebsiella pneumoniae*	-	7.00 ± 0.00
MDR3	*Acinetobacter baumannii*	-	9.00 ± 0.00
MDR4	*Enterobacter aerogenes*	-	8.00 ± 0.58
MDR5	*Serratia odorifera*	-	-
MDR6	*Proteus vulgaris*	-	13.00 ± 0.00
MDR7	*Streptococcus pneumoniae*	7.00 ± 0.58	13.00 ± 0.00
MDR8	*Staphylococcus aureus* MRSA	-	-
MDR9	*Staphylococcus aureus* MRSA + MDR	-	-
MDR10	*Providencia rustigianii*	-	-
MDR11	*Achromobacter* sp.	-	-

"-": No activity (<6 mm); *The data is given as the mean values of three replicates with standard errors
A standard error of 0 indicates that all three parallels yield the same result

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Table 2. *Positive controls against all strains except MDR strains (inhibition zones diameters in mm)*

Lab. code	1	2	3	4	5	6	7	8	9	10	11	12	13
ST1	30	26	36	44	34	37	38	41	36	44	20	52	42
ST2	12	13	-	-	-	-	-	-	-	-	-	-	-
ST3	24	18	30	14	-	26	21	-	-	16	-	9	24
ST4	12	8	19	14	-	19	29	14	-	-	15	28	29
ST5	22	20	7	18	-	22	-	6	6	6	6	16	12
ST6	28	24	27	24	11	25	-	23	-	-	23	34	32
ST7	15	22	18	-	-	9	-	-	-	-	-	-	-
ST8	13	12	19	10	8	22	-	14	-	-	16	26	26
ST9	21	15	24	23	-	28	27	16	-	16	-	28	31
ST11	21	14	22	31	24	21	16	25	22	29	16	30	27
ST12	22	20	34	37	35	33	26	24	26	32	21	45	32
FI1	11	13	24	36	30	8	29	28	22	26	24	37	32
FI2	28	15	28	40	30	11	31	32	24	33	26	43	34
FI3	19	23	30	-	-	22	6	6	6	6	-	9	6
FI4	13	15	18	14	-	23	-	13	-	-	16	28	33
CI1	22	18	23	-	-	-	-	-	-	-	-	-	-

"-": No activity (<6 mm); 1: Gentamicin; 2: Tobramycin; 3: Ciprofloxacin; 4: Cefazolin; 5: Clindamycin; 6: Chloramphenicol; 7: Ceftriaxone; 8: Ampicillin; 9: Cephalothin; 10: Cefuroxime; 11: Vancomycin; 12: Amoxicillin/Clavulanic acid; 13: Trimethoprim/Sulfamethoxazole

Table 3. *Positive controls against MDR strains (inhibition zones diameters in mm)*

Lab. code	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
MDR3	-	-	-	-	-	9	-	-	8	-	8	-	-	-	-	-	-
MDR4	16	18	32	11	-	31	32	-	-	18	-	-	30	15	33	15	-
MDR5	7	9	23	-	-	28	8	-	-	-	8	13	-	-	16	22	10
MDR6	11	11	42	-	9	22	23	9	-	20	-	9	30	10	37	32	12
MDR7	10	8	42	-	9	22	26	9	-	19	9	10	8	10	40	31	15
MDR8	-	7	-	-	45	27	8	12	-	-	26	11	24	14	-	11	12
MDR9	22	21	27	26	38	30	19	22	28	31	19	25	30	15	-	23	23

"-": No activity (<6 mm); 1: Gentamicin; 2: Tobramycin; 3: Ciprofloxacin; 4: Cefazolin; 5: Clindamycin; 6: Chloramphenicol; 7: Ceftriaxone; 8: Ampicillin; 9: Cephalothin; 10: Cefuroxime; 11: Vancomycin; 12: Amoxicillin/Clavulanic acid; 13: Trimethoprim/Sulfamethoxazole; 14: Clarithromycin; 15: Aztreonam; 16: Piperacillin/Tazobactam; 17: Ampicillin/Sulbactam

Table 3. *Continued*

Lab. code	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
MDR3	-	10	-	-	-	-	8	16	8	9	-	13	10	-	-	-
MDR4	31	8	8	-	-	8	29	14	-	28	9	13	25	8	8	30
MDR5	21	9	-	8	-	8	18	14	19	29	10	10	23	8	-	-
MDR6	25	13	-	24	11	8	26	12	12	26	16	-	12	8	-	22
MDR7	27	11	-	24	13	9	29	10	10	30	13	9	13	8	-	14
MDR8	8	-	-	9	40	19	14	-	-	9	7	8	23	-	-	-
MDR9	19	36	17	21	33	18	25	9	20	56	20	8	17	-	8	22

"-": No activity (<6 mm); 18: Ceftazidime; 19: Rifampicin; 20: Oxacillin; 21: Piperacillin; 22: Linezolid; 23: Teicoplanin; 24: Amicasin; 25: Polymyxin B; 26: Cefoxitin; 27: İmipenem; 28: Sulbactam/Cefoperazone; 29: Colistin sulfate; 30: Furazolidone; 31: Optochin; 32: Bacitracin; 33: Cefotaxime

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Table 4. *DPPH• free radical scavenging activity of SFEt*

Concentrations (µg/mL)	**SFEt** (%)	**Ascorbic acid** (%)
200.000	89.80	94.67
100.000	76.21	93.39
50.000	52.37	92.08
25.000	39.07	90.09
12.500	16.89	69.94
6.250	11.52	35.79
3.125	9.33	17.70
1.075	13.97	8.74

Analysis of the biochemical composition of SFEt

The GC-MS analysis of SFEt with its major components, which were observed higher than 1%, and their composition percentages are given in *Table 5*. The GC-MS chromatogram of SFEt is given in *Figure 1*.

Table 5. *Biochemical composition of SFEt*

No	**Retention time**	**Components**	**Formula**	**Molecular weight (g/mol)**	**Area (%)**
1	14.520	1,8-Cineole	$C_{10}H_{18}O$	154.249	2.19
2	19.193	D-camphor	$C_{10}H_{16}O$	152.233	20.27
3	19.809	Isopinocamphone	$C_{10}H_{16}O$	152.233	3.52
4	20.064	(+)-borneol	$C_{10}H_{18}O$	154.249	9.59
5	21.260	Myrtenol	$C_{10}H_{16}O$	152.233	2.24
6	24.585	Bornyl acetate	$C_{12}H_{20}O_2$	196.286	3.60
7	29.223	Caryophyllene	$C_{15}H_{24}$	204.351	8.11
8	34.465	Caryophyllene oxide	$C_{15}H_{24}O$	220.350	2.20
9	51.456	13-Epimanool	$C_{20}H_{34}O$	290.483	2.65
10	58.172	UNKNOWN	-	-	1.12
11	58.998	Cycloeucalenyl acetate	$C_{32}H_{52}O_2$	468.754	3.17
12	60.374	UNKNOWN	-	-	1.18
13	62.177	Isocarnosol	$C_{20}H_{26}O_4$	330.418	1.50
14	63.395	UNKNOWN	-	-	1.44
15	67.998	Isocarnosol	$C_{20}H_{26}O_4$	330.418	1.50
16	84.634	UNKNOWN	-	-	3.33
17	90.428	Ursa-9(11),12-dien-3-ol	$C_{30}H_{48}O$	424.702	2.09
18	91.019	Tetrapentacontane	$C_{54}H_{110}$	759.451	1.91
19	91.396	Sitosterol	$C_{29}H_{50}O$	414.707	2.69
20	92.404	β-Amyrin	$C_{30}H_{50}O$	426.717	1.06
21	94.219	Lupeol acetate	$C_{32}H_{52}O_2$	468.754	6.28
22	100.788	Humulane-1,6-dien-3-ol	$C_{15}H_{26}O$	222.366	1.49
23	102.246	24-Methylenecycloartan-3-one	$C_{31}H_{50}O$	438.728	1.98

"-": No information

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According to *Table 5*, D-camphor (20.27%), (+)-borneol (9.59%), Caryophyllene (8.11%), and Lupeol acetate (6.28%) are mainly found in the composition of SFEt.

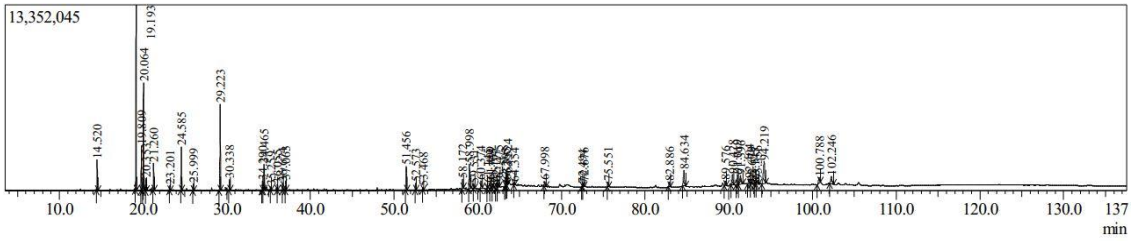

Figure 1. *GC-MS chromatography of SFEt*

Discussion

Ali-Shtayeh et al. (1998) reported the antimicrobial activity of aqueous and ethanolic extracts of *S. fruticosa* against *E. coli*, *K. pneumonia*, *P. aeruginosa*, *P. vulgaris*, *S. aureus*, and *C. albicans*. According to our results, SFEt is more effective against *C. albicans*, *E. coli*, and *P. aeruginosa*. Abdulla-Eltawaty et al. (2021) showed the antimicrobial activity of aqueous, chloroform, ethanol, and methanol extracts of *S. fruticosa* leave and bark against *B. subtilis*, *E. coli*, *P. aeruginosa*, *S. aureus*, and *C. albicans*. When we compared two studies with each other, it appears that SFEt has high antimicrobial activity against *B. subtilis*, *P. aeruginosa*, and *S. aureus*. Canlı et al. (2019) researched the antimicrobial activity of *Lavandula stoechas* (Lamiaceae) ethanol extract against 22 bacteria strains and 1 fungus strain. These strains were the same as tested strains in this study. The authors found that *L. stoechas* ethanol extract showed antimicrobial activity against 21 of 23 strains. Our results presented that SFEt is more effective in all strains except *S. typhimurium* SL 1344 (ST10), *S. infantis* (FI5), *S. kentucky* (FI6), *E. coli* (FI7), and *A. baumannii* (MDR3).

The SFEt extract exhibited greater effectiveness against *E. coli* ATCC 25922 than Ciprofloxacin, Ampicillin, Cephalothin, Cefuroxime, and Vancomycin. Similarly, SFEt showed enhanced results compared to Ceftriaxone, Ampicillin, Cephalothin, Cefuroxime, and Trimethoprim/Sulfamethoxazole when tested against *K. pneumoniae* (FI). SFEt also outperformed Gentamicin, Tobramycin, Ciprofloxacin, Cefazolin, Ampicillin, and Vancomycin when tested against *L. innocua* (FI). Moreover, the SFEt extract exhibited higher activity against *P. vulgaris* (MDR) than Gentamicin, Tobramycin, Clindamycin, Ampicillin, Amoxicillin/Clavulanic acid, Clarithromycin, Ampicillin/Sulbactam, Linezolid, Polymyxin B, Cefoxitin, and Furazolidone, with equal effectiveness to Rifampicin. SFEt also displayed increased activity against *S. pneumoniae* (MDR) compared to Gentamicin, Tobramycin, Clindamycin, Ampicillin, Vancomycin, Amoxicillin/Clavulanic acid, Trimethoprim/Sulfamethoxazole, Clarithromycin, Rifampicin, Teicoplanin, Polymyxin B, Cefoxitin, Colistin sulfate, and Optochin, while showing the same effectiveness as Linezolid, Sulbactam/Cefoperazone, and Furazolidone. Although most synthetic antibiotics yield more effective results, it is essential to note that the SFEt extract is a mixture rather than a single compound. Future studies can focus on purification and determining whether the antimicrobial potential of the plant arises from a single compound or synergistic effects, thereby providing a clearer understanding of the plant's antimicrobial potential.

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The SFEt extract demonstrated a significant 13 mm inhibition zone against *S. pneumoniae* (MDR), which is noteworthy considering the increasing prevalence of antibiotic resistance associated with community-acquired pneumonia (CAP). CAP is responsible for over 3 million annual fatalities worldwide, and antibiotic resistance poses a considerable challenge to the medical community. Streptococcus pneumoniae, the most frequently isolated bacterium during CAP, exhibits increasing resistance to commonly prescribed antibiotics, rendering it a drug-resistant strain. As resistance continues to grow, it is crucial to explore alternative treatments that show potential in combating these resistant strains (Aliberti et al., 2019). The promising results obtained from the SFEt extract may represent a valuable contribution in this context, highlighting the need for further research and development of natural products as potential therapeutic agents to address the issue of antibiotic resistance.

The data were standardized by calculating z-scores, the z-scores of the positive controls and SFEt were compared, and notable z-scores are presented in *Table 6*.

There are some researches in the literature using z-scores to evaluate and compare the antimicrobial activity of natural products and synthetic compounds against a panel of microorganisms (Dhawan et al., 2020).

Table 6. *Notable z-scores of SFEt and positive controls*

Lab. code	50 µL	200 µL	1	2	3	4	5	6	7	8	9	10	11	12	13
ST6	1.21	1.16	1.49	1.45	0.07	-0.03	-0.87	0.40	-	0.42	-	-	0.98	0.50	0.53
ST7	-1.01	0.17	-0.53	1.07	-0.94	-	-	-1.54	-	-	-	-	-	-	-
ST11	0.77	0.66	0.40	-0.48	-0.49	0.56	0.25	-0.08	-1.13	0.61	0.30	0.62	-0.10	0.22	0.03
ST12	1.44	2.15	0.56	0.68	0.85	1.06	1.19	1.38	0.08	0.52	0.66	0.88	0.67	1.29	0.53
FI1	-0.56	0.66	-1.16	-0.67	-0.27	0.98	0.76	-1.66	0.44	0.90	0.30	0.35	1.13	0.72	0.53
FI2	1.44	1.41	1.49	-0.28	0.18	1.31	0.76	-1.30	0.69	1.28	0.48	0.97	1.44	1.15	0.74
FI4	-0.56	1.65	-0.85	-0.28	-0.94	-0.86	-	0.16	-	-0.53	-	-	-0.10	0.08	0.63
CI1	0.99	-0.08	0.56	0.29	-0.38	-	-	-	-	-	-	-	-	-	-
MDR6	-	0.17	-1.16	-1.06	1.74	-	-1.04	0.04	-0.28	-0.91	-	-0.19	-	-1.28	0.33
MDR7	-1.23	0.17	-1.31	-1.64	1.74	-	-1.04	0.04	0.08	-0.91	-	-0.27	-1.18	-1.21	-1.90

"-": No activity (<6 mm); 1: Gentamicin; 2: Tobramycin; 3: Ciprofloxacin; 4: Cefazolin; 5: Clindamycin; 6: Chloramphenicol; 7: Ceftriaxone; 8: Ampicillin; 9: Cephalothin; 10: Cefuroxime; 11: Vancomycin; 12: Amoxicillin/Clavulanic acid; 13: Trimethoprim/Sulfamethoxazole

While positive controls generally displayed higher activity than SFEt, the z-scores - a statistical measure of how many standard deviations a data point is away from the mean - revealed promising results for SFEt, particularly against ST 11 (*S. aureus* ATCC 25923), ST12 (*S. epidermidis* DSMZ 20044), FI4 (*L. innocua*), and CI1 (*S. aureus*), which exhibited higher z-scores compared to the standard antibiotics given in *Table 6* indicating that these microorganisms were more sensitive to the treatment.

In addition, SFEt showed higher z-scores than the antibiotics for some other microorganisms given in *Table 6*, with a few exceptions. Specifically, SFEt had higher z-scores except Ciprofloxacin against MDR7 (*S. pneumoniae*), except Tobramycin against ST7 (*P. aeruginosa* DSMZ 50071), except Gentamycin and Tobramycin against ST6 (*L. monocytogenes* ATCC 7644), except Gentamycin and Vancomycin against FI2 (*E. faecium*), and except Ciprofloxacin and Trimethoprim/Sulfamethoxazole against MDR6 (*P. vulgaris*).

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Finally, it is worth noting that SFEt exhibited higher z-scores than several commonly used antibiotics, including Gentamicin, Tobramycin, Ciprofloxacin, Chloramphenicol, Ceftriaxone, Cephalothin, Cefuroxime, and Trimethoprim/Sulfamethoxazole against FI1 (*E. durans*).

Pasias et al. (2010) identified that *S. fruticosa* ethanol extract showed antioxidant activity with 95% inhibition at 600 μg/mL. Senol et al. (2016) revealed that the highest antioxidant activity of the twelve *Salvia* species studied was observed in Salvia verticillata, with 95% inhibition at 2000 μg/mL. In our study, the antioxidant activity was observed with 89.80% inhibition even at a lower concentration than in other studies. It is clearly understood that SFEt has a higher antioxidant potential.

D-camphor (camphor, (+)-2-Bornanone) shows antimicrobial, anticancer, antiviral, antitussive, analgesic, anti-infective, antipruritic, anticoccidial, anti-nociceptive, insecticidal, stimulant, and carminative activity (Chen et al., 2013). (+)-borneol (borneol, endo-borneol) demonstrates analgesic, antimicrobial, anti-inflammatory, anti-ulcer, and antiviral activity (Sokolova et al., 2017). Caryophyllene (beta-caryophyllene, (-)-β-caryophyllene) exhibits analgesic, anticancer, anti-inflammatory, antimicrobial, and antioxidant activity (Fidyt et al., 2016). Lupeol acetate (Lup-20(29)-en-3-ol, acetate, (3.beta.)-, 3-Acetyllupeol) has antimicrobial, antioxidant, anticancer, antivenom, antifertility, and hypotensive activity (Gallo and Sarachine, 2009). Also, 1,8-cineole, isopinocamphone, myrtenol, bornyl acetate, caryophyllene oxide, 13-epimanool, cycloeucalenyl acetate, ursa-9(11),12-dien-3-ol, and sitosterol present antimicrobial and antioxidant activity (Hendry et al., 2009; Schmidt et al., 2010; Sen et al., 2012; Buchbauer and Ilic, 2013; Musini et al., 2015; Venditti et al., 2015; Fidyt et al., 2016; Salem et al., 2016; Zubair et al., 2017; Maione et al., 2022; Mssillou et al., 2022). In the light of these data, it is considered that D-camphor, as the main component, is responsible for antimicrobial and antioxidant activity. It is also believed that other major and minor components singularly or synergistically contributes to antimicrobial and antioxidant activities.

Conclusion

SFEt was found to be antimicrobial against a wide range of tested strains. High susceptibility was observed in about 34% of these strains. 23 components were identified in SFEt, the main one is D-camphor (20.27%). Purification and further studies are recommended to determine whether the antimicrobial activity is caused by a single known biochemical component, such as D-camphor, or by a synergistic effect. SFEt showed antioxidant activity almost as high as Ascorbic acid. In addition, it should be investigated in detail whether the antioxidant activity is caused only by Caryophyllene or whether it has a synergistic effect with other components. In addition, some of the components that are found in the extract do not match the library. For this reason, it is proposed that this medicinal plant contains some unknown molecules, and they should be identified and their 3D structure should also be determined. The unknown component, which consists of 7.07%, should be analyzed in detail. Also, the mode of action(s) of the extract should be determined in further studies.

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