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Original Article

Spectral Characterization, Antioxidant and Antibacterial Activities of Cold Methanolic Extract of Sweet Basil (*Ocimum basilicum* L.) Leaves Growing in Jazan, Kingdom of Saudi Arabia

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ABSTRACT

Background: This study evaluated the chemical composition, antioxidant activity and antibacterial properties of methanol extracts of *Ocimum basilicum* L. leaves that were gathered from the Kingdom of Saudi Arabia's Jazan province. **Materials and Methods:** The extract was examined using FTIR and GC-MS techniques, which revealed the presence of terpenes (53.14%), Linalool (24.5%), sulfoxides (4.19%), cinnamic acid (5.23%), fatty acid esters (1.45%) and phthalic acid esters (0.72%). **Results:** The extract contained significant levels of total phenolic compounds and demonstrated a DPPH radical scavenging capacity of 33.70%, which is considered to be good. The antibacterial activity spectrum of *O. basilicum* leaves' methanolic extract was found to be broader. The extract was tested against eight different bacteria strains, including *S. cholestasis, S. aureus, S. epidermidis, K. pneumoniae, E. coli, P. mirabilis, E. faecalis*, and *S. cholestasis*. **Conclusion:** With an inhibition zone of 21 mm, *S. aureus* exhibited the highest level of antibacterial activity, whereas *P. aeruginosa* demonstrated the lowest value, with an inhibition zone of 15 mm. Nonetheless, the activity range was more constrained than that of regular ciprofloxacin discs.

Keywords: *Ocimum basilicum*, Sweet Basil, Ftir Spectroscopy, GC-MS, Antioxidant Activities, Antibacterial Activities.

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INTRODUCTION

Ocimum species have various essential oils that are abundant in phenolic compounds and other natural products, such as polyphenols like flavonoids and anthocyanins. The Lamiaceae family's *Ocimum* genus is one of the most extensive genera, with over 150 species.[1,2] Sweet basil, scientifically known as *O. basilicum* L., is an herb that grows once a year in numerous regions across the globe. It is widely utilized in different food as well as oral hygiene commodities. Basil is regarded as one of the most essential herbs globally and is even referred to as the 'King of herbs.' Basil's essential oils are abundant in natural products, polyphenols including anthocyanin and flavonoids and phenolic compounds.[3] Also, it is important to note that the timing of the harvest plays a significant role in obtaining the maximum percentage of essential oil.[4] "The composition and quantity of the distinct aromatic compounds present in essential oils are determined based on the chemo diversity.[5,6] Gas Chromatography coupled with Mass Spectrometry (GC-MS) is a widely used technique for analysing essential oils, but it takes a lot of time and complicated instrument assembly.[7,8] However, spectroscopic methods based on Raman and FTIR are faster and non-destructive. These techniques can be used to differentiate closely related plant varieties.[9-11]

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Extracted from *Ocimum* plants, these oils are renowned for their remarkable biological properties and have been historically used in perfumery and aromatherapy to prevent the growth of microorganisms for food preservation.[12] Notably, Sweet basil, one of the most commonly used culinary herbs, is recognized for its potent antioxidant and antimicrobial activities, which are attributed to its phenolic acids and aromatic compounds. As a result, there is a growing demand for essential oils as food preservatives, which have been shown to be biologically effective against food-borne pathogens such as listeria mono-cytogenesis, salmonella typhimurium, clostridium per fringes, *Staphylococcus aureus* and *Pseudomonas putida*. Basil leaves are also utilised in traditional medicine to treat a range of illnesses, including epilepsy, bronchitis, nausea, cancer, diarrhoea, gout, sore throat and convulsion.[13] The relationship between the chemical components of essential oils and the aroma of different types of basil is quite fascinating. According to Vina and Murillo,[14] the distinctive aromatic smell of basil is caused by varying ratios of major chemical components found in the essential oils. Some of the popular compounds such as eugenol, estragole or methyl chavicol and linalool are responsible for giving essential oils their unique flavors and tastes. Oxidative stress is a significant risk factor for chronic diseases. Researchers are exploring the antioxidant and antimicrobial potentials of essential oils from *O. canum, O. sanctum, O. basilicum, and O. gratissimum species.*[15-20] Traditional medicine has utilised *O. basilicum*, also referred to as basil, to cure a variety of ailments such as headaches, coughs and diarrhoea. Studies have demonstrated that this herb is abundant in phenolic antioxidant compounds and flavonoids, making it a valuable natural source of antioxidants.[21] The current study conducted in the Jazan region aimed to investigate the phytochemical composition of Sweet Basil and evaluate its antioxidant and anti-bacterial properties.

MATERIALS AND METHODS

Chemicals and Reagents

The study utilized chemicals, solvents and reagents of chromatographic grade that were bought from Sigma Aldrich (USA).

Study area, collection and identification

The plant's aerial portion was procured from Jazan in Saudi Arabia and transported to the lab in bio-hazard bags. The leaves underwent a washing process to eliminate impurities. Jazan University Herbarium's (JAZUH) curator, Dr. Remesh Moochikkal, confirmed the plant's authenticity by examining the specimen (Ref. number JAZUH 1316). *O. basilicum* leaves were isolated from the branches, cleansed using Millipore water and air-dried for 30 min. After that, the aerial portion was dried for 10 days in a place with good ventilation. For additional analysis, the dried aerial portions were gathered, crushed and kept correctly.

Organic Solvent extraction

The extraction of bioactive components from *O. basilicum* leaves was carried out using a cold methanol maceration process. The powder of dried leaves was dissolved in methanol in a quantity of 100 mL. The mixture was then kept refrigerated at 4°C for an overnight duration. Next week, the mixture was stirred again with the help of a magnetic stirrer and centrifuged at a rate of 2000 g for 10 min by using a Sigma tabletop centrifuge. Whatman filter paper number one was used to filter the supernatant solution, which was then kept for later use at 4°C.

GC-MS analysis of methanolic extract of *O. basilicum* leaves

Sample preparation

Dried methanolic extract of leaf of *O. basilicum* (10 mg) was dissolved in methanol (1 mL), vortex-mixed for 10 min, filtered through 0.2 nylon filter and injected 2 L of sample in the GCMS.

Instrumentation

O. basilicum leaf methanolic extract components were identified using Thermo Scientific GC-MS equipped with an AS 3000 autosampler, Trace Ultra-GC and ISQ detector.

Method A: A 0.25 m nylon filter was used to filter the sample before 2 µL was injected in split-less mode into a TR-5MS capillary column (30 mx0.25 mm IDx0.25 µm. The carrier gas used was helium, which had a flow rate of 1.2 mL/min. The oven was set with a ramping program having initial temperature set at 50°C for 10 min and subsequently ramped to 70°C, 100°C, 150°C, 200°C, 250°C, 270°C, 290°C at a rate 5°C/min with holding time of 10 min (total run time of 113 min). The MS-detector ISQ was set to identify the molecular masses ranging from 40-650 amu at 70 eV in positive ion mode. 3 min was the delay period used to record the spectra in order to avoid the appearance of any first solvent peaks. The temperatures of the injector port, MS transfer and ion source were adjusted to 250°C, 270°C and 280°C, respectively.

ATR-FTIR Analysis

Thermo Fischer Scientific, USA, produced the Nicolet iS10 FT-IR spectrophotometer, which was used to record the spectra of methanolic preparations of *O. basilicum* leaves. The samples were precisely positioned on a zinc selenide crystal and 64 scans were carried out spanning the transmittance range of 4000-400 cm^{-1} in order to get the spectra at 8 cm-1 resolution.[22]

Antioxidant Activity

The total free radical scavenging capacity of the plant sample extracts was assessed using the stable 1,1-Diphenyl-2-Pic rylhydrazyl (DPPH) radical in accordance with the procedure outlined by Brand-Williams W.[23] 2.4 mg of DPPH were dissolved in 100 mL of methanol to produce the radical solution. 3.9 mL

of methanolic DPPH were added to the test solution and it was thoroughly agitated. Following that, the mixture was kept in the dark at room temperature for 30 min. At 517 nm, the absorbance of the reaction mixture was determined using spectrophotometry. Additionally, the absorption of the "blank," or DPPH radical, in the absence of antioxidants was investigated. The blank contained 80% (v/v) methanol. The determination was repeated three times. The percentage of antioxidants was calculated using the formulae:

$$\text{DPPH Scavenged (\%)} = ((AB - AA)/AB) \times 100$$

where AA is the sample's absorbance following a 30 min incubation period with antioxidants and AB is the sample's absorbance at t=0 min.

In vitro antibacterial activity

Bacterial strains used and standardization of bacterial cultures

Numerous bacterial strains were used in the experiment, including *K. pneumoniae* ATCC 700603, *S. cholestasis* ATCC 10708, *P. mirabilis* ATCC 299, *E. faecalis* ATCC 29212, *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922, *S. epidermidis* ATCC 12228 and *S. aureus* ATCC 25923.

Determination of antibacterial susceptibility

The antimicrobial susceptibility test was performed using the Moni *et al* method.[24] Muller Hinton agar plates were set up in order to conduct the antibacterial investigation. After a 24-hr incubation period, bacterial subcultures were extracted from the stock culture and used in antibacterial investigations. Agar well diffusion was used to test the sample analytes and disc diffusion was used to test the standard ciprofloxacin disc (5 mcg/disc). A sterile cotton brush dipped in the standardised (CFU/mL) culture of each different organism separately was used to evenly distribute the culture on the MH agar plate after the petri dish was rotated. Before sample analytes were administered, the plates were allowed to dry for around ten min. Using a conventional sterile stainless-steel borer, holes were punched on the inoculated MH agar plates in order to implement the agar well diffusion procedure. Following a 24 hr incubation period at 37°C, the development of inhibitory zones was examined to determine the antibacterial spectrum. Table 1 lists the diameter of the inhibition zones, which are directly correlated with the spectrum of activity.

Statistical analysis

The research was carried out using the Prism 9 software system, which was created by Graph Pad Instat in the United States. The data were analysed using a one-way ANOVA and then *post hoc* Tukey's test. For all analyses, we considered $p<0.05$, $p<0.01$ and $p<0.001$ as statistically significant.

RESULTS

GC-MS analysis

Data Analysis

Software-generated match factors (SI) and Reverse match factors (RSI) with thresholds of 900 and above were used to ascertain the composition of the methanolic leaf extract. The spectrum matching was performed to compare the fragmentation pattern of the EI mass spectrum with the reference mass spectra in the library. Based on the best (forward or reverse-search) mass spectrum matching score, a list of the most probable identities is generated. By dividing each component's peak area by the entire peak area, the relative content (%) of each component was determined using the Xcalibur software. The peak area was calculated without any internal standard and is uncorrected. Table 1 and Figure 1 provides information on all observed compounds, including their relative concentration (%), molecular weight, molecular formula, retention time and chemical class.

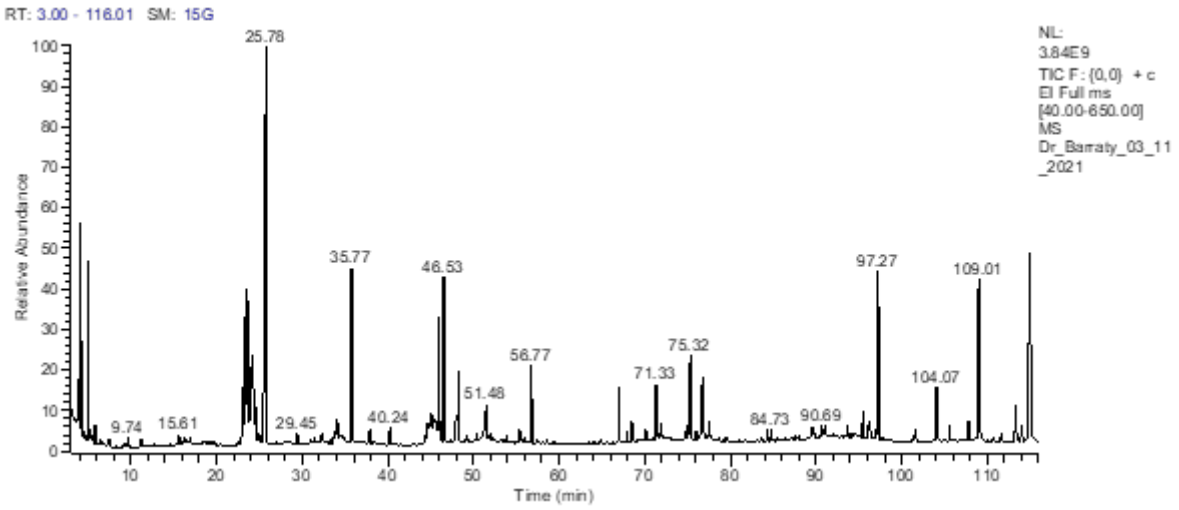

Figure 1: GC-MS Total ion chromatogram of methanolic leaf extract of *Ocimum basilicum*.

Table 1: Chemical compounds of methanolic leaf extract of *O. basilicum* by Gas Chromatography-Mass Spectrometry (GCMS).

Sl. No.	RT	Name of Compound	MF	MW	% Relative content	Chemical Class
1	4.34	Methyl (methylsulfinyl)methl sulfide (FAMSO).	$C_3H_8OS_2$	124	1.56	Sulfoxide
2	5.02	Methyl benzyl sulfoxide.	$C_8H_{10}OS$	154	2.63	Sulfoxide
3	5.83	Isopropyl acetate	$C_5H_{10}O_2$	102	0.34	Aliphatic ester.
4	6.39	Tyrosine	$C_9H_{11}NO_3$	181	0.09	Aromatic amino acid.
5	9.18	3,3-Dimethoxy-2-butanone	$C_6H_{12}O_3$	132	0.10	Aliphatic ketone.
6	9.50	2-Ethyl-1,3-dioxolane-4-methanol	$C_6H_{12}O_3$	132	0.09	Dioxolane derivative.
7	9.75	1,3:2,4-di-O-Benzylidene-ribitol	$C_{19}H_{20}O_5$	328	0.20	Pentose alcohol derivative.
8	10.55	Xylene	C_8H_{10}	108	0.09	Aromatic hydrocarbon.
9	16.93	beta-Pinene	$C_{10}H_{16}$	136	0.18	Bicyclic monoterpenoid(Essential oils plant).
10	19.38	Eucalyptol (Cineole)	$C_{10}H_{18}O$	154	0.06	Monoterpenoid bicyclic ether.
11	22.46	cis-Linalool oxide	$C_{10}H_{18}O_2$	170	0.09	Monoterpenoid (oxolanes, a tertiary alcohol and an olefinic compound).
12	25.78	Linalool	$C_{10}H_{18}O$	154	24.64	Monoterpenoid with tertiary alcohol.
13	29.45	Camphor	$C_{10}H_{16}O$	152	0.21	(Monoterpenoid)Cyclic monoterpene ketone.
14	31.43	4-Terpinenol	$C_{10}H_{18}O$	154	0.11	Monoterpenoid isomer.
15	32.20	2,6-Dimethyl-3,7-octadiene-2,6-diol	$C_{10}H_{18}O_2$	170	0.13	Tertiary alcohol
16	32.33	Alpha-Terpineol	$C_{10}H_{18}O$	154	0.22	Monoterpenoid isomer
17	33.26	Fenchyl acetate	$C_{12}H_{22}O_2$	196	0.10	Monoterpenoid.
18	33.69	Geraniol (Lemonol).	$C_{10}H_{18}O$	154	5.10	Monoterpenoid with primary alcohol.
19	37.88	Borneol acetate	$C_{12}H_{20}O_2$	194	0.35	Bicyclic monoterpenoid.
20	44.67	Linalool formate	$C_{11}H_{18}O_2$	182	0.42	Monoterpenoid tertiary with alcohol ester.
21	45.96	Geraniol acetate	$C_{12}H_{20}O_2$	196	2.25	Monoterpenoid with primary alcohol ester.
22	46.53	Cinnamic acid methyl ester.	$C_{10}H_{10}O_2$	162	5.23	Aromatic ester.
23	48.26	Alpha-Bergamotene.	$C_{15}H_{24}$	204	1.31	Sesquiterpene bridged compound, with a polycyclic olefin.
24	49.21	Alpha-Caryophyllene (alpha-Humulene).	$C_{15}H_{24}$	204	0.19	Sesquiterpene
25	49.50	Bicyclosesquiphellandrene.	$C_{15}H_{24}$	204	0.07	Sesquiterpene, a member of octahydronaphthalenes.
26	50.38	trans-beta-Farnesene.	$C_{15}H_{24}$	204	0.11	Sesquiterpene
27	51.48	Cadinene	$C_{15}H_{24}$	204	0.84	Sesquiterpene, a member of octahydronaphthalenes.
28	52.30	Dihydroactinidiolide	$C_{11}H_{16}O_2$	180	0.08	Benzofuran derivative

29	53.48	n-Dodecanoic acid (lauric acid)	$C_{12}H_{24}O_2$	200	0.05	Saturated medium-chain fatty acid
30	53.83	Spathulenol	$C_{15}H_{24}O$	220	0.11	Sesquiterpenoid
31	55.42	Cubenol	$C_{15}H_{26}O$	222	0.31	Sesquiterpenoid with tertiary alcohol, a member of octahydronaphthalenes.
32	56.77	Cedrelanol	$C_{15}H_{26}O$	222	1.61	Cadinane sesquiterpenoid, a carbobicyclic compound, a tertiary alcohol and a member of octahydronaphthalenes.
33	57.03	Methyl jasmonate	$C_{13}H_{20}O_3$	224	0.04	It is a jasmonate ester, a methyl ester and a member of Jasmonate derivatives.
34	58.12	Bulnesol	$C_{15}H_{26}O$	222	0.08	Sesquiterpenoid
35	61.52	Methyl tetradecanoate	$C_{15}H_{30}O_2$	242	0.08	Fatty acid methyl ester.
36	64.09	Tetradecanoic acid (Myristic acid).	$C_{14}H_{28}O_2$	228	0.10	Saturated long-chain fatty acid.
37	66.98	3,7,11,15-tetramet hyl-1-Hexadecen-2-ol.	$C_{20}H_{40}O$	296	1.56	Unsaturated fatty alcohol.
38	70.13	Palmitic acid methyl ester.	$C_{17}H_{34}O_2$	270	0.24	Fatty acid methyl ester.
39	71.33	n-Hexadecanoic acid.	$C_{16}H_{32}O_2$	256	1.29	Saturated long-chain fatty acid.
40	74.71	Geranyl isovalerate	$C_{15}H_{26}O_2$	238	0.30	Fatty alcohol ester.
41	71.95	Hexadecanoic acid ethyl ester	$C_{18}H_{36}O_2$	284	0.24	Fatty acid ethyl ester.
42	75.04	Linolenic acid methyl ester.	$C_{19}H_{32}O_2$	292	0.51	Fatty acid methyl ester.
43	75.32	Phytol	$C_{20}H_{40}O$	296	1.76	Acyclic diterpene alcohol.
44	76.43	Linoleic acid ethyl ester.	$C_{20}H_{36}O_2$	308	0.10	Fatty acid ethyl ester.
45	76.74	Linolenic acid	$C_{18}H_{30}O_2$	278	1.82	Omega-3 fatty acids.
46	77.53	Linolenic acid ethyl ester.	$C_{20}H_{34}O_2$	306	0.38	Fatty acid ethyl ester.
47	86.33	9-Octadecenamide	$C_{18}H_{35}NO$	281	0.08	Fatty acid amide.
48	87.19	Octacosane	$C_{28}H_{58}$	394	0.12	straight-chain alkane.
49	87.65	2,2'-Methylenebis(4-methyl-6-tert-butylphenol).	$C_{23}H_{32}O_2$	340	0.15	Diarylmethane
50	90.69	Diisooctyl phthalate	$C_{24}H_{38}O_4$	390	0.19	Phthalic acid ester
51	91.98	Hexacosane	$C_{26}H_{54}$	366	0.11	Straight-chain alkane
52	94.22	Heptacosane	$C_{27}H_{56}$	380	0.09	Straight-chain alkane
53	94.49	1-Mono-linolenin	$C_{21}H_{36}O_4$	352	0.13	Monoglyceride
54	95.46	Bis(2-ethylhexyl) isophthalate	$C_{24}H_{38}O_4$	390	0.53	Phthalic acid ester
55	97.27	Squalene	$C_{30}H_{50}$	410	3.43	Triterpene
56	101.64	Geranylgeraniol	$C_{20}H_{34}O$	290	0.40	Diterpenoid
57	107.80	alpha-Tochopherol	$C_{29}H_{50}O_2$	430	0.46	Vitamin E
58	110.69	Campesterol	$C_{28}H_{48}O$	400	0.15	Phytosterol
59	111.60	Stigmasterol	$C_{29}H_{48}O$	412	0.21	Phytosterol
60	114.02	Sitosterol	$C_{29}H_{50}O$	414	0.46	Phytosterol

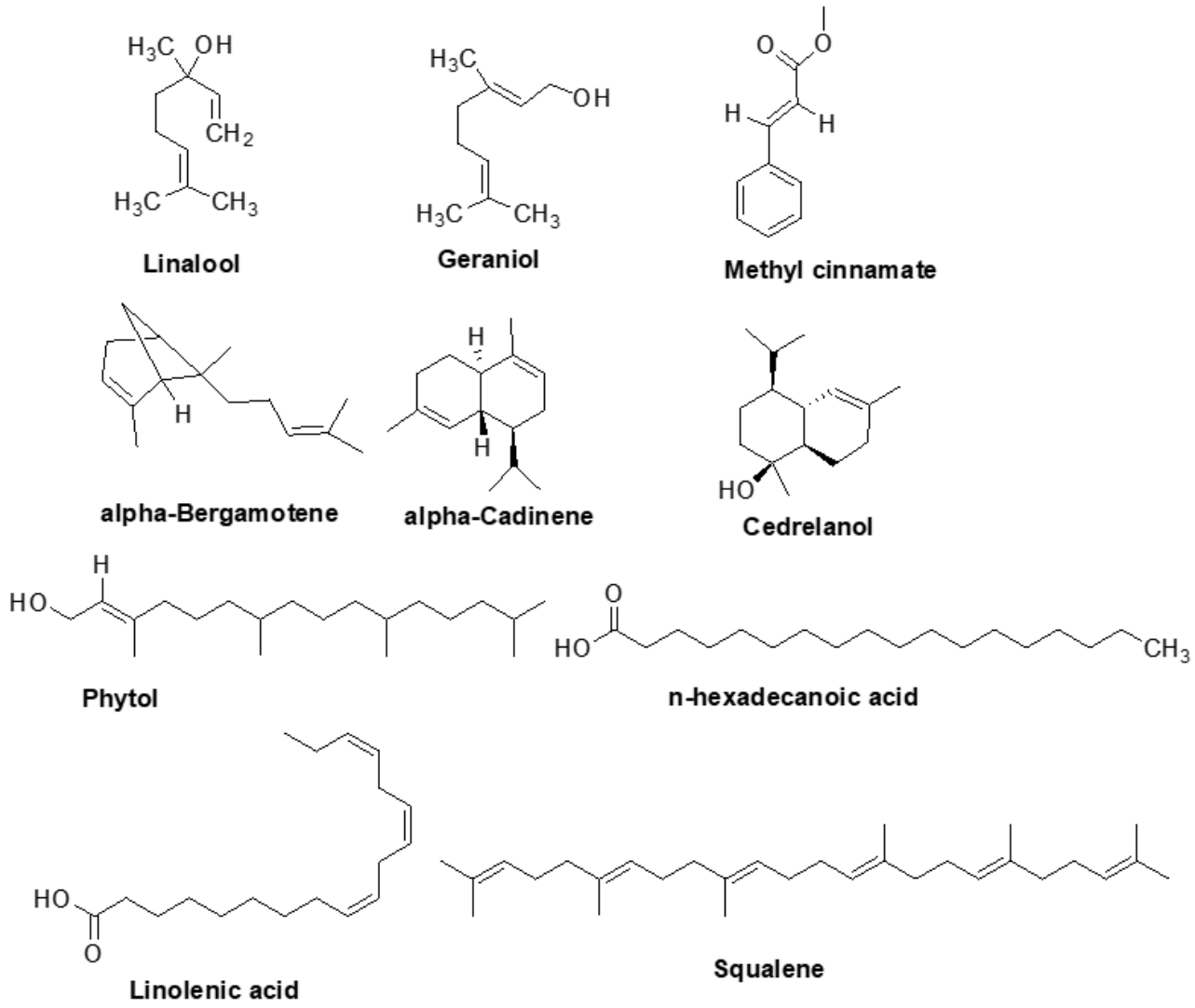

Figure 2: GC-MS detection of bioactive compounds of methanolic leaf extract of *O. basilicum*.

The methanolic leaf extract of *O. basilicum* appeared as a dull green colored semisolid material with sweet aromatic odor. The GC-MS analysis showed the presence of terpenes (53.14%) as the major class among all the chemical constituents. Linalool (24.5%), a monoterpenoid with tertiary alcohol was most abundant component in the leaf extract followed by Geraniol having the primary alcoholic group (5.10%). Both linalool and geraniol were also present in their formate (0.42%) and acetate ester (2.25%) forms respectively. Overall, the monoterpenoid contents were found to be 33.73%. Esters represents the next major class (8.18%) followed by sulfoxides (4.19%). Methyl ester of cinnamic acid (5.23%) was the major ester component. Besides cinnamic acid esters, fatty acid esters (1.45%) and phthalic acid esters (0.72%) were also present. Linolenic acid (1.82%), an essential fatty acid belonging to the omega-3 fatty acids group was also present in the sample. Besides this, phytosterols (0.82%), alpha-tocopherol (0.46%), straight chain alkanes (0.20%), pentose alcohol (0.20%), aliphatic ketones (0.10%) and fatty alcohols (1.56%) were also found in the leaf extract Overall, a total of 60 compounds were identified with a mass balance of 63.88%. The details of these compounds are given in Table 1 and Figure 1. Structures for the most abundant bioactive compounds of methanolic leaf extract of *O. basilicum* are presented in Figure 2.

FT-IR spectroscopy analysis

All the methanolic components mentioned above were confirmed using FTIR Spectroscopy measurement. *O. basilicum* leaf extract showed 12 prominent peaks, as shown in Figure 3 and Table 2. The absorption bands were observed in two wavenumber regions: 400-2000 cm^{-1} and 2000-4000 cm^{-1}. The first peak at 3309 cm^{-1} represents the alcohol and hydroxyl compound class, which is distinguished by OH stretches that are internally bonded, tertiary alcohol OH stretches and OH stretches found in phenols. The peak at 2971 cm^{-1} represents the Olefinic (alkene) compound and the C-H stretch. Stretching Methylene C-H asym./sym. is identified by the peaks at 2918 cm^{-1} and 2846 cm^{-1}, respectively.

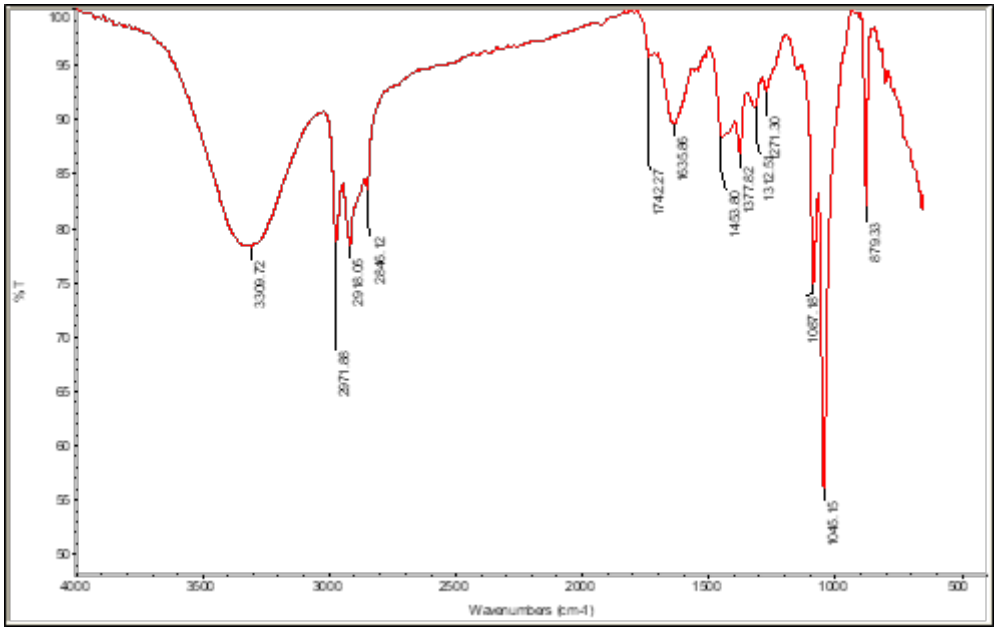

Figure 3: FT-IR spectra of methanolic leaf extract of *O. basilicum*.

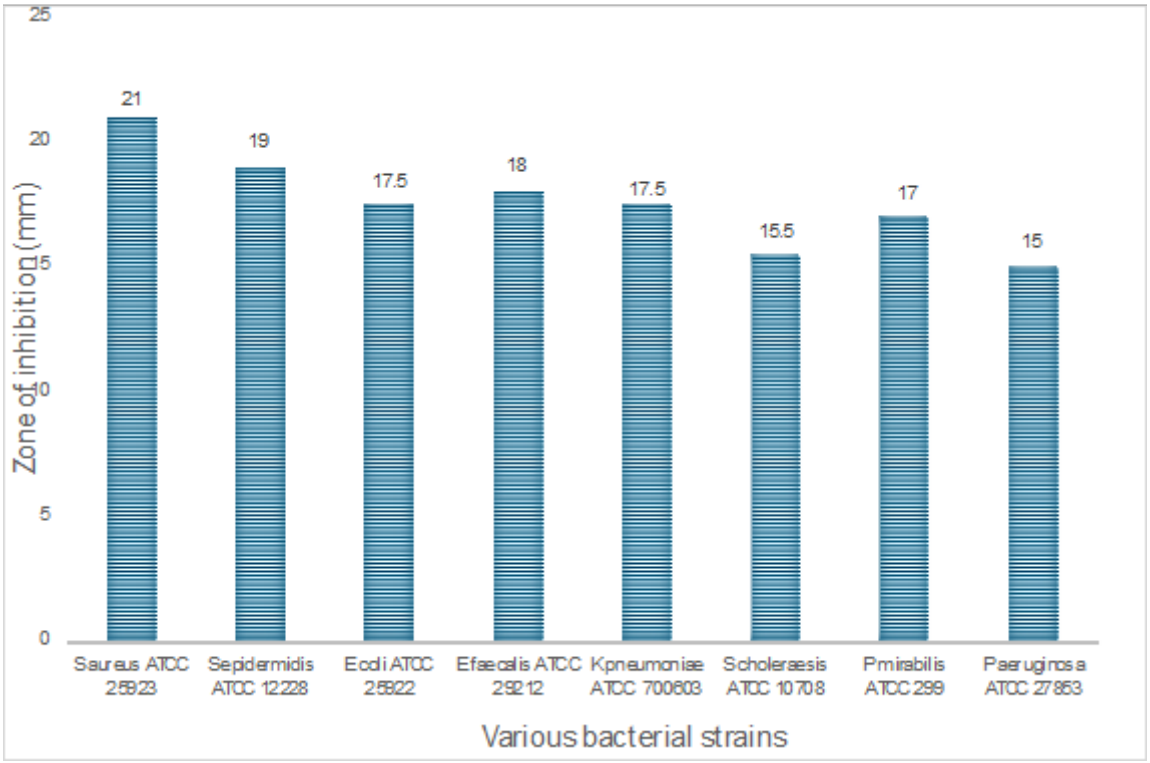

Figure 4: The antibacterial spectrum of cold methanolic extract of *Ocimum basilicum* leaves against screened bacterial organisms.

The peak at 1742 cm^{-1} is a strong one that is characterized by C=O stretching and NH bending.

It is noticeable from the spectral data that the exocyclic C=C stretch and Olefinic (alkene) correspond to the band at 1635 cm^{-1}. The band at 1453 cm^{-1} is marked by methyl C-H asym./sym bending. The O-H bending vibration is characterized by the peaks at 1377 cm^{-1}. Furthermore, the peaks at 1271, 1087 and 1045 cm^{-1} indicate CN, C-O and S=O stretching vibrations, respectively. The peak at 879 cm^{-1} is found as stretching Aromatic C-H out-of-plane bend.

Antioxidant Effects

Table 3 represents the antioxidant activity of *O. basilicum* methanolic extract and was found to be 33.70% using DPPH free radical scavenging assay. Free-radical DPPH absorbs most odd

Table 2: Chemical composition of methanolic leaf extract of *O. basilicum* from FTIR Spectroscopy.

Wave number (cm^{-1})	Intensity Estimation	Functional group	Type of vibration	Possible Compounds
3309	Medium	O-H	Stretching	alpha-Tochopherol, Campesterol, Stigmasterol, Sitosterol, Phenolic compounds.
29712918	Strong	C-H	Asymmetrical Stretching	Methyl benzyl sulfoxide, squalene, Camphor.
2846	Weak	CH_2 str. (Symmetrical)	Stretching	Alkanes, Carboxylic acids and Derivatives, beta-Pinene, Camphor, Octacosane.
1742	Strong	C=O, N-H	C=O StretchingNH bending	Isopropyl acetate, 3,3-Dimethoxy-2-butanone, Camphor, Fenchyl acetate, Borneol acetate, Linalool formate, Geraniol acetate, Cinnamic acid methyl ester, Tetradecanoic acid (Myristic acid), Palmitic acid methyl ester, n-Hexadecanoic acid, Geranyl isovalerate, Linolenic acid methyl ester, Linoleic acid ethyl ester, Linoleic acid, Bis(2-ethylhexyl) isophthalate, 1-Mono-linolenin.
1635	Strong	C=C	Stretching	1,3:2,4-di-O-Benzylidene-ribitol, beta-Pinene, Linalool, Geraniol (Lemonol), Geraniol acetate, Cinnamic acid methyl ester, Alpha-Bergamotene, Alpha-Caryophyllene (alpha-Humulene), trans-beta-Farnesene, Cadinene, Cubenol, Geranylgeraniol.
1453	Strong	C-H	Asymmetrical bending	Methyl group (Aliphatic compounds), Xylene, Linalool, 4-Terpinenol, Geraniol (Lemonol), Alpha-Bergamotene, Alpha-Caryophyllene (alpha-Humulene), Phytol, Camphor.
1377	Medium	O-H	Bending	2-Ethyl-1,3-dioxolane-4-methanol, 1,3:2,4-di-O-Benzylidene-ribitol, 2,6-Dimethyl-3,7-octadiene-2,6-diol, Linalool, 4-Terpinenol, alpha-Terpineol, Geraniol (Lemonol), Spathulenol, Cubenol, Cedrelanol, 3,7,11,15-tetramethyl-1-Hexade cen-2-ol, Phytol, Geranylgeraniol.
1271	Medium	C-N	Stretching	Amine, 9-Octadecenamide.
1087	Strong	C-O	Stretching	2-Ethyl-1,3-dioxolane-4-methanol, 1,3:2,4-di-O-Benzylidene-ribitol.
1045	Strong	S=O	Stretching	Methyl (methylsulfinyl)methl sulfide, Methyl benzyl sulfoxide.
879	Strong	C-H	Out of plane bending	Arenes, Amines, Camphor.

electrons at 517 nm (purple color). Due to its lower hydrogen content, DPPH, formed by a free-radical scavenger antioxidant, has a lower absorbance than DPPH. As electrons are gathered, this radical state decolorizes (yellow) unlike the DPPH-H state. Further quantitative analysis of antioxidant activity was conducted and the results are depicted in % DPPH scavenging capacity (Table 3).

Antibacterial Effects

The results are summarized in Table which self-exemplary that the samples shows good activity and sequenced as *S. aureus*>*S. epidermidis*>*E. faecalis*>*E. coli*>*K. pneumoniae*>*P. mirabilis*>*S. choleraesis*>*P. aeruginosa*. The activity spectrum of the sample against screened organisms was satisfactory, as depicted in Figure 4 and Table 4.

DISCUSSION

In GCMS study, the reported percentage content of terpenoids in the leaf extract was less than those reported in the oil samples in the literature.[25-27] Other terpene derivatives such as cerdrelanol (1.61%, sesquiterpenoid), phytol (1.76%, diterpenoid) and squalene (3.43%, triterpene) were also found in the leaf extract. These terpene derivatives were reported in lesser quantities in the oil samples reported in the literature.[25-27]

Table 3: Qualitative and quantitative estimation of Antioxidant activity by DPPH.

Sl.No.	Samples	Antioxidant	% Antioxidant at (100 µL)
1	Samples-1 MSA-Rh Ocimum basilicum	+	33.70±02

R=No of replicates three.

Table 4: Antibacterial study of the cold methanolic extract of *Ocimum basilicum* leaves.

Organisms	Zone of inhibition (mm)	
	Extract	Ciprofloxacin (5 µg/Disc)
S. aureus ATCC 25923	21±1.5	33.66±1.20
S. epidermidis ATCC 12228	19±2	33.3±1.20
E. coli ATCC 25922	17.5±2.5	35.66±1.0
E. faecalis ATCC 29212	18.0±1.5	25.33±0.5
K. pneumoniae ATCC 700603	17.5±2	26±1.5
S. choleraesis ATCC 10708	15.5±0.5	33.3±1.20
P. mirabilis ATCC 299	17±2	23.66 + 1.25
P. aeruginosa ATCC 27853	15±2	33.3±1.0

* For statistical analysis, GraphPad Prism 9 was utilised. The student *t*-test was utilised to analyse the data, which is the mean of three batches. Comparing the test results to the standard value, they are considerably lower at $p>0.05$.

Notably, the results obtained from FTIR spectral data validate the findings of Gas chromatography and vibrational spectroscopic work conducted for characteristic components. Similar strong bands owing to CH stretching can be seen in our sample of *O. basilicum* at 2930 and 2933 cm-1, along with a peak at 1638 belonging to the C=C aromatic component.[28] According to Michelina *et al.*,[29] the strongest band in the FTIR spectrum at 1742 cm^{-1} belongs to the carbonyl stretching (C=O) of camphor, while the strongest band at 1453 cm^{-1} correlates to methylene deformation.[30] Furthermore, our FTIR spectrum data of camphor is consistent with the prior FTIR spectral data of the compound, which corresponds to the peaks at 2873, 2959, 1448, 1046 and 751.[31]

In antioxidant activity, the qualitative results show that sample is positive in antioxidant activity. The sample's antibacterial effectiveness was notably lower in comparison to standard ciprofloxacin disc (5 mcg/disc). The sample exhibited a promising activity spectrum when tested against both Gram-positive and Gram-negative bacteria.

CONCLUSION

The principal components of *O. basilicum* species have been identified and distinguished successfully using FTIR and GCMS techniques. The spectral data obtained from both techniques has been consistent with prior data on individual principle components present in the methanolic extract sample. *O. basilicum* can be utilized for various purposes based on the aroma and principal essential oil compounds, which are primarily terpenes and linalool. This species is ideal for value addition and perfumery. The overall phenolic contents of the basil extracts are noticeable and they have shown good DPPH radical scavenging ability. Furthermore, against a range of Gram-positive and Gram-negative bacteria, *O. basilicum* L. leaf extract has shown strong antibacterial activity. It is advisable to extract and segregate the bioactive molecules responsible for this antibacterial activity.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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ABBREVIATIONS

DPPH: 1,1-diphenyl-2-picrylhydrazyl; **SI:** Software-generated match factors; **RSI:** Reverse match factors; **GCMS:** Gas chromatography-mass spectrometry.

SUMMARY

The study examined the chemical composition, antioxidant activity and antibacterial properties of methanol extracts of *Ocimum basilicum* L. leaves from the Jazan province in Saudi Arabia. The extract contained terpenes, Linalool, sulfoxides, cinnamic acid, fatty acid esters and phthalic acid esters. It exhibited good antioxidant activity with a DPPH radical scavenging capacity of 33.70%. The extract also showed broad-spectrum antibacterial activity against eight bacteria strains. *S. aureus* displayed the highest antibacterial activity with a 21 mm inhibition zone.

REFERENCES

1. Holm Y. Bioactivity of basil. In: Hiltunen R, Holm Y, editors. Basil. The genus *Ocimum*. Amsterdam: Harwood Academic Publishers; 1999. p. 113-36.
2. Imad HH, Hussein JH, Muhanned AK, Nidaa SH. Identification of five newly described bioactive chemical compounds in methanolic extract of *Mentha viridis* by using gas chromatography-mass spectrometry (GC-MS). J Pharmacogn Phytother. 2015b;7(7):107-25. doi: 10.5897/JPP2015.0349.
3. Joshi V, Bothara B, Surana J. Evaluation of aqueous extract of *Ocimum sanctum* in experimentally induced parkinsonism. J Chem Pharm Res. 2011;3(1):478-87.
4. Yeşil M, Özcan MM. Effect of harvest stage and diurnal variability on yield and essential oil content in *Mentha x piperita* L. Plant Soil Environ. 2021;67(7):417-23. doi: 10.17221/114/2021-PSE.
5. Tyagi A, Yadav AK, Yadav A, Saini L, Kumar V, Jain P, *et al.* Vibrational spectroscopic methods for the identification and distinction of essential oils in genus *Ocimum* L.: A chemometric approach. J King Saud Univ Sci. 2022;34(8):102355. doi: 10.1016/j.jksus.2022.102355.
6. Satyal P, Jones TH, Lopez EM, McFeeters RL, Ali NA, Mansi I, *et al.* Chemotype characterization and biological activity of *Rosmarinus officinalis*. Foods. 2017;6(3):20. doi: 10.3390/foods6030020, PMID 28273883.
7. Grayer RJ, Kite GC, Goldstone FJ, Bryan SE, Paton A, Putievsky E. Infraspecific taxonomy and essential oil chemotypes in sweet basil, *Ocimum basilicum*. Phytochemistry. 1996;43(5):1033-39. doi: 10.1016/s0031-9422(96)00429-3, PMID 8987875.
8. Joshi RK. GC-MS analysis of essential oil of *Ocimum gratissimum* L. growing desolately in South India. Acta Chromatogr. 2017;29(1):111-19. doi: 10.1556/1326.2017.29.1.10.
9. Wiwart M, Kandler W, Suchowilska E, Krska R. Discrimination between the grain of spelt and comm0on wheat hybrids and their potential forms using Fourier Transform Infrared-Attenuated Total Reflection. Int J Food Prop. 2015;18(1):54-63. doi: 10.1080/10942912.2013.814665.
10. Chen Y, Chen Y, Feng X, Yang X, Zhang J, Qiu Z, *et al.* Variety identification of Orchids using Fourier transform infrared spectroscopy combined with stacked spares auto-encoder. Molecules. 2019;24(13):2506. https:. doi: 10.3390/molecules24132506, PMID 31324007.
11. Kolašinac S, Pećinar I, Danojević D, Stevanović ZD. Raman spectroscopy coupled with chemometric modelling approaches for authenticathin of different paprika varieties at physiological maturity. LWT Food Sci Technol. 2022;162. doi: 10.1016/j.lwt.2022.113402.
12. Mohanad JK, Azhar AS, Imad HH. Evaluation of anti-bacterial activity and bioactive chemical analysis of *Ocimum basilicum* using Fourier transform infrared (FT-IR) and gas chromatography-mass spectrometry (GC-MS) techniques. J Pharmacogn Phytother. 2016;8(6):127-46. doi: 10.5897/JPP2015.0366.
13. Duke JA. Handbook of medicinal herbs. CRC press; 2002.
14. Viña A, Murillo E. Essential oil composition from twelve varieties of Basil (*Ocimum* spp.) grown in Columbia. J Braz Chem Soc. 2003;14(5):744-49. doi: 10.1590/S0103-50532003000500008.
15. Politeo O, Jukic M, Milos M. Chemical composition and antioxidant capacity of free volatile aglycones from basil (*Ocimum basilicum* L.) compared with its essential oil. Food Chem. 2007;101(1):379-85. doi: 10.1016/j.foodchem.2006.01.045.
16. Koba K, Poutouli PW, Raynaud C, Chaumont JP, Sanda K. Chemical composition and antimicrobial properties of different basil essential oils chemotypes from Togo. Bangladesh J Pharmacol. 2009;4(1):1-8. doi: 10.3329/bjp.v4i1.998.
17. Zhang JW, Li SK, Wu WJ. The main chemical composition and *in vitro* antifungal activity of essential oils of *Ocimum basilicum* Linn. var. pilosum (Willd.) Benth. Molecules. 2009;14(1):273-8. doi: 10.3390%2Fmolecules14010273.
18. Imad HH, Lena FH, Sabreen AK. Analysis of bioactive chemical compounds of *Aspergillus niger* by using gas chromatography-mass spectrometry and Fourier-transform infrared spectroscopy. J Pharmacogn Phytother. 2015a;7(8):132-63. doi: 10.5897/JPP2015.0354.
19. Altameme HJ, Hameed IH, Abu-Serag NA. Analysis of bioactive phytochemical compounds of two medicinal plants, Equisetum arvense and *Alchemila valgaris* seed using gas chromatography-mass spectrometry and Fourier-transform infrared spectroscopy. Malays Appl Biol. 2015;44(4):47-58.
20. Ameera OH, Ghaidaa JM, Mohammed YH, and IH. Phytochemical screening of methanolic dried galls extract of *Quercus infectoria* using gas chromatography-mass spectrometry (GC-MS) and Fourier transform-infrared (FT-IR). J Pharmacogn Phytother. 2016;8(3):49-59. doi: 10.5897/JPP2015.0368.
21. Juliani HR, Simon JE. Antioxidant activity of basil. In: Janick J, Whipkey A, editors. Trends in new crops and new uses. Alexandria: ASHS Press; 2002. p. 575-79.
22. Patel MR, Patel RB, Parikh JR, Patel BG. Formulation consideration and skin retention study of microemulsion containing tazarotene for targeted therapy of acne. J Pharm Investig. 2016;46(1):55-66. doi: 10.1007/s40005-015-0213-0.
23. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT Food Sci Technol. 1995;28(1):25-30. doi: 10.1016/S0023-6438(95)80008-5.
24. Moni SS, Alam MF, Safhi MM, Jabeen A, Sanobar S, Siddiqui R, *et al.* Potency of nano-antibacterial formulation from *Sargassum binderi* against selected human pathogenic bacteria. Braz J Pharm Sci. 2018;54(4):e17811. doi: 10.1590/s2175-97902018000417811.
25. Amal MA, Ladwani SM, Abdel Hameed ES. Chemical composition of *Ocimum basilicum* L. essential oil from different regions in the Kingdom of Saudi Arabia by using Gas chromatography mass spectrometer. J Med Plants Stud. 2018;6(1):14-9.
26. Ahmed AF, Attia FA, Liu Z, Li C, Wei J, Kang W. Antioxidant activity and total phenolic content of essential oils and extracts of sweet basil (*Ocimum basilicum* L.) plants. Food Sci Hum Wellness. 2019;8(3):299-305. doi: 10.1016/j.fshw.2019.07.004.
27. Rasha KA. Volatile oil composition of *Ocimum basilicum* (Rehan) Leaf extract and antibacterial activity against bacterial pathogens in Sudan. Elixir Biosciences; 2018;125(52306):52306-8.
28. Wang LH, Sung WC. Rapid evaluation and quantitative analysis of eugenol derivatives in essential oils and cosmetic formulations on human skin using attenuated total reflectance-infrared spectroscopy. Spectroscopy. 2011;26(1):43-52. doi: 10.1155/2011/176163.
29. Michelina C, Naviglio D, Gallo M, Severina P. FT-IR and GC-MS analysis of an oxidant leaf essential oil from sage plants cultivated as an alternative to tobacco production. J Essent Oil Res. 2018;31(9):1-7. doi: 10.1080/10412905.2018.1540364.
30. Gudi G, Krähmer A, Krüger H, Schulz H. Attenuated Total Reflectance- Fourier transform infrared Spectroscopy on intact dried leaves of Sage (*Salvia officinalis* L.) – accelerated chemotaxonomic discrimination and analysis of essential oil composition. J Agric Food Chem. 2015;63(39):8743-50. doi: 10.1021/acs.jafc.5b03852, PMID 26360136.
31. Nunes S, Ramacciotti F, Neves A, Angelin EM, Ramos AM, Roldão É, *et al.* A diagnostic tool for assessing the conservation condition of cellulose nitrate and acetate in heritage collections: quantifying the degree of substitution by infrared spectroscopy. Herit Sci. 2020;8(1). doi: 10.1186/s40494-020-00373-4.

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