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Research Article

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Phytochemical analysis of *Mentha spicata* plant extract using UV-VIS, FTIR and GC/MS technique

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ABSTRACT

The present study was carried out to characterize the bioactive constituents present in plant extract of M. spicata using UV-VIS, FTIR and GC/MS technique. The plant extract was scanned in the wavelength ranging from 300-800nm by using UV-VIS spectrophotometer and the characteristic peaks were detected. The UV-VIS profile showed different peaks ranging from 300-800nm with different absorption respectively. The FTIR spectrum confirmed the presence of alcohols, phenols, alkanes, alkenes, carbonyl, carboxylic acids and aromatic compounds in extract. The results of the GC/MS analysis of methanolic plant extract provide different peaks determining the presence of 42 phytochemical compounds. The major phytoconstituents were pentadecanoic acid (7.47%), 7-oxabicyclo[4.1.0]heptane (9.56%),3-penten-2-one,4-(2,2,6-trimethyl-7-oxabicyclo[4.1.0] hept-1-yl)-,(E)-(12.20%), Stigmast-4-EN-3-one (18.99%). The results shows that important bioactive compounds are present in plant extract and these constituents may be responsible for pharmacological activities.

Keywords: *M. spicata*, Plant extract, UV-VIS Spectroscopy, FTIR Spectroscopy, GC/MS analysis.

INTRODUCTION

Mentha spicata L. (spearmint) is a creeping glabrous, rhizomatous and perennial herb with a strong aromatic odor and a member of the Labiatae family is originated from Eastern Asia. This family is a rich source of polyphenolic compounds and hence could possess strong antioxidant properties. It contains volatile oil, carvone, limonene, cis-carveol, 1,8 cineol, cis-dihydrocarvone, carvyl acetate, cis-sabinene hydrate of which carvone is the most important constituent of *M. spicata* [1]. The fresh and dried plants and their essential oils are widely used in food, chewing gum, cosmetic, toothpaste, confectionary and pharmaceutical industries [2,3]. Leaves, flowers and the stem of Mentha species are frequently used in herbal teas or as additives in commercial spice mixtures for many foods to offer aroma and flavor [4]. It is well documented that the essential oil from mentha species posses antimicrobial and antioxidant properties due to the presence of active constituents like menthone, menthol, rosmarinic acid and carvone [1,2]. The aim of the present work was to study the presence of bioactive constituents in *Mentha* spicata plant extract.

EXPERIMENTAL SECTION

Preparation of plant extract

Mentha spicata dried plants were used to make the alcoholic extract. *Mentha spicata* plants purchased from local market, Indore, (M.P.), India. The plant material was thoroughly washed individually under running tap water to remove any traces of soil particles and other dirt and dried in shade. The dried plant material is stored in the laboratory at room temperature (298K) and in the shade before the extraction then the leaves, steam and roots were separated, air dried for complete drying.

The dried plant material was powdered using a heavy duty blender and powder was extracted with methanol [5]. 500g material extracted with 10 liters of methanol at reflux temperature for 15 hours and cool to room temperature, filtered concentrated under vacuum. The yield of the extract is 490 grams[6].

Characterization of *M. spicata* extract

UV-VIS and FTIR Spectroscopic analysis

UV-visible spectrophotometric analysis was conducted on the *M. spicata* extract using a UV-visible spectrophotometer (Perkin Elmer, USA Model: Lambda 950) with a slit width of 2nm, using a 10-mm cell at room temperature. The extract was examined under visible and UV light in the wavelength ranging from 300-800nm for proximate analysis. For UV-VIS spectrophotometer analysis, the extract was centrifuged at 3000 rpm for 10 min and filtered through Whatman No. 1 filter paper. The sample is diluted to 1:10 with the same solvent [**7**].

Fourier transform infrared (FTIR) was used to identify the characteristic functional groups in the extract. It provides the information about the structure of a molecule could frequently be obtained from its absorption spectrum. A small quantity of the *Mentha spicata* extract was mixed in dry potassium bromide (KBr). The mixture was thoroughly mixed in a mortar and pressed at a pressure of 6 bars within 2 min to form a KBr thin disc. Then the disc was placed in a sample cup of a diffuse reflectance accessory. The IR spectrum was obtained using Bruker, Germany Vertex 70 infrared spectrometer. The sample was scanned from 4000 to 400 cm^{-1} [5]. The peak values of the UV-VIS and FTIR were recorded.

GC/MS analysis

To identify the presence of active constituents and the chemical composition of *M. spicata* plant extract was characterized by the use of gas chromatography and mass spectrometry (GC/MS). The GC/MS analysis of *M. spicata* extract was performed by using GC/MS Shimadzu QP-2010 plus with thermal desorption system TD 20. The column used was Rtx-5 of 30m X 0.25mm X 0.25μm size. The initial column temperature was 100^0C rising 280^0C at a rate of 5^0C/min and the temperature was maintained for 3 minutes. The temperature was further increased to 280^0C at a rate of 15^0C/min with a hold time of 35 minutes. The ion source of mass spectrometer was held at 230^0C with an interface temperature of 270^0C. Detection was performed in full scan mode from m/z 40 to 650. The identification of the compounds was achieved by comparing obtained mass spectra of unknown peaks with those stored in the NIST (National Institute of Standards and Technology) and Wiley mass spectral electronic libraries [8,9].

RESULTS AND DISCUSSION

UV-VIS Analysis

The UV-VIS analysis preformed for identification of phytoconstituents present in methanolic extract of *M. spicata*. The UV-visible spectra were performed to identify the compounds containing σ-bonds, π-bonds and lone pair of electrons, chromophores and aromatic rings.

The qualitative UV-VIS profile of methanolic extract of *M. spicata* was taken at the wavelength of 300 nm to 800 nm due to the sharpness of the peaks and proper baseline. The profile showed the peaks at 353, 407, 504, 535, 609 and 665 nm with the absorption 1.309, 1.463, 0.1, 0.066, 0.108 and 0.625 respectively. Figure 1 shows the absorption spectrum of *M. spicata* extract and these are almost transparent in the wavelength region of 300-800 nm. Absorption bands observed pertaining to *M. spicata* plant extract are displayed in Table 1.

In the UV-VIS spectra the appearance of one or more peaks in the region from 200 to 400 nm is a clear indication of the presence of unsaturated groups and heteroatoms such as S, N, O [8]. The spectrum for *M. spicata* extract shows two peaks at positions 353 nm, and 407 nm. This confirms the presence of organic chromophores within the *M. spicata* extract. Nevertheless, the use of UV-visible spectrophotometery in the analysis of complex media is limited by the inherent difficulties in assigning the absorption peaks to any particular constituents in the system. Thus, UV–VIS findings must be supplemented with some other analytical technique such as GC/MS etc, to enable proper extract characterization and constituent identification[7]. The obtained data of UV-VIS spectroscopic analysis in the methanolic extract of *M. spicata* is given as follows in table 1.

Table 1: UV-VIS peak values of extract of *Mentha spicata*

S. No.	Wavelength (nm)	Absorbance
1	353	1.309
2	407	1.467
3	504	0.1
4	535	0.062
5	609	0.108
6	665	0.630

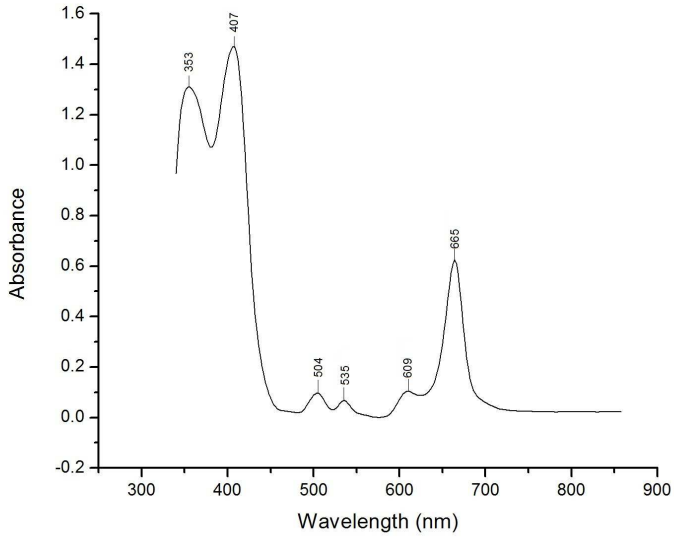

Figure 1: UV-VIS spectra of pure methanolic *Mentha spicata* extract

FTIR Analysis

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The FTIR spectrum of the *M. spicata* plant extract in the form of KBr pallet is shown in Figure 2. The absorption at 3349.81 cm^{-1} is due to the stretching of hydroxyl groups that are present in the extract (table 2). The band at 2927.23 cm^{-1} is due to the symmetric stretching of saturated (sp^3) carbon. The band at 1633.44 cm^{-1} is assigned to the bending mode of absorbed water, since plant extracts are known to have a strong affinity for water. The band at 1537.09 cm^{-1} is due to C=C stretching associated with the aromatic skeletal mode of the extracts. The vibrational absorption band at 1384.66 cm^{-1} was assigned to rocking of methyl group. A notable band at 1253.97 and 1054.89 cm^{-1} can be assigned to C-O stretching. A band at 599.76 cm^{-1} represent the aromatic H out of plane bending[5,10,11].

Table 2: Structural features of the *M. spicata* extract by FTIR spectrum

Wave numbers (cm^{-1})	Assignments
3349.81 cm^{-1}	-OH stretch
2927.23 cm^{-1}	C-H stretch
1633.44 cm^{-1}	Alkene C=C
1537.09 cm^{-1}	C=C stretching
1384.66 cm^{-1}	C-H bending
1253.97 cm^{-1}	C-O stretch
1054.89 cm^{-1}	C-O stretch
599.76 cm^{-1}	aromatic ring

GC/MS Analysis

The chemical constituents identified by the GC/MS analysis of the methanolic plant extract of *Mentha spicata* (L.) was enumerated along with their molecular formula, retention time, and peak area (Table 3). The GC/MS analysis of *Mentha spicata* (L.) revealed the presence of 48 compounds identified in the methanolic extract.

The chromatogram (Figure 3) of methanolic plant extract shows 9 prominent peaks as Phytol ($C_{20}H_{40}O$) with retention time of 17.648 and peak area of 2.96%, Methyl Commate A ($C_{32}H_{52}O_4$) with retention time of 39.520 (4.0%), cis,cis,cis-7,10,13-Hexadecatrienal ($C_{16}H_{26}O$) with retention time of 17.900 (5.23%), Stigmast-5-EN-3-OL, (3.Beta.)-($C_{29}H_{50}O$) with retention time of 24.894(4.80%), 7Oxabicyclo[4.1.0]Heptane, 1-(2,3-Dimethyl-1) ($C_{15}H_{24}O$) with retention time of 25.320 (9.56%), Cholest-4-en-3-one ($C_{27}H_{44}O$) with retention time of 30.084 (2.73%), gamma.-Sitosterol ($C_{29}H_{50}O$) with retention time of 37.724 (2.45%), Pentadecanoic acid ($C_{15}H_{30}O_2$) with retention times of 16.143 and peak area of 7.47%, 7-Oxabicyclo[4.1.0]Heptane ($C_{15}H_{24}O$)with retention time 25.320

(9.56%), 3-Penten-2-one, 4-(2,2,6-Trimethyl-7-Oxabicyclo[4.1.0]HEPT-1-YL)-, (E)- ($C_{14}H_{22}O_2$) with retention time of 28.836 (12.20%) , Stigmast-4-EN-3-one ($C_{29}H_{48}O$) with retention time of 42.643 (18.99%).

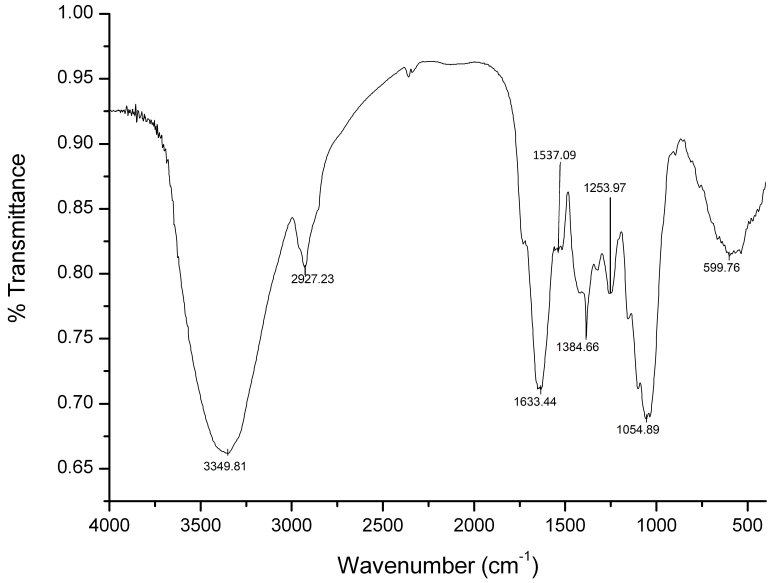

Figure 2: FTIR spectra of pure methanolic *Mentha spicata* extract (dried solid mass, KBr)

It contains nine major peaks along with many small peaks indicating presence of major compounds (Table 3). Structural assignment of GC retention data of compounds is based on spectral matching with NIST library (National Institute of Standards and Technology).The small peaks may be attributed to the compounds present in small quantities as well as disintegrated major compounds. The peaks related to low retention times are mainly low polar plant compounds[12].

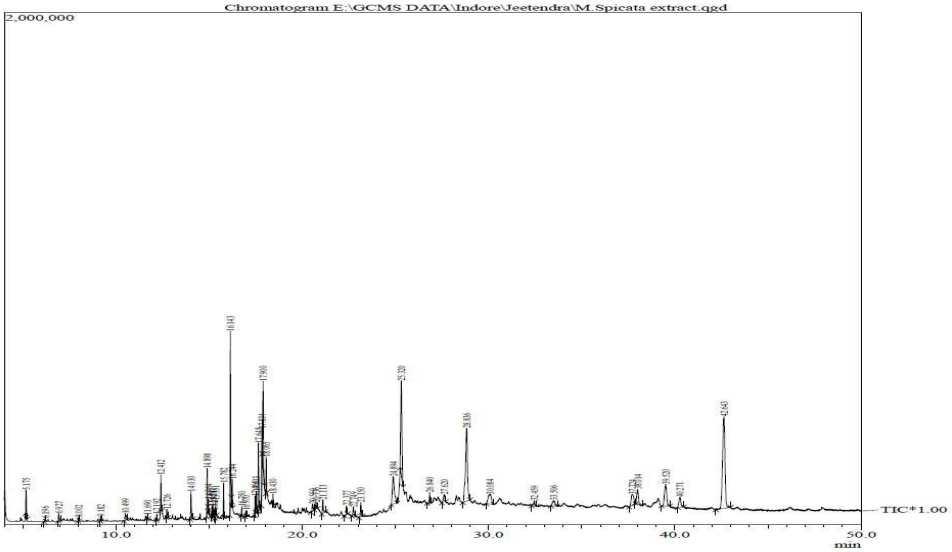

Figure 3: GC/MS chromatogram of *Mentha spicata* plant extract

Table 3: Chemical constituents present in the methanolic extract of *Mentha spicata*

Peak#	R. Time	Area%	Molecular formula	Name
1	5.175	1.32	$C_9H_{12}O_2$	Benzaldehyde dimethyl acetal
2	6.196	0.17	$C_{13}H_{22}O_3$	(2R,5R,6R)-2-T-BUTYL-5-ALLYL-5,6-Dimethyl-1,3-Dioxan-4-One
3	6.927	0.47	$C_8H_{10}O_2$	ETHANOL, 2-PHENOXY-
4	8.002	0.16	$C_{10}H_{14}O$	PHENOL, 5-METHYL-2-(1-METHYLETHYL)
5	9.182	0.16	$C_{18}H_{32}O_4$	Succinic acid, dec-4-enyl isobutyl ester
6	10.499	0.55	C10H16O	Cyclononanone, 5-Methylene-
7	11.690	0.15	$C_{10}H_{20}O_2$	Decanoic Acid
8	12.192	0.20	$C_{12}H_{14}O_4$	1,2-Benzenedicarboxylic Acid, Diethyl Este
9	12.412	1.47	$C_{10}H_{18}O$	2H-Pyran, 2-ethenyltetrahydro-2,6,6-trimethyl-
10	12.726	0.48	$C_{10}H_{16}O$	Bicyclo[3.3.1]non-2-en-9-ol, 9-methyl-
11	14.030	1.38	$C_{11}H_{16}O_3$	2(4H)-Benzofuranone, 5,6,7,7A-Tetrahydro-6-
12	14.898	1.96	$C_{20}H_{38}$	2,6,10-Trimethyl,14-Ethylene-14-Pentadecne
13	14.964	0.37	$C_{18}H_{36}O$	2-Pentadecanon, 6,10,14-Trimethyl-
14	15.158	0.30	$C_{20}H_{38}$	2,6,10-Trimethyl,14-Ethylene-14-Pentadecne
15	15.211	0.42	$C_{10}H_{18}O_2$	2,3aDimethylhexahydrobenzofuran-7a-ol
16	15.277	0.22	$C_{16}H_{22}O_4$	1,2-Benzenedicarboxylic Acid, BIS(2-Methyl
17	15.351	0.78	$C_{20}H_{38}$	2,6,10-Trimethyl,14-Ethylene-14-Pentadecne
18	15.782	1.11	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester
19	16.143	**7.47**	$C_{15}H_{30}O_2$	Pentadecanoic Acid
20	16.244	0.77	$C_{18}H_{24}O_6$	1,2-Benzenedicarboxylic Acid, 2-Butoxy-2-O
21	16.750	0.28	$C_{19}H_{38}O_2$	Isopropyl palmitate
22	16.970	0.22	$C_{19}H_{40}O_2Si$	Hexadecanoic acid, trimethylsilyl ester
23	17.466	0.60	$C_{18}H_{34}O$	9,12-Octadecadien-1-OL
24	17.533	1.02	$C_{19}H_{32}O_2$	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-
25	17.648	**2.96**	$C_{20}H_{40}O$	Phytol
26	17.834	1.70	$C_{18}H_{32}O_2$	9,12-Octadecadienoic Acid (Z,Z)-
27	17.900	**5.23**	$C_{16}H_{26}O$	cis,cis,cis-7,10,13-Hexadecatrienal
28	18.065	1.45	$C_{18}H_{36}O_2$	Octadecanoic acid
29	18.430	0.33	$C_{20}H_{36}O_2$	Ethyl (9Z,12Z)-9,12-Octadecadienoate #
30	20.559	0.65	$C_{10}H_{18}O_2$	2,7-Naphthalenediol, decahydro-
31	20.736	0.50	$C_{10}H_{16}O_2$	2,3-Dioxabicyclo[2.2.2]Oct-5-ENE, 1-Methyl-4-(
32	21.113	1.03	$C_{18}H_{36}O$	Octadecanal
33	22.377	0.56	$C_{19}H_{28}O_3$	4-Androsten-11.beta.,17.beta.-diol-3-one
34	22.749	0.80	$C_{17}H_{32}O$	16-Heptadecenal
35	23.150	1.05	$C_{24}H_{38}O_4$	1,2-Benzenedicarboxylic Acid
36	24.894	**4.80**	$C_{29}H_{50}O$	Stigmast-5-EN-3-OL, (3.Beta.)-
37	25.320	**9.56**	$C_{15}H_{24}O$	7Oxabicyclo[4.1.0]Heptane, 1-(2,3-Dimethyl-1
38	26.840	0.53	$C_{30}H_{50}$	Squalene
39	27.620	0.98	$C_{20}H_{34}O_2$	Duvatriendiol
40	28.836	**12.20**	$C_{14}H_{22}O_2$	3-PENTEN-2-ONE, 4-(2,2,6-Trimethyl-7-Oxabicyclo[4.1.0]HEPT-1-YL)-, (E)-
41	30.084	**2.73**	$C_{27}H_{44}O$	Cholest-4-en-3-one
42	32.459	0.61	$C_{29}H_{50}O_2$	Vitamin E
43	33.506	1.24	$C_{14}H_{22}O_2$	3-Penten-2-one, 4-(2,2,6-Trimethyl-7-Oxabicyc
44	37.724	**2.45**	$C_{29}H_{50}O$	.gamma.-Sitosterol
45	38.014	1.93	$C_{30}H_{48}O$	4 ,4,6A,6B,8A,11,11,14B-Octamethyl-1,4,4A,5,6,6A,6
46	39.520	4.00	$C_{32}H_{52}O_4$	Methyl Commate A
47	40.271	1.72	$C_{29}H_{46}O$	4,22-Stigmastadiene-3-one
48	42.643	**18.99**	$C_{29}H_{48}O$	Stigmast-4-EN-3-one
		100.00		

CONCLUSION

This investigation has given preliminary information to determine the chemical composition of *M. spicata* using UV-VIS, FTIR and GC/MS techniques. In the present study 42 chemical constituents have been identified from methanolic plant extract of *Mentha spicata* by Gas Chromatogram / Mass spectrometry (GC/MS) analysis. The presence of these bioactive compounds in *M. spicata* plants lends credence to its use by the human community. It also holds for the production of novel drugs with isolation of specific compounds. It could be concluded that *M. spicata* contains various bioactive compounds. So it is recommended as a plant of phytopharmaceutical importance. However, further studies will need to be undertaken to ascertain fully its bioactivity, toxicity profile, effect on the ecosystem and agricultural products.

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