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Phytochemical Screening by GCMS Analysis of Leaf Extract of *Ocimum sanctum* and *Mentha arvensis* in Different Organic Solvents

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ABSTRACT

According to estimations from the World Health Organization (WHO), certain Asian and African people already utilize herbal medicine for some component of basic healthcare. In the current work, it was intended to perform phytochemical and FTIR analyses on the leaves of *Mentha arvensis* and *Ocimum sanctum* in various organic solvents. The findings of this study make it abundantly evident that *Ocimum sanctum* and *Mentha arvensis* leaves have saponins, flavanoids, steroids, alkaloids, phenols, Tannins, glycosides, and terpenoids in them when they underwent preliminary phytochemical examination. The current work uses FTIR spectroscopy to investigate the leaf extracts of two medicinal plants, *Ocimum sanctum* and *Mentha arvensis*, using water, ethanol, hexane, ethyl acetate, and benzene as solvents. The FTIR spectroscopy analyses identified numerous functional chemicals in the extracts with distinct distinctive peak values. The presence of amide, alcohols, phenols, alkanes, carboxylic acids, aldehydes, ketones, alkenes, primary amines, aromatics, esters, ethers, alkyl halides, and aliphatic amines compounds, which showed major peaks, was confirmed by FTIR analysis of ethanol and hexane leaf extracts of *Ocimum sanctum* and *Mentha arvensis*.

Keywords: *Mentha arvensis*, *Ocimum sanctum*, Phytochemical examination, Organic solvents, etc.

INTRODUCTION

Since most medicinal plants have less adverse effects than synthetic medications, they are frequently utilised as complementary treatment for human and animal illnesses. Knowing the chemical makeup of the phytochemicals found in medicinal plants can help us understand the many functional groups behind those compounds' therapeutic effects[1].

Due to their capacity to heal a range of illnesses, medicinal plants play a vital part in Siddha, Ayurveda, and the Unani school of medicine. The traditional medical system employs plant sources for medications that have no adverse effects on the human body, according to recent research employing animal models[2]. Glucosides are a key metabolite involved in cellular activities. Secondary metabolites, which develop in response to stress and have a more complex

structural composition, include alkaloids, flavonoids, terpenoids, steroids, and saponins[3]. A critical phase of the drug development process is the screening for phytochemicals. Phytochemicals present in plants, such as saponins, flavanoids, steroids, alkaloids, phenols, Tannins, glycosides, and terpenoids, are a great source of new and exciting medications[4–7]. The current work, was intended to perform phytochemical and FTIR analyses on the leaves of *Mentha arvensis* and *Ocimum sanctum* in various organic solvents.

Methods of extraction

The plants were gathered from the neighbourhood and washed thoroughly with water. The leaves were dried in shade and powdered for extraction. The dried powder of *Mentha arvensis* and *Ocimum sanctum* leaves was kept in contact with the different solvents in a stoppered container for 24 h with frequent agitation until the soluble matter is dissolved. This method is best suitable for use in case of the thermolabile drugs.

Parameters for characterization

The different solvent extracts of *Mentha arvensis* and *Ocimum sanctum* leaves were analysed for the following parameters as shown in Table 1.

Table 1: Experimental methods for phytochemical analysis[8–14]

S. No	Experiment	Observation	Inference	
1	Test for Alkaloids			
	Mayer's Test	Plant extract+2 drops of Mayer's reagent along the sides of the test tube	White creamy ppt.	Alkaloid present
	Wagner's Test	Plant extract+2 drops of Wagner's reagent along the sides of the test tube	Reddish brown ppt.	Alkaloid present
2	Test for Glycosides			
	Borntrager's Test	Plant extract is hydrolysed with concentrated HCl for 2 h on a water bath and filter. Filtered hydrolysate+3 mL of chloroform and shake well+10% ammonia solution when chloroform layer is separated	Pink colouration	Glycosides present
	Legal's Test	Pyridine dissolved plant extract+sodium nitroprusside solution+10% NaOH	Pink colouration	Glycosides present
3	Test for Phenolic compounds			
	Gelatin Test	Plant extract+5 mL distilled water+2 mL 1% gelatin+10% NaCl solution	White ppt.	Phenolic compound present
	Lead acetate Test	Plant extract+distilled water+3 mL 10% lead acetate	Bulky white ppt.	Phenolic compound present
4	Test for Tannins			
	Ferric Chloride Test	Dissolve 50mg of pulverized sample in 5 mL distilled water and filter+1% $FeCl_3$ solution	Brownish green or Blue black colouration	Tannins present
5	Test for Flavonoids			
	Alkaline Reagent Test	Plant extract+distilled water+10% NH_4OH solution	Yellow fluorescence	Flavonoids present
	Magnesium and HCl Reduction Test	Alcoholic plant extract+a few fragments of Mg ribbon and concentrated HCl dropwise	Pink to crimson colouration	Flavanol glucosides present
6	Test for Phytosterols			
	Libermann-Burchard's Test	Plant extract+2 mL acetic anhydride+2 few drops of concentrated H_2SO_4 along the test tube's sidewalls	An array of colour change	Phytosterols present
		Ethanolic plant extract+5 mL hot water and allowed to stand for 1 hours and filtered. Using a separating funnel and 2.5 mL of chloroform, the filtrate was extracted 0.5 mL of chloroform extract + 1 mL concentrated H_2SO_4	brownish-red interface	Steroids present
7	Test for Terpenoids			
		Ethanolic plant extract+5 mL of boiling water is added, let to stand for 1 h, and then filtered. A separating funnel was used to extract the filter with 2.5 mL of chloroform. On a water bath, 0.5 mL of chloroform extract is evaporated to dryness+3 mL concentrated H_2SO_4+heat	Grey colouration	Terpenoids present
8	Test for Saponins			
		Plant extract+20 mL distilled water and boil in the water bath. After 50% reduction shakes vigorously to obtain stable persistent froth. Froth+olive oil1	Emulsion formation	Saponins present

RESULTS AND DISCUSSIONS

Phytochemical analysis

Tables 2 and 3 in the current study present the results of phytochemical analyses of the following plants: *Ocimum sanctum* and *Mentha arvensis*. The positive sign (+) denotes the presence of a specific phytochemical component in plants, whereas the negative sign (-) denotes the absence of that component in plants. Depending on the different extraction solvents (water, ethanol, hexane, ethyl acetate and benzene) utilised, these phytochemical components in various plants exhibit varying results. Saponins, flavanoids, steroids, alkaloids, phenols, Tannins, glycosides, and terpenoids are thought to indicate strong therapeutic value.

Table 2: Phytochemical analysis for different *Ocimum sanctum* leaf extracts

S. No		1. *Ocimum sanctum* leaf extract				
	Phytochemicals	Ethanol	Hexane	Water	Ethyl acetate	Benzene
1	Saponins	+	+	+	+	+
2	Flavanoids	+	+	+	+	+
3	Steroids	+	+	-	-	-
4	Alkaloids	-	+	+	-	-
5	Phenols	+	+	-	+	-
6	Tannins	+	-	-	-	-
7	Glycosides	+	+	+	+	+
8	Terpenoids	+	+	+	+	+

Table 3: Phytochemical analysis for different *Mentha arvensis* leaf extracts

S. No		2. *Mentha arvensis* leaf extract				
	Phytochemicals	Ethanol	Hexane	Water	Ethyl acetate	Benzene
1	Saponins	+	+	+	+	+
2	Flavanoids	+	+	+	+	+
3	Steroids	-	+	-	-	-
4	Alkaloids	+	+	+	+	+
5	Phenols	+	-	+	-	-
6	Tannins	+	+	+	-	-
7	Glycosides	+	+	-	+	+
8	Terpenoids	+	+	+	+	+

The presence of saponins, flavanoids, steroids, alkaloids, phenols, Tannins, glycosides, and terpenoids indicates high medicinal value. The phytochemical analysis of all the plants indicate that all the selected plants have medicinal value and strong potential to be valuable treatments for improving human health. Using chromatographic and spectroscopic methods, the current work leads to future research in the separation and identification of the active compound from the selected plants. Considering the presence of maximum phytochemicals in ethanol and hexane extracts, the following FTIR analysis was done for ethanol and hexane extracts only.

GCMS Analysis

The GCMS result show no presence of any heterocyclic compounds as in case of alkaloids and carbohydrates. The presence of –O– linkage in the different structures obtained indicates the presence of glycosides. None of the extracts contain steroids and Tannins. The presence of alkaloids is confirmed in mentha plant by the presence of amines.

The GCMS study for all the plant extracts except the Ethanol extract of *Ocimum* shows the presence of long chain hydrocarbons indicating the presence of higher fatty acids and higher esters.

The GCMS chromatograms obtained for ethanol and hexane extracts of *Ocimum* and *Mentha* plant are shown in the following tables respectively.

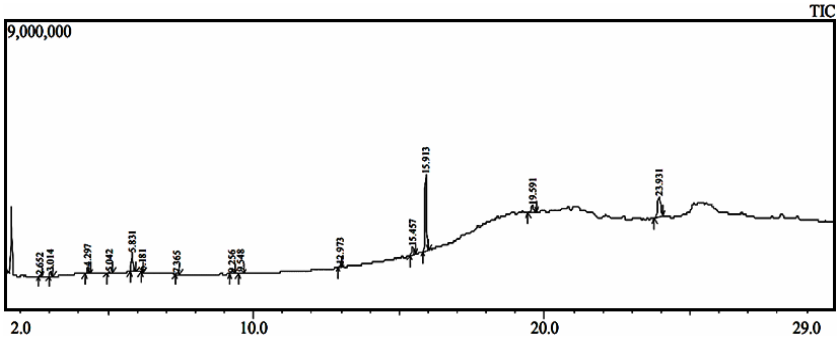

Table 1: Ocimum ethanol extract analysis

S. No	Name	Molecular Weight	R.Time
1	Benzyl 2 chloroethyl sulfone; Idomethyl benzene	218	12.973
2	2-methoxy-3-(2-propenyl)-phenol; Eugenol	164	15.457
3	Methyl eugenol	178	15.914
4	Caryophyllene oxide	220	19.591
5	Neophytadiene; 3,7,11,15-tetramethyl-2-hexadecan-1-ol; phytol acetate	278, 296, 338,	23.931

The peak at 15.457 min indicates the presence phenolic compound, flavonoids and the glycosidic linkage. The presence of flavonoids is further confirmed by the peak obtained at 15.914 minutes. The peaks obtained at 19.591 and 23.931 min indicate the presences of terpenoids and saponins.

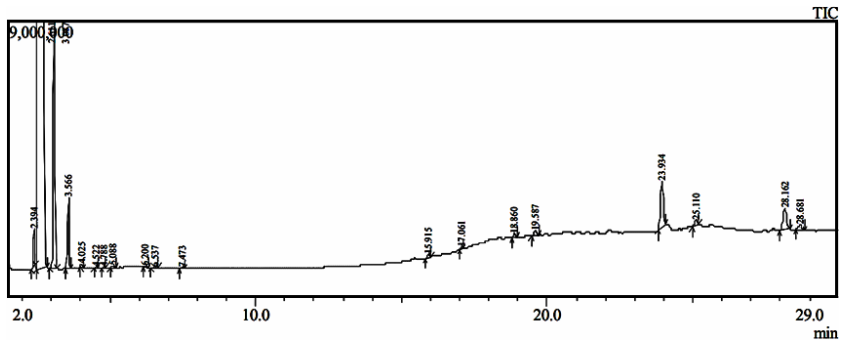

Table 2: *Ocimum* hexane extract analysis

S. No	Name	Molecular Weight	R.Time
1	Methyl eugenol	178	15.916
2	Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl; 4-hydroxy-2,6, 6-trimethylcyclohex-1-ene carbaldehyde	150,168	17.061
3	Cyclopropane-1-chloro-2,2-dimethyl-3-(3,3-dimethyl-1-butynl); 1-cyclohex-1-enyl-1-phenyl ethanol	184, 202	18.86
4	Caryophyllene oxide; Methyl (z)-5,11,14,17-eicosateraenoate	220, 318	19.588
5	Neophytadiene; 3,7,11,15-tetramethyl-2-hexadecan-1-ol; phytol acetate	278, 296, 338	23.934
6	Neophytadiene; 3,7,11,15-tetramethyl -2-hexadecan-1-ol; phytol acetate	278, 296, 338	25.11
7	Ethyl 13-methyl-tetradecanoate; Hexadecanoic acid ethyl ester	270, 284	28.162
8	Nonadecene; 9-Tricosene(z); Behenic alcohol	266, 322, 326	28.681

The peaks obtained at 15.916 and 17.061 min clearly indicates the presence of flavonoids. The peak with molecular weight 168 indicates the presence of phenolic compound. The peaks at run time 19.588 and 23.934 shows the presence of terpenoids and saponins. The presence of glycosidic linkages can be confirmed from peaks at 15.916, 19.588 and 28.162.

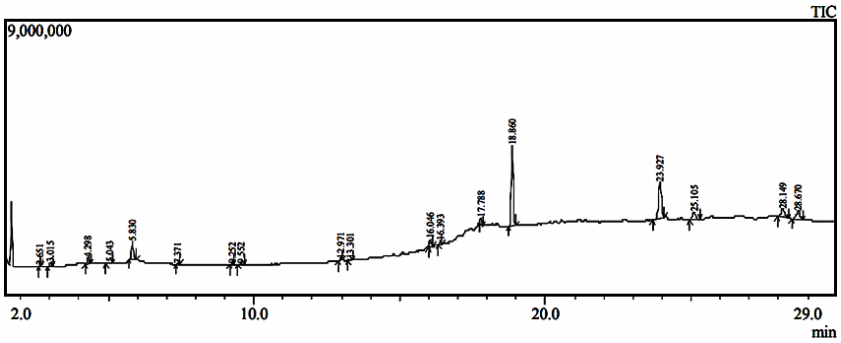

Table 3: Mentha ethanol extract analysis

S. No	Name	Molecular Weight	R.Time
1	Propanamide, 2-methoxy-N-methyl-; 2,2-diethoxy-propane	117,132	5.043
2	2,2-dimethyl cyclopropoane carboxylic acid, cyanomethyl ester; 2-Butenamide, N-(aminocarbonyl)- 2-ethyl-(z)-; Cyclopropane carboxylic acid, 3-formyl-2,2-diethyl-, ethyl ester	153,156,170	9.552
3	Benzyl 2 chloroethyl sulfone	218	12.971
4	8,9-dehydrothymol; Propanal, 2-methyl-3-phenyl-;Benzene, 1-methoxy-2-(1-methylethenyl)-	148	13.301
5	Cyclopentene, 1-isopropyl-2,3-dimethyl-; Bicyclo[3.2.1]octan-3-one,6-(2-hydroxyethyl)-endo-	138,168	16.046
6	2-cyclopentene-1-one, 2-(2-butenyl)-4-hydroxy-3-methyl-(z)-	166	16.394
7	Propanoic acid, 2-bromo-ethyl ester; 2-Octene-1-ol, 7-ethoxyl-3,7-dimethy-(E)-;5,6-dihydroxy-5-isopropyl-6-methyl-hept-3-en-2-one	166,200	17.789
8	Cyclopropane-1-chloro-2,2-dimethyl-3-(3,3-dimethyl-1-butynl); Myrtene acid chloride	184	18.86
9	Neophytadiene; 3,7,11,15-tetramethyl-2-hexadecan-1-ol; phytol acetate	278,296,338	23.927
10	Neophytadiene; 3,7,11,15-tetramethyl-2-hexadecan-1-ol;phytol acetate	278,296,338	25.105
11	Ethyl 13-methyl-tetradecanoate; Hexadecanoic acid ethyl ester	270,284	28.149
12	Nonadecene; n-Nonadecanol-1;) 9-Tricosene(z)	266,284,322	28.67

The peaks at 5.043 and 9.552 min shows the presence of alkaloid whereas peak at 3.301 min indicate the presence of phenol. The presence of glycosidic linkages can be seen in the peaks obtained at run time 5.043, 13.301, 17.789 and 28.149 minutes. Flavanoids are obtained at 13.301, 16.394 and 17.789 minutes. The presence of terpenes is clearly indicated by the peaks at 13.301, 16.046, 18.86 and 25.105 minutes. The peak obtained at 28.67 min shows the presence for long chain hydrocarbons.

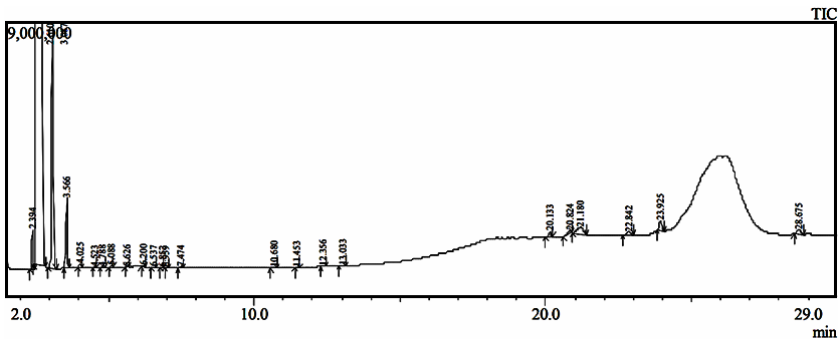

Table 4: Mentha hexane extract analysis

S. No	Name	Molecular Weight	R.Time
1	1-Undecene, 4-methyl-; Decane, 3,7-dimethyl-; Undecane, 4,7-dimethyl	168, 170, 184	10.688
2	Octane, 6-ethyl-2-methyl-; Dodecane, 2,6,11-trimethyl-; Sulfurous acid, nonyl-2-propyl ester	156, 212, 250	11.458
3	Benzenamine, 2,5-dimethyl-	121	12.354
4	Azulene, Naphthalene	128	13.042
5	Ar-Tumerone; Cinnamyl angelate, E-	216	20.125
6	n-propyl, 9,12-hexadocadienoate; Linolcic acid ethyl ester	294, 308	20.833
7	9,12,15-Octadecatrienoic acid ethyl ester	306	21.188
8	Ethyl 13 methyl-tetradecanoate; Octadecanoic acid, 17-methyl-methyl ester	270, 312	22.833
9	Neophytadiene; 3,7,11,15-tetramethyl-2-hexadecan-1-ol; phytol acetate	278,296,338	23.917
10	Nonadecene; Pentacos-1-ene	266,350	28.667

From the above table it is found that the peaks at 10.688, 11.458 and 23.917 min confirms the presence for terpenoids and saponins. The peaks at 20.125, 20.833, 21.188 and 22.833 min indicate the presence of glycosidic linkages. The alkaloid is shown by the peak at 12.354 minutes. The peak obtained at 28.667 min shows the presence for long chain hydrocarbons.

CONCLUSION

The phytochemicals that medicinal plants generate may end up being effective therapeutic agents for promoting human health. More and more, research on the phytochemical composition of plants is being used as the initial step in the search for powerful medications. The current research sets the door for future research utilizing the GCMS analysis to isolate and identify the active chemical from the selected plants. The two plants *Ocimum sanctum* and *Mentha arvensis* have very strong medicinal potential.

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Conflict of interest

The authors show no conflict of interest.

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