

Phytochemical Screening and GC- MS Analysis of Three Indian Traditional Medicinal Plants

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Abstract

The investigation of current findings on phytochemicals and gas chromatography mass spectroscopy analysis (GCMS) of selected three Indian traditional medicinal plants namely Basil (*Ocimum basilicum*), Leucas (*Leucas aspera*) and Marigold (*Tagetes erecta*) of leaves of methanolic extract. All the plant extracts were screened to know their principle agents in qualitative phytochemical analysis. The qualitative phytochemical screening of *O. basilicum* leaves methanol extract shown positive results of flavonoids, terpenoids, glycosides, alkaloids, phenols, in *L. aspera* leaves shows all are present and in *T. erecta* leaves shown presence only for alkaloids, flavonoids, phenols and tannins whereas absence for terpenoids, glycosides and saponins. Out of three plants, *L. aspera* was selected for the spectroscopic studies by GCMS due to its active principles showed all the metabolites, in the GCMS results found 38 volatile compounds in the methanol extract of leaves. These plant extracts may possess insecticidal or larvicidal property due to strong aromatic property of the plants and also observed these volatile compounds in the one of the plant extract and may utilize it in agro-horticulture application as biocontrol agents.

Keywords: Phytochemical analysis, GCMS, *Ocimum basilicum*, *Leucas aspera*, *Tagetes erecta*, Biocontrol agents

Introduction

Medicinal plants are the hidden treasure of phytochemicals and form the large group of economically important plants for wide range of applications. The secondary metabolites of medicinal plants has exhibit in support of insects growth and development also against as detrimental, which inturn manifested in various levels including sterility, morbidity, toxicity, mortality, growth inhibitor, anti-feedant, reduction of fecundity, CNS depressants, fertility regulation and many of the reproductive activity inhibition (1-2). Leaves extracts of various native and exotic medicinal plants were reported from the ancient time, that to be in aromatic plants having been reported to have toxicity with various organ systems also inhibition potential in gravimetric as well as histometric in insect models or any other model organisms (3-4). Various findings have been revealed for plant phytochemicals in qualitative and quantitative approach to explore the potential biomolecules for the application of agro-horticulture. These phytochemicals are depends upon the plant part extraction and method of extraction to identify their distri-

bution of specific molecules and exhibit such biological activity depends on their lethality level based on concentrations of the involved active agents may differ from one plants to others (5-7).

In this scenario, current study focused on to screen the phytochemicals of three selected aromatic medicinal plants qualitatively and based on the presence of metabolites study further restricted to explore the one plant extract to quantify the volatile compounds by GCMS studies.

Material and Methods

Plant material

Medicinal plant commonly called as Basil (*Ocimum basilicum*), Leucas (*Leucas aspera*) and Marigold (*Tagetes erecta*) of leaves were collected from the botanical garden of Jyoti Nivas College and the plant samples were authenticated at Botany Department, Jyoti Nivas College Autonomous, Bengaluru, India.

Preparation of plant extracts

The leaves of *O. basilicum*, *L. aspera* and *T. erecta* were collected, washed thoroughly in running tap water, sprinkled 70% ethanol and dried in room temperature to avoid contamination and moisture free sample. Dried samples were crushed, powdered in electric mixer and stored in polybag at in the refrigerator prior to follow extraction. Each plant sample 500 gm stored powder used for extraction in methanol highly polar solvent. The solvent of the each extracts were removed by rotary evaporator to get solvent free crude extract. Standard stock of each extract solutions was prepared using acetone solvent in 1% of extract residues for dissolution. After saturation, dissolved extract residues were filtered, and concentrated to dryness in 400°C - 500°C using vacuum evaporator of each 100 gm of plant extract concentrated to obtain 100% output of concentration. Then further extract was diluted in double distilled water five times in graded concentration from low at (0.2%), mid at (0.4%), moderate at (0.6%) and

high at (0.8%) of each samples and at (0%) used for the control.



Figure 1: Morphology of the aromatic medicinal plants

Qualitative Phytochemical screening

Qualitative screening of phytochemicals of 3 aromatic medicinal plants methanol extracts were carried out in order to know their active constituents presence in the leaves, which further aimed to finalize the plant extract potential in the GCMS study to identify the quantitative volatile compounds and their characterization.

Methanol extract of selected aromatic medicinal plants leaves subjected to standard phytochemical qualitative tests described by Sharangouda and Patil, (8), Harnborne, (9) and Fransworth, (10) to determine the presence or absence of flavonoids, terpenoids, glycosides, tannins, alkaloids, phenols and saponins. Preparation of reagents for phytochemical assay followed standard protocol of Harnborne (11). To make the concentration to obtain the proper solution of methanol extract was re-suspended to obtain 100% dissolution used MilliQ water then filtered for the studies.

GCMS analysis

Gas chromatography and mass spectroscopy

Preparation of extract

10mg/ml aromatic medicinal plant methanolic

extract of *L. aspera* leaves were prepared in universal solvent methanol and for which 1µl plant extract was employed to quantify the volatile compounds by GCMS analysis.

Instruments and chromatographic conditions

GCMS study of *L. aspera* methanol extract was assessed by Thermo GCMS of model Clarus 500. For the mass spectrum detection, used the mode of MS DSQ II electron ionization with energy rate of 70 eV, at the mass range of m/z 50-650. Capillary column in the range of 30m x 0.25mm size covering the film thickness 0.25 of Restek RtxR-5MS column model by using solvent diphenylamine and dimethyl polysiloxane at the ratio of 5 and 95 in the analysis. The base temperature of column was pre-defined at 60°C/5min, GC injector and MS temperatures was configured at 280°C and 290°C in transfer line respectively. GC was assessed in the mode of splitless to get maximum hits in the sample. Helium at the flow rate per minute described it as 1.0ml and also it was applied as carrier gas in GC studies in the volume of 1.0 µL injection was used. The extracted plant sample dissolved in methanol solvent and filtered to apply it further for polymeric solid phase extraction (SPE) column to detect different constituents in GCMS. As a result of constituents presence searched in the data base of NIST REFPROP 9.1 Version by comparing one another to enlist the volatile compounds in the extract by the GCMS studies.

Identification of volatile compounds

Result interpretation of GCMS data was assessed with the help of the database of National Institute Standard and Technology (NIST) due to vast depiction of more than 62,000 compound patterns. The comparative assessment helped to identify unknown compounds when compared with stored NIST library to explore the available data of plant extract. The characters like name, formula, weight and structure of the volatile compounds of the sample was ascertained to derive the molecular chemical data.

Results and Discussion

Phytochemicals screening for methanolic three aromatic plant extracts

The screening of phytochemicals of methanol extract of three aromatic medicinal plants of leaves showed result depends on plants and found maximum positive in *Lecuas aspera* only. In *O. basilicum* extract showed positive for flavonoids, terpenoids, glycosides, alkaloids, phenols and negative for tannins and saponins. In *L. aspera* extract showed positive for flavonoids, terpenoids, glycosides, alkaloids, phenols, tannins and saponins. In *T. erecta* extract showed positive only for alkaloids, flavonoids, phenols and tannins whereas absence for terpenoids, glycosides and saponins (Table1). Similar studies were reported by many of the researchers from the background of medicinal plants using various types of plant parts and their extracts (12-17).

Table: 1: Screening of phytochemicals on aromatic medicinal plants in the methanol extract of leaves

Qualitative Screening of Phytochemical Analysis			
Tests	O. basilicum	L. aspera	T. erecta
Flavanoids	+	+	+
Terpenoids	+	+	-
Glycosides	+	+	-
Tannins	-	+	+
Alkaloids	+	+	+
Phenols	+	+	+
Saponins	-	+	-

Note: + = Positive; - = Negative

GCMS analysis of methanolic extract of leucas aspera leaves
















The GCMS study of the methanolic extract results were shown in figure 2 and it has eluted 38 different metabolites. Identification of volatile compounds were assessed with the comparison of standards of NIST and Wiley
















9.1. Most of the phytochemical were characterized by their respective area (%) depends on the availability and elution on particular retention time. The obtained peaks of the chromatograms, shown highest % of area by (1Ar-(1alpha,5abeta,9ar(*)))5a,9,9-trimethyloctahydrobenzo(d)cycloprop(c)oxepin-2,4- (55.94), Cyclohexanecarboxylic acid, 2-[[bis(2-methylpropyl)amino]carbonyl]-, 4-nitrophenyl este (11.51), Benzene, 1,2-dimethoxy-4-(2-propenyl)- (5.48), Bicyclo[2.2.1]heptane-1-methanesulfonic acid, 7,7-dimethyl-2-oxo-, (.+/-)- (4.12), Thunbergol (3.76), 4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-oc (1.63), alpha-Amyrin (1.63),

Stigmasterol (1.46), gamma.-Sitosterol (1.24) 2-(3-Pentyl)pyridine (1.22), 2,4,5,5,8a-Pentamethyl-4a,5,6,7,8,8a-hexahydro-2H-chromene (1.05) and rest other 28 volatile compounds shown less than 1 % of area as per the elution on particular retention time. As per the literature many of the volatile compounds which were present in the plant shown potent cytotoxicity activities and also several researchers reported for various pharmacological applications on these compounds in different plants, hence these volatile compounds listed with various characters to understand their biological action in table 2.

Table 2: Quantification of volatile compounds by GCMS of methanolic extract leaves of *Leucas aspera* plant and their various characters.

Peak No.	Retention time	Peak area (%)	Molecular formula	Molecular Weight (g/mol)	Name of the molecules	Molecular structure
1	6.345	0.21	C ₁₂ H ₂₆	170.33	Dodecane	
2	10.611	5.48	C ₁₁ H ₁₄ O ₂	178.23g/mol	Benzene, 1,2-dimethoxy-4-(2-propenyl)-	
3	10.980	0.37	C ₁₅ H ₂₄	204.35g/mol	Caryophyllene	
4	13.230	0.23	C ₁₅ H ₂₄ O	220.35g/mol	1,4-Methanoazulen-9-one, decahydro-1,5,5,8a-tetramethyl-, [1R-(1.alpha.,3a.beta.,4.alpha.)]	
5	13.434	4.12	C ₂₈ H ₃₈ O ₈ S ₂	566.7g/mol	Bicyclo[2.2.1]heptane-1-methanesulfonic acid, 7,7-dimethyl-2-oxo-, (.+/-)-	
6	13.655	0.19	C ₁₀ H ₁₅ BrO	231.13g/mol	Bicyclo[2.2.1]heptan-2-one, 5-bromo-1,7,7-trimethyl-, (1R-exo)-	
7	13.864	0.22	C ₂₀ H ₃₄ O	290.5g/mol	Thunbergol	
8	16.362	0.31	C ₁₃ H ₂₂ O	194.313	1a,2,5,5-Tetramethyl-cis-1a,4a,5,6,7,8-hexahydro-gamma-chromene	

9	16.791	0.45	C ₁₅ H ₃₀ O ₂	242.4g/mol	Pentadecanoic acid	
10	18.296	0.44	C ₂₀ H ₄₀ O	296.5g/mol	Phytol	
11	18.525	0.97	C ₁₆ H ₂₆ O	234.38g/mol	cis,cis,cis-7,10,13-Hexadecatrienal	
12	18.757	0.24	C ₁₈ H ₃₂ O	264.4g/mol	17-Octadecen-14-yn-1-ol	
13	19.056	0.18	C ₂₇ H ₄₄ O	384.6g/mol	Cholest-4-en-3-one	
14	19.539	0.76	C ₁₅ H ₂₄ O ₂	236.35g/mol	Spiro[4.5]decan-7-one, 1,8-dimethyl-8,9-epoxy-4-isopropyl-	
15	20.794	0.45	C ₁₅ H ₂₆	206.37g/mol	2,4a,8,8-Tetramethyldecahydrocyclopropa[d]naphthalene	
16	21.195	0.35	C ₁₂ H ₂₀ O	180.29g/mol	1H-Inden-1-ol, 2,4,5,6,7,7a-hexahydro-4,4,7a-trimethyl-	
17	21.676	55.94	C ₁₄ H ₂₀ O ₃	236.31g/mol	(1Ar-(1a alpha,5a beta,9a*)))-5a, 9,9-trimethyl-octahydrobenzo(d) cycloprop(c)oxepin-2,4-	
18	21.994	3.76	C ₂₀ H ₃₄ O	290.5g/mol	Thunbergol	
19	22.206	0.59	C ₃₀ H ₅₀ O ₂	442.7g/mol	Betulin	
20	22.352	1.05	C ₁₄ H ₂₄ O	208.34g/mol	2,4,5,5,8a-Pentamethyl-4a,5,6,7,8,8a-hexahydro-2H-chromene	
21	22.489	0.68	C ₂₀ H ₂₈ OS	316.5g/mol	Bicyclo[4.1.0]heptane, 1-(3-oxo-4-phenylthiobutyl)-2,2,6-trimethyl-	
22	22.754	0.63	C ₂₇ H ₄₆ O ₃	418.7g/mol	3.alpha.,7.beta.-Dihydroxy-5.beta.,6.beta.-epoxycholestane	
23	23.420	0.31	C ₁₆ H ₂₆ O ₃	266.38g/mol	2,5-Furandione, 3-decyl-	

24	23.793	1.22	$C_{10}H_{15}N$	149.23g/mol	2-(3-Pentyl)pyridine	
25	23.975	0.39	$C_{30}H_{50}$	410.7g/mol	2,6,10,14,18,22-Tetracosahexaene,	
26	24.265	0.39	$C_{19}H_{26}O_4$	318.4g/mol	2,6,10,15,19,23-hexamethyl-, (all-E)-2-Cyclohexene-1-carboxylic acid, 1,3-dimethyl-2-(3-methyl-7-oxo-1,3-octadienyl)-4-oxo	
27	24.470	0.24	$C_{15}H_{26}O$	222.37g/mol	3,3,7,11-Tetramethyltricyclo[5.4.0.0(4,11)]undecan-1-ol	
28	24.557	0.64	$C_{30}H_{50}O_2$	442.7g/mol	Betulin	
29	24.964	0.95	$C_{17}H_{24}N_2O_5$	336.4g/mol	4-Aminobenzoylglutamic acid penta-methyl derivative	
30	25.726	0.18	$C_{10}H_{16}N_2OSi$	208.33g/mol	N-Methyl nicotinimide, O-trimethylsilyl	
31	26.574	0.17	$C_8H_5F_4NO$	207.12g/mol	Acetamide, N-(4-fluorophenyl)-2,2,2-trifluoro-	
32	27.484	11.51	$C_{12}H_{20}ClN_3O_4$	305.76g/mol	Cyclohexanecarboxylic acid, 2-[[bis(2-methylpropyl)amino]carbonyl]-, 4-nitrophenyl este	
33	28.479	1.46	$C_{29}H_{48}O$	412.7g/mol	Stigmasterol	
34	29.269	1.24	$C_{29}H_{52}O_2$	432.7g/mol	.gamma.-Sitosterol	
35	30.323	1.63	$C_{30}H_{50}O_2$	442.7g/mol	4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-oc	
36	30.728	1.63	$C_{30}H_{50}O$	426.7g/mol	alpha-Amyrin	
37	31.460	0.19	$C_{10}H_{28}O_4Si_3$	296.58g/mol	Silicic acid, diethyl bis(trimethylsilyl) ester	
38	31.950	0.21	$C_{24}H_{36}O_2Si_2$	412.7g/mol	4-Methyl-2,4-bis(4'-trimethylsilyloxyphenyl)pentene-1	

GCMS studies of *Leucas aspera* leaves methanolic extract (Figure 2) exhibited total 38 volatile compounds as per the detection of peaks and their retention time and these are indicating the presence of thirty eight different volatile compounds which have various biological properties. These compounds identified by their characterization in differentiating retention time in each compounds along with their peak (%) in the studied plant extract in GCMS analysis. These characters classified by name of the individual molecules with molecular formula, weight and structures as per the retention time and concentration of the peak area elution in (%) the spectrum (Table 2). The characterized results represented as per the peak and percent area of the volatile compounds, i.e., (1Ar-(1aalpha,5abeta,9ar(*)))-5a,9,9-trimethyloctahydrobenzo(d)cycloprop(c)oxepin-2,4- (55.94), Cyclohexanecarboxylic acid, 2-[[bis(2-methylpropyl)amino]carbonyl]-, 4-nitrophenyl este (11.51), Benzene, 1,2-dimethoxy-4-(2-propenyl)- (5.48), Bicyclo[2.2.1]heptane-1-methanesulfonic acid, 7,7-dimethyl-2-oxo-, (+/-)- (4.12), Thunbergol (3.76), 4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-oc (1.63), alpha.-Amyrin (1.63), Stigmasterol (1.46), gamma-Sitosterol (1.24) 2-(3-Pentyl)pyridine (1.22), 2,4,5,5,8a-Pentamethyl-4a,5,6,7,8,8a-hexahydro-2H-chromene (1.05) and remaining 28 volatile compounds shown less than 1 % with respect retention time and peak area.

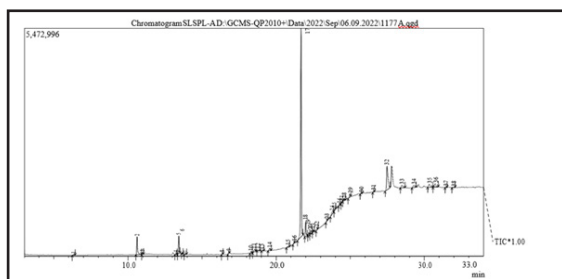


Figure 2. Spectrum of GCMS analysis and their volatile compounds representation of *Leucas aspera* leaves methanolic extract.

The *L. aspera* leaves of methanol extract found

with potent volatile compounds in GCMS studies, in that identified all the present compounds are carbohydrate derivatives and fatty acid mixtures. From the obtained data, we can expect the maximum purity and characterized compound such as (1Ar-(1aalpha,5abeta,9ar(*)))-5a,9,9-trimethyloctahydrobenzo(d)cycloprop(c)oxepin-2,4- (55.94%) found higher concentration and percent area, other 9 compounds altogether shown (27.64%) moderate concentration and rest all shown (16.4%) lower concentration covering 28 compounds. Patil *et al.*, (33) extensively worked on seed oil of *Citrus medica* and resulted in GCMS findings A8 volatile compounds i.e., higher rate of concentration with hexadecanoic acid, 9,12-octadecanoic acid, β -Sitosterol and oleic acid as an eluted mixture of fatty acids (18). Eramma and Patil, (19) revealed 41 distinct volatile compounds from crude and TLC fractions in *Flacourtia indica* root extract of methanol, GCMS analysis indicated the presence of, including Heneicosane (25.945), Squalene (20.51), Cholesterol (33.525), Cycloheptasiloxane, tetradecamethyl-(14.864), 2,4-Di-tert-butylphenol-(16.032), Cycloheptasiloxane hexadecamethyl (16.848), Cyclononasiloxane octadecamethyl (20.733), and n-Hexadecanoic acid (22.092). Kolgi *et al.*, (20, 21) reported the two metabolites such as alkaloid and flavonoid also revealed antioxidant and anticancer property in *Leucas aspera* leaves of chloroform and ethanol extracts. Similar compounds also reported for antioxidant property of *Simarouba glauca* seed extracts of petroleum and ethanol and revealed their qualitative and quantitative phytochemistry (22, 23).

Conclusion

Medicinal plants are the natural service provider for the treatment of any human diseases, and they have super healing power due to the richness of active ingredient of phytochemicals. Selected three Indian traditional medicinal plants namely *Ocimum basilicum*, *Leucas aspera* and *Tagetes erecta* of leaves of methanolic extract shown various types of phytochemicals, but in the *L. aspera* shown all the phytochem-

cials and it is further elucidated with spectroscopy study and found 38 volatile compounds by the GCMS characterization. Compare to other two plants, *L. aspera* found potential, they possess pharmacological properties and may have valuable biological action. The identified active volatile compounds may exhibit various biological properties and they act as future drug therapeutic molecule for agro-horticulture as larvicidal or insecticidal biocontrol agent. The co-relation of phytochemicals and volatile compounds resulted in various biological actions and may become novel future drugs for the various level of therapeutics.

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Conflict of Interest

The authors declare no conflict of interest.

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