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Research Article

Phytochemical Screening of Methanolic extract of Tulsi (*Ocimum sanctum L.*)

Doli J. Jain, Suchita K. Rajurkar*

Department of Botany, Deogiri College, Aurangabad, Maharashtra, India.

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***Corresponding author E-mail address:** *biokasliwal@gmail.com*

ABSTRACT
Ocimum sanctum L. is serving as medicine in Indian medicinal system from ancient time and today. This plant is potential source of bioactive molecules .In recent years, the indigenous system of medicine are getting more importance because of therapeutic value of these medicinal plants. In present study primary biochemical analysis followed by HRLCMS analysis leads to the identification of 98 secondary metabolites, in which Triparanol has cholesterol lowering effect. Quercitrin is antioxidant and antileshmanial agent. Khivorinis is an aromatic compound. Nivalenol is important glucoside. Ketotifen useful in eyes disorder and Tuberonic acid and Ecgonine are various chemical compounds getting eluted from chemical profile of methanol extract of Tulsi leaf.

KEYWORD
Aromatic plant, therapeutic compounds, bioactive molecule, secondary metabolites.

1. INTRODUCTION

Ocimum sanctum L. plant is serving as medicine from Ancient time. This plant is potential source of antimicrobial and bioactive molecule. *Ocimum sanctum* L. is known for its Antimicrobial activity against *E.coli* and *Staphlylococus aureus*[1]. *Ocimum sanctum* L. source of many industrial Antimicrobial compounds [2]. This plant is commonly known as tulsi belongs to family Lamiaceae. Tulsi is an incredible herb revered in Indian mythology known for its medicinal properties. Tulsi is branched shrub 30-60 cm tall with hairy stem. This plant is used to prevent cough, cold, fever, asthma, hepatic disease and much skin disease [3]. It is also known as aromatic plant. Plant derived drugs forms an important part of the modern medicinal system. Tulsi is considering as a natural resources for biological research. Extract of tulsi is useful for management of many infections and pathogens. Present investigations were monitor Methanolic extract of tulsi. Extract showed presence of secondary metabolites like alkaloid, phenols, glycoside flavonoids and amino acids. These metabolites are shows many biological activity.

2. MATERIALS AND METHODS

2.1. Collection of plant material

Ocimum sanctum L. was collected from Aurangabad.

2.2. Ethanol extract

40 gm powder of fresh and shad dry leaves extracted by Soxhlet extraction.

2.3. High Resolution Liquid Chromatography - Mass Spectroscopy (HRLC- MS)

Samples were analyzed on a LC-ESI-Q-TOF-MS (Agilent Technologies 6550 i-Funnel) system equipped with a G4220B pump, G4226A auto sampler and G1316C, and a diode array detector (DAD). The elution solvent consisted of a gradient system of 0.1% formic acid in water (A) and acetonitrile (B) at a flow rate of 0.3 ml/min. The gradient system started with 95% A: 5% B reaching 5% A: 95% B in 50 minutes, then back to initial composition 95% A: 5% B in 10 min which was held at same composition for 5 minutes. The MS analysis was carried out by ESI positive ionization mode. MS source conditions were as follows: capillary voltage 3500 V, Gas temperature 250 C, drying gas flow 13 L/min, sheath Gas temp 300, sheath Gas Flow 11, nebulizing gas pressure 35 (psig), fragmentor 175 V, Skimmer 65 V, Octopole RFPeak 750 V, and mass range m/z 50-1000. The resolution was 40,000 FWHM. Metlin database was used to structure confirmation.

3. RESULTS AND DISCUSSION

HRLC-MS analysis lead to the identification of various phytochemical compounds from HR fractions of methanol extract of tulsi leaves .Secondary Metabolite were identified through mass spectrometry attached with HR which showed the presence of 98 secondary metabolites and results of present phytochemical screening are tabulated in (Table 1).

Phytochemicals identified from methanol extract of tulsi leaves *i.e.* D-4-Hydroxyphenylglycine, Isotectorigenin, 7-Methyl Ether, Pteroyl-D-glutamic acid, Nivalenol, Triparanol, Ketotifen, 2-Methylbutyrylglycine, Tuberonic acid, Ecgonine, 3H-1,2,4-Triazol-3-one, 5ethyl-2,4-dihydro-2-

(3-Hydroxypropyl)-4-(2-phenoxyethyl)-,Alprazolam Quercitrin, amcinonide, cefamandolenafate,1,4,5,8-Tetrahydroxy-2,6-Dimethylanthroquinone, ulfolithocholylglycine, Tryptophan, Protorifamycin, Protoveratrine- A, Picrotoxinin, Baeomycesic Acid, Khivorin, Oleamide and Ramiprilglucuronide. The retention time, mass, molecular formula and M/Z value, DB difference (ppm) of 26 major metabolites was evaluated (Table 1). Mass to charge ratio (M/Z) was calculated from spectra (Table 1). HRLC-MS chromatogram of the extract showed relative concentrations of various chemical compounds. Heights of peak indicate relative concentrations of components. Nature, molecular formula and structure of chemical compounds were analyzed by using Mass spectrometer at different times. Large compound splits into small ones giving rise to appearance of peaks at various m/z ratios. These HRLC-MS spectra are fingerprint of that compound and can be identified with the help of data library.

The main chemical constituent of Tulsi are Oleanolic acid, Ursolic acid, Rosmarinic acid was reported [4]. In present study Tuberonic acid,4-Hydroxypelargonic acid,5-Phenylaleric acid and Usnic acid were observed. The leaves of Tulsi contain 0.7%volatile oil, comprising about 71% Eugenol and 20% methyl Eugenol. The oil also contains Carvacrol and Sesquiterpine Caryophyllene [5]. Two Flavonoids Orientin and Andvicenin isolated from aqueous leaf extract of tulsi [6-10]. In present study observed that leaves extract of *Ocimum sanctum* found to have Pharmacological constituent.

Table 1. Phytochemicals present in Tulsi Leaf.

Sr. no	Name of compounds	RT	Mass	Formula	M/Z
1	myo-Inositol	0.967	180.0618	$C_6 H_{12} O_6$	203.0511
2	3-Amino-L-Tyrosine	0.999	196.0808	$C_9 H_{12} N2 O_3$	197.0881
3	D-4-Hydroxyphenylglycine	1.012	167.0544	$C_8 H_9 N O_3$	168.0617
4	Ile Pro	1.303	228.1459	$C_{11} H_{20} N_2 O_3$	229.1531
5	Doxylamine	1.341	270.1776	$C_{17} H_{22} N_2 O$	293.1684
6	6,8,10,12-pentadecatetraenal	1.361	218.1636	$C_{15} H_{22} O$	241.1529
7	GlyLeu	1.437	188.1146	$C_8 H_{16} N_2 O_3$	189.1219
8	Isotectorigenin, 7-Methyl Ether	1.486	328.0922	$C_{18} H_{16} O_6$	329.0996
9	Pteroyl-D-glutamic acid	1.488	473.1648	$C_{20} H_{23} N_7 O_7$	474.1721
10	Nivalenol	1.506	312.1201	$C_{15} H_{20} O_7$	313.1296
11	Allysine	1.541	145.0723	$C_6 H_{11} N O_3$	168.0615
12	Thr Pro	1.769	216.1093	$C_9 H_{16} N_2 O_4$	239.0985
13	Triparanol	1.821	143.0957	$C_7 H_{13} N O_2$	166.085
14	Ketotifen	2.01	309.1195	$C_{19} H_{19} N O S$	310.1267
15	GlyAsnGln	2.024	317.135	$C_{11} H_{19} N_5 O_6$	340.1246
16	Pro Val Ser	2.025	301.1615	$C_{13} H_{23} N_3 O_5$	324.1506
17	Heteropeucenin, Methyl	2.072	274.1148	$C_{16} H_{18} O_4$	297.1037

	Ether				
18	1-alkenyl-2-acylglycerophospho-ethanolamine	2.238	267.0488	$C_8 H_{14} N O_7 P$	268.0564
19	3'-Oxopentobarbitone	2.399	240.1093	$C_{11} H_{16} N_2 O_4$	241.1166
20	5-azauridine	2.804	245.0668	$C_8 H_{11} N_3 O_6$	268.0562
21	2-Methylbutyrylglycine	3.165	159.0882	$C_7 H_{13} N O_3$	182.0774
22	PICEID	4.335	404.1436	$C_{21} H_{24} O_8$	427.1332
23	AsnGlnGln	4.339	388.1703	$C_{14} H_{24} N_6 O_7$	411.1594
24	Gln Met Lys	4.35	405.1968	$C_{16}H_{31}N_5O_5S$	406.2038
25	1-Cyclohexene-1-acrylic acid, 2,6,6-trimethyl-3-oxo-	4.522	208.1092	$C_{12} H_{16} O_3$	209.1165
26	Tuberonic acid	4.523	226.119	$C_{12} H_{18} O_4$	227.1263
27	Hydroxyamobarbital	4.556	242.1272	$C_{11} H_{18} N_2 O_4$	265.1164
27	Chloramphenicol palmitate	4.608	560.2407	$C_{27}H_{42}C_{l2}N_2O$	561.2478
28	Artemether	4.903	298.175	$C_{16} H_{26} O_5$	321.1645
29	Lys PheGly	4.992	350.1911	$C_{17} H_{26} N_4 O_4$	373.1804
30	5,8,11-heptadecatriynoic acid	5.004	258.156	$C_{17} H_{22} O_2$	259.1633
31	Undecylic acid	5.067	186.1626	$C_{11} H_{22} O_2$	209.1519
32	8alpha-3beta-hydroxy-estra-1,3,5(10)-trien-17-one	5.111	270.1559	$C_{18} H_{22} O_2$	271.1632
33	11-Dehydrocorticosterone	5.141	344.1939	$C_{21} H_{28} O_4$	367.1832
34	GluArg Asp	5.239	418.1809	$C_{15} H_{26} N_6 O_8$	441.1697
35	Tyr Pro	5.307	278.1247	$C_{14} H_{18} N_2 O_4$	279.1319
36	3H-1,2,4-Triazol-3-one, 5ethyl-2,4-dihydro-2-(3-Hydroxypropyl) -4-(2-phenoxyethyl)-	5.648	291.1587	$C_{15} H_{21} N_3 O_3$	14.1479
37	Phe Lys	5.761	293.174	$C_{15} H_{23} N_3 O_3$	316.1631
38	Ecgonine	5.807	185.1041	$C_9 H_{15} N O_3$	186.1114
39	cefamandolenafate	5.957	462.077	$C_{18}H_{18}N_6 O_5S_2$	463.0838
40	10-Deoxymethymycin	6.042	453.3133	$C_{25} H_{43} N O_6$	476.3028
41	Galbeta1-3GalNAcalpha-Thr	6.175	540.2168	$C_{21} H_{36} N_2 O_{14}$	563.206
42	Quercitrin	6.321	448.0969	$C_{21} H_{20} O_{11}$	449.1042
43	Kaempherol	6.325	286.0457	$C_{15} H_{10} O_6$	287.053
44	Temazepamglucuronide	6.422	476.0924	$C_{22}H_{21}ClN_2O_8$	477.0993
45	Cosmosiin	6.529	432.1028	$C_{21} H_{20} O_{10}$	433.1101

46	LeuLeuTrp	6.988	430.2538	$C_{23}H_{34}N_4O_4$	453.243
47	1,4,5,8-Tetrahydroxy-2,6-Dimethylanthroquinone	7.02	300.0614	$C_{16}H_{12}O_6$	301.0687
48	4-hydroxy pelargonic acid	7.116	174.1266	$C_9H_{18}O_3$	197.1158
49	5-Phenylvaleric acid	7.116	178.0981	$C_{11}H_{14}O_2$	179.1054
50	Tacrolimus metabolite M-IV	7.196	819.4576	$C_{44}H_{69}NO_{13}$	842.4472
51	Arg Asp Pro	7.386	386.1907	$C_{15}H_{26}N_6O_6$	409.1798
52	Penbutololglucuronide	7.416	467.2411	$C_{24}H_{37}NO_8$	490.2305
53	His Ser Lys	7.476	370.1967	$C_{15}H_{26}N_6O_5$	393.1857
54	undecanal	7.489	170.1681	$C_{11}H_{22}O$	193.1573
55	Arg Pro Thr	7.489	372.2125	$C_{15}H_{28}N_6O_5$	395.2016
56	3E,5E-tridecadienoic acid	7.49	210.1609	$C_{13}H_{22}O_2$	211.1683
57	2H-Indol-2-one, 1,3-dihydro-7-hydroxy-4-[2(propylamino)ethyl]-glucuronide	7.491	410.1689	$C_{19}H_{26}N_2O_8$	411.1764
58	3-Phenoxypropionic acid	7.584	166.0617	$C_9H_{10}O_3$	167.069
59	amcinonide	7.64	502.2378	$C_{28}H_{35}FO_7$	525.2266
60	3"-HydroxyPravastatin	7.758	440.2352	$C_{23}H_{36}O_8$	441.2427
61	Alprazolam	7.798	308.0777	$C_{17}H_{13}ClN_4$	309.0847
62	AsnGly His	7.96	326.1342	$C_{12}H_{18}N_6O_5$	349.1232
63	ArgGlu Ile	7.971	416.2381	$C_{17}H_{32}N_6O_6$	439.2272
64	2-tridecenal	7.973	196.1835	$C_{13}H_{24}O$	219.1727
65	6b,11b,16a,17a,21Pentahydroxypregna-1,4-diene-3,20-dione 16,17-acetonide	7.973	432.2121	$C_{24}H_{32}O_7$	455.2015
66	Sulfolithocholylglycine	8.013	513.2752	$C_{26}H_4NO_7S$	514.2825
67	GluLeuArg	8.103	416.2382	$C_{17}H_{32}N_6O_6$	439.2274
69	amcinonide	8.707	502.238	$C_{28}H_{35}FO_7$	525.2273
70	His Ser Lys	9.015	370.1967	$C_{15}H_{26}N_6O_5$	393.1855
71	Propanoic acid, 2-hydroxy-3-[(4-hydroxy-1-naphthalenyl)oxy]-	9.483	248.0692	$C_{13}H_{12}O_5$	271.0585
72	1,4,5,8-Tetrahydroxy-2,6-Dimethylanthroquinone	9.682	300.0614	$C_{16}H_{12}O_6$	301.0686
73	Tryptophan	9.908	204.0911	$C_{11}H_{12}N_2O_2$	227.0803
74	Dihydrodeoxystreptomycin	10.095	567.2856	$C_{21}H_{41}N_7O_{11}$	568.2926
75	Thiopental	10.202	242.1066	$C_{11}H_{18}N_2O_2S$	265.0956

76	(+)-6-methyl caprylic acid	10.583	158.132	$C_9 H_{18} O_2$	181.1213
77	Protorifamycin I	10.59	639.3061	$C_{35} H_{45} N O_{10}$	640.3135
78	Protoveratrine A	10.75	793.4271	$C_{41} H_{63} N O_{14}$	816.4164
79	Picrotoxinin	10.773	292.095	$C_{15} H_{16} O_6$	315.0842
80	(1R,2R)-3-oxo-2-pentylcyclopentanehexanoic	10.861	268.2042	$C_{16} H_{28} O_3$	291.1934
81	(-)-Usnic acid	10.969	344.0872	$C_{18} H_{16} O_7$	345.0944
82	Baeomycesic acid	11.619	374.0976	$C_{19} H_{18} O_8$	375.1048
83	Lys Cys His	11.941	386.1706	$C_{15} H_{26} N_6 O_4 S$	387.1775
84	Fipexide	12.712	388.1133	$C_{20} H_{21} Cl N_2 O_4$	389.1204
85	QuercetinTetramethyl (5,7,3',4') Ether	13.575	358.1025	$C_{19} H_{18} O_7$	359.1098
86	Docosanedioic acid	13.664	370.3164	$C_{22} H_{42} O_4$	371.3238
87	Phthalic acid Mono-2-ethylhexyl Ester	15.852	278.1499	$C_{16} H_{22} O_4$	279.157
88	26,26,26,27,27,27-hexafluoro-1alpha,25-dihydroxy-23,23,24,24-tetradehydro-vitamin D3	16.24	520.2414	$C_{27} H_{34} F_6 O_3$	543.2308
89	GPGro(16:0/0:0)[U]	17.554	484.2788	$C_{22} H_{45} O_9 P$	485.2861
90	Khivorin	18.658	586.2769	$C_{32} H_{42} O_{10}$	609.2663
91	Oleamide	18.713	281.27	$C_{18} H_{35} N O$	282.2773
92	Ramiprilglucuronide	19.05	592.2642	$C_{29} H_{40} N_2 O_{11}$	593.2717
93	Stearamide	20.111	283.2856	$C_{18} H_{37} N O$	284.2929
94	Trandolaprilglucuronide	20.354	606.2796	$C_{30} H_{42} N_2 O_{11}$	607.2872
95	12beta-Hydroxy-3-oxo-5betacholan-24-oic Acid	20.992	390.2747	$C_{24} H_{38} O_4$	391.2815
96	1,2di(9Z,12Z,15Zoctadecatrienoyl 3-O-Beta-Dgalactosyl-sn-glycerol	22.236	774.5218	$C_{45} H_{74} O_{10}$	797.5109
97	2,4,6-trimethyl-2,15-tetracosadienoic acid	23.329	406.3797	$C_{27} H_{50} O_2$	429.369
98	N-(2hydroxyethyl)hexacosanamid	25.404	439.4352	$C_{28} H_{57} N O_2$	440.4425

4. CONCLUSION

HRLC-MS analysis lead to the identification of various phytochemical compounds from HR fractions of methanol extract of Tulsi leaf estimated phytochemical constituents include alkaloids, flavonoids, phenolics, essential oils, tannins and saponins it also contains volatile substances. The plant truly deserves the title 'Elixir of Life' due to its Ethano- pharmacological properties. The active constituent present in *Ocimum sanctum L.,* has been found to be largely responsible for the therapeutic potentials of Tulsi like antifertility, anticancer, antidiabetic, antifungal, antimicrobial, hepatoprotective, cardioprotective, antiemetic, antispasmodic, analgesic, and diaphoretic actions. *Ocimum sanctum* is also known for its antibacterial, antioxidant, antiulceric, antimalarial, anti-inflammatory, antilipidemic, anticancer and immunomodulatory properties due to presence of 98 metabolites.

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