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Original Article

ANALYSIS OF BIO-ACTIVE COMPOUNDS PRESENT IN THE LEAVES AND STEM OF *TRICHOSANTHES* ROXB. USING GC-MS TECHNIQUE WITH RESPECT TO ITS ANTI-INFLAMMATORY ACTION

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ABSTRACT

Objective: Structural elucidation studies on *Trichosanthes lobata* ethyl acetate and methanol extracts of leaf and stem parts through Gas Chromatography-Mass Spectrometry (GC-MS) technique with respect to anti-inflammatory potential.

Methods: Extracts obtained with shade dried and powdered samples in successive solvent extraction using ethyl acetate and methanol by Soxhlet apparatus and subjected to GC-MS analysis and interpreted for its anti-inflammatory compounds.

Results: The study revealed that the extraction solvent used was able to recover compound of classes such as organic acid esters and conjugated alkaloids in larger quantities than other classes of compounds and they varied with leaf and stem and also with the polarity of solvents used. In total compounds identified, GC-MS profile of the Ethyl Acetate leaf extract of *T. lobata* contained 41 compounds, stem extract contained 45 compounds which have reported bioassays in PubChem. Whereas GC-MS profile of methanol leaf extract of *T. lobata* contained 66 compounds and stem extract contained 46 compounds having bioassay reports in PubChem. A large number of phytochemical peaks with good area percentage were found in methanolic extract. We were also able to find out potent anti-inflammatory compounds including Octanoic acid, Dodecanoic acid, Octadecane, Enoic acid, Hexanoic acid, Quinazolin-8-one, Ilicic acid, Pentadecanoic acid, Oxaspiro, Benzeneacetic acid, etc. from the extracts.

Conclusion: *T. lobata* contains phytocompounds against inflammation which may serve as a new drug lead of natural products origin in future and make it employable in modern pharmacological practices.

Keywords: Ethyl Acetate, Methanol, *Trichosanthes lobata,* GC-MS, Phytochemicals, Leaf, Stem

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INTRODUCTION

Basically, nature is the first source of medicinal compounds, from which bigger and accurate molecules are being formed. Since ancient times the natural substrates have been used as a source of various products applied in food, drug, cosmetic, textile and energy [1]. These naturally available products of higher plants serve as traditional medicine, possibly due to scientifically not proven mechanisms of action. The natural compounds exhibit various beneficial biological activities such as antimicrobial, antioxidant, anticancer, anti-inflammatory, anti-obesity, anti-angiogenic, neuroprotective activities, etc. Therefore, various phytochemicals isolated from plant sources have attracted much attention in the field of pharmacology [2]. *Trichosanthes* species belongs to Cucurbitaceae family. Generally, fruits of this species are proved to have good sources of phenolics and antioxidants, also possesses anticancer, antiproliferative, cardioprotective, antioxidant properties, etc. Many research studies have reported that it contains important protein called Ribosome Inactivating Protein which helps in the formulation of anticancer agents. In general, biological components present in plants are considered as a stimulant for its medical properties like anti-inflammatory, antidiabetic, antiulcer and cardioprotective activities. Similarly, there are various herbal preparations from *Trichosanthes* species being prescribed widely for the treatment of inflammatory conditions. One such plant is *Trichosanthes lobata* Roxb. Commonly called as *patolam*, *kaypanpatolam*, *kattupatolam* and *peppatolam*. It is a monoecious climber, an indigenous species therapeutically used in the tribes of Idukki hills, Kerala, India. These species have shown the presence of active constituents like flavonoids, phenolics, carotenoids, saponins, triterpenoids, etc. Although the extracts of this plant have been traditionally used in the treatment of inflammations, there is no scientific evidence which supports this therapeutic use. Our earlier research has documented its acute and chronic *in vivo* anti-inflammatory activities. The overall results obtained confirmed that the extracts of *Trichosanthes lobata* contain anti-inflammatory substances.

Inflammation refers to the reaction of living tissues to overcome injury, infection or irritation. Lysosomal enzymes are released during inflammation producing a variety of disorders leading to tissue injury by damaging the macromolecules and lipid peroxidation of membranes. These activities are presumed to be responsible for certain pathological conditions such as heart attacks, septic shocks, rheumatoid arthritis, etc. The extracellular activity of these enzymes is said to be related to acute or chronic inflammation [3]. Thus, there is a need for natural anti-inflammatory agents to achieve increased pharmacological response and less side effects. In this regard, complementary, alternative and traditional medicine play a pivotal role in medication. While proving them through scientific methods gives more authentication to the practices followed. Fulzule *et al.* [4] studied anti-inflammatory activity of *Trichosanthes cucumerina* and it was evaluated by use of the carrageenan-induced paw edema model in Wistar rats. Also, the underlying mechanism by which *T. cucumerina* mediates the anti-inflammatory activity was assessed by determining its effects on membrane stabilizing activity and nitric oxide inhibitory activity. Inhibitions of nitric oxide (NO) production and membrane stabilization activities are probably the mechanisms by which ligand molecules mediate its anti-inflammatory actions. Those findings rationalized the traditional practice of this plant as an anti-inflammatory agent. They suspect that membrane stabilizing abilities and the absence of inhibitory action are possible mechanisms through which *T. cucumerina* arbitrates its anti-inflammatory action.

In this study, we have analysed the chemical profiling of ethyl acetate and methanol leaf and stem extracts of *T. lobata,* with special reference to their anti-inflammatory effects using by Gas Chromatography-Mass Spectrometry (GC-MS). The obtained compounds were checked for its anti-inflammatory properties in PubChem and tried to report the possible effect of plant extracts against inflammation.

MATERIALS AND METHODS

Plant collection and preparation of plant extracts

Fresh, healthy and matured leaves and stem of *Trichosanthes lobata* Roxb. were collected from Vattavada Panchayat (10 °10'38.7"N 77 °15'33."E), Koviloor Post, Idukki District, Kerala state of India. The authenticity of the selected plant species was confirmed from the Botanical Survey of India, Southern Circle, Coimbatore (Vide No: BSI/SRC/5/23/2016/Tech./213). The leaves and stem were cleaned, dried in shade and ground well to a fine powder. About 50 g of dry powders were subjected to successive solvent extraction with ethyl acetate and methanol using Soxhlet apparatus. The filtered and dried extracts were subjected for GC-MS analysis.

Gas chromatography-mass spectrometry (GC-MS) analysis

The GC-MS analysis was carried out using the instrument Thermo GC-Trace Ultra Ver: 5.0, Thermo MS DSQ II with Column: DB 35-MS Capillary Standard Non-Polar Column possessing Dimension: 30 Mts, ID: 0.25 mm, Film: 0.25 µm. The initial temperature of the instrument was set to 110 °C and maintained for 2 min. At the end of this period, the oven temperature was raised up to 260 °C, at the rate of an increase of 6 °C/min, and maintained for 9 min. The temperature of injection port was ensured as 250 °C and the flow rate of Helium as 1 ml/min. An injection volume of 1 µl of sample is considered in the analysis. The ionization voltage was maintained to be 70 eV and samples were injected in split mode in the ratio 10:1. Mass Spectral scan range was set at 45-450 (MHz).

The chemical constituents were identified by GC-MS. The fragmentation patterns obtained from mass spectra were compared and analysed with spectrometer database using National Institute of Standards and Technology Mass Spectral database (NIST-MS). From the relative peak area of each component in the chromatogram the percentage of each component was calculated.

Table 1: Showing the list of compounds having pharmaceutical importance obtained from GC-MS profile of Ethyl acetate leaf extract of *Trichosanthes lobata*

S. No.	RT (min)	Compound name	Molecular formula	Mol. weight (g/Mol)	Area %	Biological activity*
1	14.43	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	7.84	Antagonistic activity against Escherichia coli
2	14.43	Octanoic acid	$C_8H_{16}O_2$	144	7.84	Prostaglandin E2 inhibitors, and Cox2
3	14.43	Dodecanoic acid	$C_{12}H_{24}O_2$	200	7.84	Antimicrobial and anticancer activity
4	14.43	D-Glucuronic acid	$C_6H_{10}O_7$	194	7.84	Apoptotic and cytotoxic activity
5	14.43	Dodecanoic acid	$C_{12}H_{24}O_2$	200	7.84	Blood pressure regulation and vascular inflammation
6	26.8	Trimethylsilyl	$C_{18}H_{34}O_4Si_3$	398	4.21	Fungicidal activity
7	26.8	Phenazine, 5,10-dioxide	$C_{12}H_8N_2O_2$	212	4.21	Anti-trypanosomal activity
8	28.8	Docosane	$C_{22}H_{46}$	310	3.06	Antimicrobial activity
9	28.8	Octadecane	$C_{18}H_{38}$	254	3.06	Immuno-stimulating properties, Anti-inflammatory activity
10	30.51	Benzoic acid	$C_{16}H_{14}O_3$	254	1.74	Drug metabolism in human liver
11	30.51	Benzopyran-4-One	$C_{16}H_{14}O_3$	254	1.74	Antiestrogenic activity
12	30.51	Fluoroquinazoline	$C_{15}H_{11}FN_2O$	254	1.74	Antifungal activity
13	33.50	Phthalic acid	$C_{23}H_{30}O_4$	370	3.19	Insecticidal, Antibacterial, Gastroprotective and cytotoxic activity
14	33.95	Diphenylpyridine	$C_{23}H_{25}N$	315	3.98	Antiviral activity, Cytotoxicity
15	33.95	Quinone	$C_{24}H_{14}O_6$	398	3.98	Biomarker for cancer chemoprevention
16	34.28	Dimethylisoquinolin-8-ol	$C_{31}H_{33}NO_4$	483	4.94	Antibacterial activity and Cytotoxicity
17	34.28	Tetramethyl	$C_{26}H_{28}O_8$	468	4.94	Antimalarial activity, cox2 inhibition
18	34.28	Benzothiazin	$C_{11}H_{13}NOS$	207	4.94	Antitubercular activity
19	34.28	Morphinane	$C_{22}H_{17}F_6N_3O_2$	469	4.94	Cell protective activity
20	35.09	Benzenepropanoic acid	$C_{11}H_{14}O_3$	194	2.73	Inhibitory activity against platelet aggregation
21	35.09	Tetrafluoroborate	$C_{18}H_{22}BF_4N_2O_4PS$	480	2.73	Anti-hemolytic, Antibacterial activity
22	35.93	Milbemycin B	$C_{32}H_{44}ClNO_7$	589	2.57	Insecticidal activity
23	35.93	Carboxylate	$C_{17}H_{23}NO_2$	273	2.57	Neuropsychiatric events
24	37.05	Propylamine	$C_{13}H_{18}FN$	207	2.35	Cytotoxicity
25	37.05	Enoic Acid	$C_{11}H_{11}FO_2$	194	2.35	Inhibitors of fatty acid and cyclooxygenase
26	37.05	Butenoic acid	$C_9H_7BrO_3$	242	2.35	Antimicrobial, Effective dose to inhibit gastric acid secretion
27	37.25	Pyridine	$C_{14}H_{11}N_3O$	253	2.20	Drug metabolism
28	37.70	Methyl Ethyl Pyrazine	$C_7H_{10}N_2$	122	4.22	Cytoprotective activity
29	37.70	Propenoate	$C_9H_{11}NO_3$	181	4.22	Antiapoptotic, Cardioprotective effect
30	37.70	Pyrimidine	$C_{15}H_{17}FN_2$	244	4.22	Drug metabolism
31	37.70	Glucopyranoside	$C_{33}H_{55}NO_7$	577	4.22	Inhibition of Saccharomyces
32	38.21	Dichloro[tri(trimethylsilyl) methyl]Silane	$C_{10}H_{28}Cl_2Si_4$	330	1.90	Inhibit Cholesterol Biosynthesis, Cytotoxicity
33	38.21	Diazepine	$C_{20}H_{17}N_3O$	315	1.90	Anticonvulsant activity
34	38.43	Carboxylate	$C_{27}H_{35}NO_6$	469	1.88	Neuropsychiatric events
35	38.43	Naphthalene	$C_{27}H_{23}N_3O_5$	469	1.88	Inhibition of COX1
36	38.43	Phenylimidazole	$C_{26}H_{24}N_6OS$	468	1.88	Cholesterol lowering effect
37	38.43	Isophthalate	$C_{11}H_8F_3IO_7S$	468	1.88	Cancer drug development
38	38.43	Thiophene	$C_{29}H_{24}O_2S2$	468	1.88	Drug metabolism
39	38.82	Quinazoline	$C_{19}H_{13}Br_2N_3$	441	3.87	Antimicrobial activity
40	39.17	Dihydrofuran	$C_{14}H_{12}O$	196	1.87	Cytotoxicity Antibacterial activity
41	39.92	Tetrahydroisoquinoline	$C_{17}H_{20}N_2O_2$	284	2.45	Drug metabolism

*Source of the Biological activities of the compound is PubChem database.

Identification of compounds

Using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns the interpretation of mass spectrum of GC-MS was conducted. The spectrum components of known compounds were compared with unknown components stored in the NIST library. The name, molecular weight and structure of the compounds were ascertained in above said way.

Identification of compounds having biological activity

The obtained list of compounds was checked for having biological properties which are reported in PubChem. All the compounds which are subjected for Bioassay are listed in tables and phytochemicals having anti-inflammatory action is considered as positive hits for our study.

RESULTS AND DISCUSSION

Characterization of secondary metabolites from medicinal plants provides an extensive resource for the isolation and identification of novel therapeutic agents for inflammation. The major non-volatile compounds can be identified by GC-MS analysis. The crude methanolic extract revealed the high peak intensity compound predominant presence of major compounds like pentadecanoic acid, 14-methyl-, methyl esters (RT-14.43) and methyl stearate among other derivatives. Minor compounds such as 10-methyl ester, methyl tetradecanoate, tetradecanoic acid, 12-methyl-methyl ester, 9-hexadecenoic acid, hexadecanoic acid, 14-methyl-methyl ester, 10-octadecenoic acid and heptadecanoic acid were also identified. These compounds are exhibited activities like antioxidant, cancer-preventive, hypercholesterolemic, nematicide, antifungal and antimicrobial. The advantage of GC-MS is its highest accuracy in the identification of derivatized compounds. The list of compounds from different extracts and plant parts are as tabulated in table 1-4 and the respective spectrum is as shown in fig. 1-4.

Table 2: Showing the list of compounds having pharmaceutical importance obtained from GC-MS profile of Ethyl acetate stem extract of *Trichosanthes lobata*

S. No.	RT (min)	Compound name	Molecular formula	Mol. weight (g/Mol)	Area %	Biological activity*
1	13.90	Dimethylbenz Oate	$C_{14}H_{20}O_4$	252	0.33	Drug Metabolism and Pathologic Process
2	13.90	Chloropyridine	$C_{11}H_8ClNO$	205	0.33	Antinociceptive Activity
3	13.90	Tetralone	$C_{13}H_{16}OS_2$	252	0.33	Parkinson's disease and depression
4	13.90	Dihydroisobenzofuran	$C_{13}H_{16}O_3$	220	0.33	Drug-protein interactions at serotonin transporter
5	13.90	Dicarboxylate	$C_{15}H_{13}ClO_6$	324	0.33	Agonist for metabotropic glutamate receptors
6	13.90	Pyrazine	$C_{12}H_{20}N_2Si$	220	0.33	Antimalarial agent
7	14.43	Acetamide	$C_{24}H_{20}BrN_3O_2S_2$	525	0.29	Radioligand for benzodiazepine receptor in brain
8	14.43	Glaucine	$C_{21}H_{25}NO_4$	355	0.29	Antioxidant and Antiviral activity
9	14.43	Quinoline	$C_{17}H_{15}Br_2NO$	455	0.29	Drug metabolism
10	19.25	Carboxylate	$C_{13}H_{18}O_5$	254	0.37	Neuropsychiatric events in influenza
11	19.25	Hexanoic acid	$C_{19}H_{34}N_4O_6$	414	0.37	Inhibit cyclooxygenase activity
12	23.00	Hexadecanoic acid	$C_{18}H_{36}O_2$	284	0.44	Promote cancer cell death
13	24.92	Glucopyranose	$C_{15}H_{19}D_3O_{10}$	362	0.34	Cytotoxicity
14	24.92	Oxodehydroabietate	$C_{21}H_{28}O_3$	328	0.34	Cytotoxicity
15	24.92	Cyclohexanol	$C_{13}H_{24}O_2$	212	0.34	MDR-reversal drug lead
16	26.00	Bromobenzene	$C_{15}H_{13}BrO_2S$	336	0.44	Anticancer, Antimicrobial Activity
17	26.00	Cyclopropane	$C_{10}H_{12}S$	164	0.44	Inhibition Assay
18	28.33	Phthalimide	$C_{19}H_{16}ClNO_2S$	357	0.38	Hypolipidemic activity
19	28.33	Colchicine	$C_{20}H_{23}NO_5$	357	0.38	Binding affinity
20	28.33	Diphenylquinoline	$C_{22}H_{17}N$	295	0.38	Metabolic stability
21	28.77	Haliclonacyclamine	$C_{32}H_{58}N_2$	470	0.54	Cytotoxicity
22	29.42	Cyanopyridine	$C_{18}H_{20}N_2$	264	0.51	Antimicrobial activity
23	29.42	Ethanone hydrochloride	$C_6H_{11}NOS_2$	177	0.51	Antiproliferative activity
24	29.42	Oxazoline	$C_{21}H_{25}NO_7$	403	0.51	Ovicidal activity
25	29.89	Pyridinedicarboxylic acid	$C_{21}H_{27}NO_5$	373	1.92	Inhibitory against Hepatitis B
26	31.74	Stigmastane	$C_{29}H_{52}$	400	0.29	Antimicrobial, Effective dose to inhibit gastric acid secretion
27	31.74	Phenylquinoxaline	$C_{15}H_{10}ClN_3O_2$	299	0.29	Binding affinity to beta-amyloid in Alzheimer's disease
28	31.74	Glucopyranosiduronic acid	$C_{29}H_{56}N_2O_{10}S$	676	0.29	Cytotoxicity
29	32.38	Methyl propyl 6-[3-(propylthiocarbonyl)benzyl]benzene-1-carboxylate-3-carboxythioic S-ester	$C_{23}H_{26}O_4S_2$	430	0.72	Analgesic activity
30	32.86	Carbaldehyde	$C_{34}H_{35}N_5O_5$	593	1.23	Inhibitory drugs for smoking reduction
31	32.86	Oxazolidinone	$C_{20}H_{27}NO_4S$	377	1.23	Antimycobacterial activity
32	32.86	Sanguinarine	$C_{22}H_{19}NO_5$	377	1.23	Antibacterial activity
33	33.31	Oxazoline	$C_{21}H_{25}NO_7$	403	0.27	Ovicidal activity
34	33.31	Tetrahydroisoquinolin-4-ol	$C_{19}H_{20}N_2O_6$	372	0.27	Potentiating and inhibiting activity
35	33.70	Imidazole	$C_{16}H_{10}Br_2N_2O$	404	0.46	Kinase Assay
36	33.70	Oxobutanoic acid	$C_{20}H_{23}FN_2O_5$	390	0.46	Stimulated Gastric Acid Secretion
37	34.23	Phenylindole	$C_{16}H_{15}NO_2$	253	1.29	Pro-apoptotic in cancer cells
38	34.23	Azepine	$C_{14}H_{14}N_2OSi$	254	1.29	Antioxidant activity
39	34.64	Acetohydrazide	$C_{24}H_{30}N_2O_6$	442	1.08	Inhibition of topoisomerase 2
40	34.64	Physodic acid	$C_{26}H_{30}O_8$	470	1.08	Drug metabolism
41	39.16	ë(2,2')-Bis(1,3-dithio[4,5-c]selenophene)	$C_{10}H_4S_4Se_2$	412	69.34	Antimicrobial activity
42	39.57	Quinolinecarboxylic acid	$C_{10}H_9NO_4$	207	3.56	Gastrointestinal disorder
43	39.57	4-Methylthio-3-quinolinesulfonamide	$C_{10}H_{10}N_2O_2S_2$	254	3.56	Antidepressant activity
44	40.24	4H-1-Benzopyran-4-one	$C_{28}H_{26}O_{10}$	522	5.24	Potent anti-estrogens.
45	40.42	N-Benzyl-2-Bromoaniline	$C_{13}H_{12}BrN$	261	1.55	Inhibition of Fibroblast

*Source of the Biological activities of the compound is PubChem database.

Table 3: Showing the list of compounds having pharmaceutical importance obtained from GC-MS profile of Methanol leaf extract of *Trichosanthes lobata*

S. No.	RT (min)	Compound name	Molecular formula	Mol. weight (g/Mol)	Area %	Biological activity*
1	15.51	Monohydroxamic acid	$C_8H_7NO_4$	181	2.78	Inhibition Of Leukemia Cells
2	15.51	Piperidine	$C_{11}H_{24}N_2O$	200	2.78	Potential cocaine antagonists.
3	15.51	Serine	$C_3H_6DNO_3$	105	2.78	Inhibit, osteoarthritis, Alzheimer's, age-related macular degeneration
4	16.84	Arsine, trimethyl-(CAS)	C_3H_9As	120	1.24	Decreased heart uptake with alkyl substitution
5	16.84	Pyridine	$C_{11}H_9NO_2$	187	1.24	Hypo-cholesteremic activity
6	19.85	Propanone	$C_{24}H_{51}NO_5Si_5$	573	1.11	Antagonist activity
7	19.85	Quinazolin-8-one	$C_{20}H_{20}N_2O_5$	368	1.11	Anti-inflammatory activity
8	19.85	Trimethylsilyl ester	$C_{20}H_{40}O_5Si_4$	472	1.11	Antiviral activity and Cytotoxicity
9	21.77	Annulene	$C_{21}H_{18}$	270	1.96	Cytotoxicity
10	21.77	Anthraquinone	$C_{15}H_{10}O_3S$	270	1.96	Anti-filarial activity, Cytotoxicity
11	21.77	Isoquinolin	$C_{28}H_{21}NO$	387	1.96	Increase of intracellular drug uptake
12	21.77	Azulene	$C_{20}H_{14}O$	270	1.96	Agonist Activity
13	22.03	Hexadecenoic acid	$C_{17}H_{32}O_2$	268	0.91	Antibacterial activity Anti-plasmodial activity
14	22.03	Hydroxy palmitate	$C_{19}H_{36}O_4$	328	0.91	Antihyperglycemic activity
15	22.87	Benzenedicarboxylic acid	$C_{16}H_{22}O_4$	278	1.09	Antitumor activity
16	22.87	phenylpropionate	$C_{13}H_{17}ClO_2$	240	1.09	Cellular uptake in human PBMC nucleus
17	22.87	Naphthalene	$C_{11}H_{19}NO_3$	213	1.09	Inhibition of HIV1 protease dimerization
18	22.87	Phenylpropan-1-one	$C_{17}H_{18}O_2$	254	1.09	Bio-reduction by bacterial ferredoxin
19	23.97	Methacrylate	$C_{21}H_{32}O_3$	332	0.86	Antiparasitic activity
20	23.97	Butyl acrylate	$C_{21}H_{32}O_3$	332	0.86	Cytotoxicity Antioxidant activity
21	23.97	1,3-Dithiolane	$C_{19}H_{30}S_2$	322	0.86	Detoxification activity
22	25.60	Octadecanoic acid	$C_{19}H_{38}O_2$	298	1.33	Highest toxicity against pathogens
23	28.25	N-phenylnitrone	$C_{16}H_{21}NO_3$	275	1.43	Cytoprotective activity
24	28.25	Carboxylic acid	$C_{13}H_{17}NO_3$	235	1.43	Anti-spermatogenic activity, Drug metabolism
25	28.25	Oxoquinoline	$C_{12}H_{12}N_2O_4S_2$	312	1.43	Antimicrobial activity
26	28.25	Methy L Dopamine	$C_{15}H_{10}Cl_3F_6N$	503	1.43	Binding affinity towards human dopamine D4 receptor
27	28.25	Glucopyranosiduronic acid	$C_{29}H_{57}NO_8Si_5$	687	1.43	Inhibition of HIV1 protease
28	28.25	Trimethylsilyl ester	$C_{29}H_{57}NO_8Si_5$	687	1.43	Antiviral activity, Cytotoxicity
29	28.88	Glutamic acid dimethyl ester	$C_7H_{12}DNO_4$	175	2.16	Inhibitory Activity Against Leukaemia Cells
30	28.88	Dihydropyrrole	$C_{11}H_5F_{16}N$	455	2.16	Mitotic arrest of ovarian carcinoma cells
31	29.73	Azetidinone	$C_{24}H_{25}NO_6S$	455	2.16	Reduction of liver cholesteryl esters
32	29.73	Quinoxalinone	$C_{27}H_{25}N_3O_4$	455	2.39	Cytotoxicity
33	29.73	Methylphenol	$C_{26}H_{35}ClN_4O$	454	2.39	Antimicrobial activity
34	29.73	Argentatin	$C_{33}H5_4O_4$	514	2.39	Inhibitory towards leukemic cell line
35	29.73	Isopropylamide	$C_{32}H_{41}NO$	455	2.39	Inhibitor of beta-galactosidases.
36	31.32	Montiporyne	$C_{15}H_{20}O$	216	5.25	Cytotoxicity
37	31.32	Chlorophenol	$C_{12}H_{13}ClO$	208	5.25	Anti-trypanosomal activity
38	31.32	Dimethylindole	$C_{10}H_{10}N_2O_3$	206	5.25	Growth inhibitory activity against pancreatic cancer cells.
39	31.32	Benzimidazole	$C_{11}H_{13}N_3S$	219	3.65	Reduced blood pressure
40	31.32	Cyclopropane	$C_{20}H_{18}N_2$	286	3.65	Potential targets for anti-tuberculous drugs
41	32.20	Aminocyclohexane	$C_{25}H_{26}BrN_2O$	512	0.82	Antidiuretic activity
42	32.20	Dimethoxy indole	$C_{33}H_{27}N_3O_3$	513	0.82	Antitumor activity
43	32.20	Methylpyrazole	$C_{20}H_{20}Br_2N_2$	510	0.82	Drug metabolism in human liver, osteoporosis
44	32.93	Dimethylcoumarin	$C_{11}H_{10}O_3$	190	0.91	Inhibition of monoamine oxidases
45	33.12	Lupeol	$C_{30}H_{50}O$	426	6.50	Reduce blood glucose, Cytotoxicity
46	33.12	Sclareol	$C_{20}H_{36}O_2$	308	6.50	Cytotoxicity, Induction of apoptosis
47	33.12	Farnesyl bromide	$C_{15}H_{25}Br$	284	6.50	Anti-plasmodial activity
48	33.12	Geranylgeraniol	$C_{20}H_{34}O$	290	6.50	Cytotoxicity
49	33.42	Illudinic acid	$C_{15}H_{18}O_4$	262	7.32	Antimicrobial activity, antitumor activity
50	34.36	Ilicic Acid	$C_{15}H_{24}O_4$	268	2.12	Anti-inflammatory activity
51	35.05	Dicarboxylic acid dimethyl ester	$C_{27}H_{32}O_5$	436	10.51	Antagonist activity
52	35.05	Phthalazin-1(2H)-one	$C_{20}H_{19}N_5OS$	377	10.51	Antibacterial activity
53	35.89	Quinazolinone	$C_{27}H_{22}N_4O_3$	450	2.01	Treatment for neurodegenerative disorders
54	35.89	Dimethoxybenzene	$C_{17}H_{29}BrO_3Si$	388	2.01	Cross allergenicity
55	35.89	Scopadulane	$C_{24}H_{38}O_4$	390	2.01	Cytotoxicity, Antiviral activity
56	36.13	Methylcrinasiadine	$C_{15}H_{11}NO_3$	253	2.31	Cytotoxic alkaloid
57	36.13	Methylpyrrole	$C_{13}H_{19}NO_4$	253	2.31	Antitumor Activity
58	36.84	Undecanolide	$C_{12}H_{20}O_3$	212	1.57	Antimicrobial activity, Cell cycle arrest in HepG2 cells
59	36.84	Galactitol	$C_{18}H_{26}O_{12}$	434	1.57	Antihyperglycemic agent
60	37.56	Aminodiphenylmethane	$C_{22}H_{21}NO_2$	331	0.98	Antimicrobial activity, Antiviral activity
61	37.8	Propargyl ether	$C_{14}H_{20}O_2S$	252	1.21	Antinociceptive tests
62	37.8	Phthalic acid	$C_{22}H_{26}O_4$	354	1.21	Insecticidal activity
63	38.41	Porphine	$C_{36}H_{42}N_4O_4$	594	10.21	Cytotoxicity
64	38.78	Glucopyranosiduronic acid	$C_{27}H_{52}N_2O_{10}S$	648	12.27	Cytotoxic activity, Anti-HIV-1 protease
65	38.78	Hydroxypregnenolone	$C_{23}H_{36}O_3$	360	12.27	Steroid-producing neurons
66	40.33	Azaanthracene	$C_{34}H_{24}N_6$	516	1.91	Activity against L1210 leukaemia cells

*Source of the Biological activities of the compound is PubChem database.

Table 4: Showing the list of compounds having pharmaceutical importance obtained from GC-MS profile of Methanol stem extract of *Trichosanthes lobata*

S. No.	RT (min)	Compound name	Molecular formula	Mol. weight (g/Mol)	Area %	Biological activity*
1	15.51	Dihydroisocoumarin	$C_{13}H_{16}O_2$	204	2.73	Aromatase inhibitor
2	16.84	Tert-butyldimethylsilyl ether	$C_{14}H_{28}O_5Si$	304	3.19	Antiviral activity, Cytotoxicity
3	19.44	Iminoisoquinoline	$C_{11}H_{12}N_2$	172	1.32	Inhibitors of nitric oxide synthase
4	19.44	Hydroxypropanoate	$C_{11}H_{22}O_3$	202	1.32	Antioxidant activity
5	21.79	Hexadecanoic acid	$C_{17}H_{34}O_2$	270	3.12	Anticancer drug target
6	21.79	Pentadecanoic acid	$C_{17}H_{34}O_2$	270	3.12	Inhibition of COX1 and prostaglandin biosynthesis
7	24.76	Oxaspiro	$C_{14}H_{20}O_3$	236	1.26	Acts against cancer and inflammation
8	25.72	Trimethylsilyl ester	$C_{20}H_{40}O_5Si_4$	404	2.22	Anti-HIV activity
9	26.80	D-glucopyranose	$C_8H_{19}ClO_6$	246	1.73	Cytotoxicity
10	26.80	Octadecanoic acid	$C_{20}H_{40}O_2$	312	1.73	Antimicrobial agent
11	27.94	Sulfoximine	$C_{23}H_{31}NO_4S_2$	449	1.21	Cytotoxicity
12	29.45	Benzeneacetic acid	$C_{10}H_{12}O_3$	180	1.60	Anti-inflammatory activity
13	29.92	Cyanoacetate	$C_{20}H_{15}N_3O_5$	377	1.63	Cytotoxicity, Antimicrobial activity
14	29.92	Phenylmethanone	$C_{27}H_{23}NO$	377	1.63	Inhibitory Activity on diabetes mellitus
15	30.36	Ethanamine	$C_9H_{13}N$	135	2.78	Antifungal activity
16	30.36	2-(Chlorovinyl)phenyl sulfide	C_8H_7ClS	170	2.78	Antimalarial design
17	30.36	Dihydrobenzothiophene	$C_{15}H_{14}S$	226	2.78	Potential treatment of Parkinson's disease
18	30.36	Phenylpropan-1-one	$C_{17}H_{18}O_2$	254	1.09	Bioreduction by bacterial ferredoxin
19	31.44	Tetraol	$C_{27}H_{44}O_4$	432	20.55	Cytotoxicity
20	31.44	ç-Sitosterol	$C_{29}H_{50}O$	414	20.55	Antiproliferative activity
21	32.20	Cycloheptatrien	$C_{10}H_{12}O_2$	164	2.44	Neuroprotective activity
22	32.52	Isopropylidene	$C_{39}H_{50}O_4Si_2$	638	3.17	Antitumor and antiviral activity
23	33.12	D-Glucopyranosiduronic acid	$C_{29}H_{56}N_2O_{10}S$	676	2.42	Cytotoxicity
24	33.12	Oxo-acetamide	$C_{24}H_{23}N_3O_3$	401	2.42	Highly potent histone deacetylase inhibitors
25	33.79	Azabicyclo	$C_{14}H_{22}N_2$	218	1.79	Muscarinic receptor antagonists
26	33.79	Quinazolinone	$C_{19}H_{23}N_3O_2$	325	1.79	Neurodegenerative disorder
27	34.17	Glucopyranosiduronic acid	$C_{29}H_{57}NO_8Si_5$	687	1.43	Inhibition of HIV1 protease
28	34.17	Porphycene	$C_{31}H_{36}N_4O_4$	528	1.24	Induction of apoptosis in human HeLa
29	34.17	Spongiadioxin	$C_{13}H_6Br_4O_3$	526	1.24	Cytotoxicity
30	34.48	Oxazolidinone	$C_{12}H_{15}NO_5$	253	1.42	Antibacterial activity
31	34.48	Cinnamate	$C_{19}H_{18}O_3S$	326	1.42	Depigmenting activity
32	34.48	Dimethyluracil	$C_{15}H_{22}N_2O_6$	326	1.42	Antibacterial activity
33	36.46	Propylpyrimidine	$C_{28}H_{28}N_2O_3$	440	16.55	Binding affinity towards Corticotropin
34	37.46	Androstane	$C_{21}H_{34}N_2O_2$	346	2.16	Inhibition of reduction of dihydrotestosterone
35	38.66	Trifluoromethanesulfonate	$C_9H_9F_3O_5S$	286	1.44	Antineoplastic activity
36	38.66	Benzenecarboximidamide	$C_{15}H_{12}F_3N_3O_3$	339	1.44	Antithrombotic
37	38.92	Norsesterterpene	$C_{24}H_{38}O_2$	358	1.62	Cytotoxicity
38	38.92	Methyl pentyl ester	$C_{20}H_{27}ClO_4$	366	1.62	Antiviral agent
39	39.72	Hydroxycodeinone	$C_{18}H_{19}NO_4$	313	3.97	Antagonistic Activity
40	39.72	Pyrimidine	$C_{22}H_{22}N_2$	314	3.97	Drug metabolism
41	39.72	Phenanthroline	$C_{19}H_{11}N_3O_2$	313	3.97	Cytotoxicity
42	39.72	Indolizine	$C_{21}H_{15}NO_2$	313	3.97	Inhibition of human EGFR autophosphorylation
43	39.72	Nitroanthracene	$C_{19}H_{23}NO_3$	313	3.97	Cancer therapy
44	40.02	Norbornadiene	$C_{14}H_{13}N$	195	1.82	Analgesic activity, Psychotomimetic activity
45	40.27	Phenanthrene	$C_{28}H_{20}Br_2$	514	1.65	Antiviral activity, Drug metabolism
46	40.27	Gomisin F	$C_{28}H_{34}O_9$	514	1.65	Cytotoxicity, Antiviral activity

*Source of the Biological activities of the compound is PubChem database.

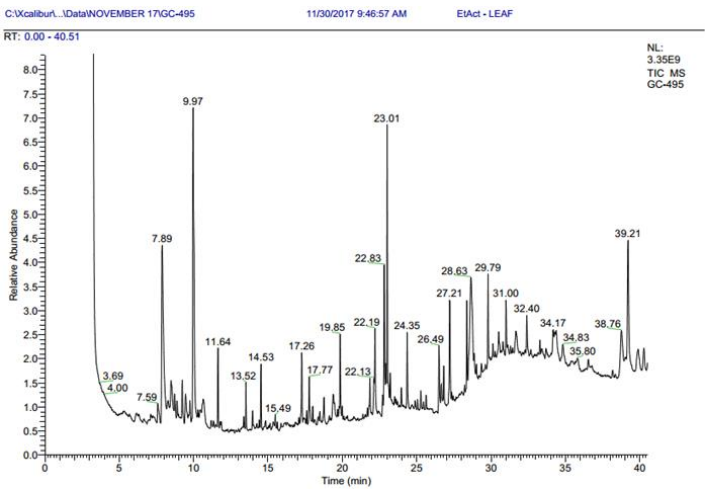

Fig. 1: Showing the GC-MS spectrum of *Trichosanthes lobata* leaf extracted from Ethyl acetate

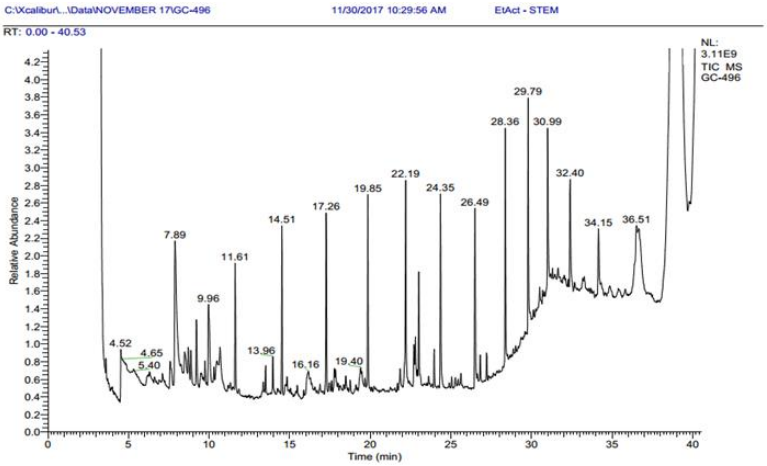

Fig. 2: Showing the GC-MS spectrum of *Trichosanthes lobata* stem extracted from Ethyl acetate

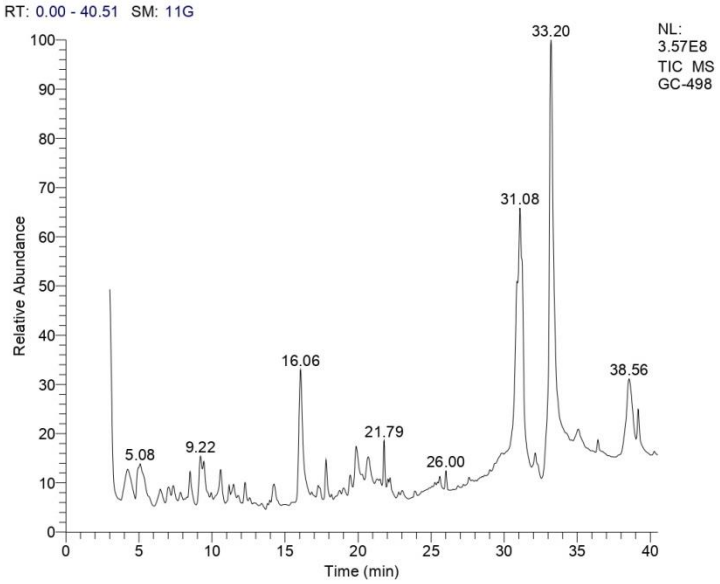

Fig. 3: Showing the GC-MS spectrum of *Trichosanthes lobata* leaf extracted from Methanol

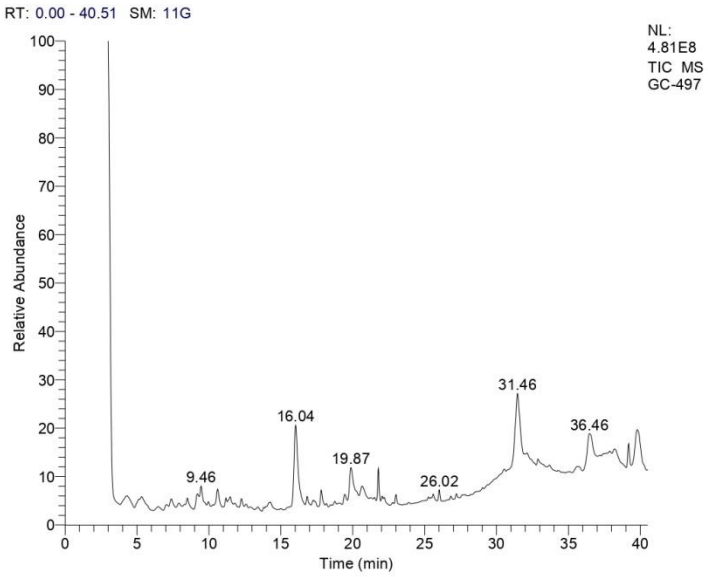

Fig. 4: Showing the GC-MS spectrum of *Trichosanthes lobata* stem extracted from Methanol

The GC-MS profile of Ethyl acetate leaf extract of *Trichosanthes lobata* showed the presence of 41 bioactive compounds, whereas its stem extract showed 45 compounds which are reported for various bioassays in PubChem. The GC-MS profile of methanol leaf extract of *T. lobata* showed 66 compounds which are biologically active and its respective stem extract contained 46 compounds having bioassay reports in PubChem. Overall, a large number of phytochemical compounds with good area percentage were found in methanolic extract, which was followed by ethyl acetate. We could also note that the number of active compounds was found more in leaf than in stem extracts. We were also able to find out potent anti-inflammatory compounds such as Octanoic acid, Dodecanoic acid, Octadecane, Tetramethyl, Enoic acid and Napthalene from ethyl acetate leaf extracts; Hexanoic acid and Methyl propyl from ethyl acetate stem extracts. Similarly, anti-inflammatory compounds such as Methylpyrazole, Quinazolin-8-one and Ilicic acid were present in the methanol leaf extract, whereas Pentadecanoic acid, Oxaspiro, Benzeneacetic acid and Norbornadiene were present in the methanol stem extract. With this we conclude that a greater number of bioactive agents against inflammation is found in extracts of leaf generated using ethyl acetate.

Many studies have proven that methanol has higher activities than that of extracts obtained from different solvents. In this case also even though methanolic extract has shown the presence of more number compounds, ethyl acetate leaf extracts stand to be effective against treating inflammation. Based on these examinations the anti-inflammatory activity of ethyl acetate and methanol leaf extracts may be attributed towards the presence of very active agents. The thorough quantitative analysis of phytochemicals using GC-MS studies reveals the presence of all components such as flavonoids, sterols, fatty acid and esters in the sample [5]. GC-MS analysis is also helpful in the determination of the chemical composition, area and molecular weight of the samples [6, 7].

In addition to anti-inflammatory activity we could notice that the extracts contain compounds which are responsible for treating other ailments too. It is reported that *T. lobata*, *T. dioica* [8, 9] *T. cucumerina* [10, 11] and *T. kirilowii* are said to contain carbohydrates, glycosides, flavonoids, tannins, proteins, steroids and saponins. *T. lobata* is used for malarial fever and liver disorders [12]. The highlighted compounds had been noted earlier for their anti-inflammatory potential. Active polyphenols, flavones, phenolic terpenes, fatty acids, sterols, amide, esters, alkaloids, flavonoids, lactones and carotenoids such as Orientin, isoorientin, isovitexin, vitexin, chlorogenic acid, catechin, palmitic, stearic, vanillic acids, sitotesnone, vinyl guaiacol, o-Tolylaldehyde, epicatechin, procyanidins, protocatechuic acid, oleanolic acids, Pomolic acid, α-amyrin, β-amyrin and derivatives of stigmasterol, oleic, linoleic, linolenic, limonene, lupeol, phytol, vellaral, cucuminoids, selinene, piperitol, camphor, quercetin, kaempferol, benzoazolinone, tocopherol, eugenol, myricetin, thymol, lutein, carotene, etc. have been proven for their anti-inflammatory potential in varied plant species [13-15]. More number of compounds present in the list are attributed towards cytotoxic activity and hence *Trichosanthes lobata* can be a potent anti-cancer agent also.

CONCLUSION

The analytical characterization of ethyl acetate and methanol extract of *Trichosanthes lobata* leaf and stem witness the presence of active metabolites. GC-MS reveals that the plant and its extract has valued agents which fulfil the pharmaceutical need. From the present study, it is confirmed that *Trichosanthes lobata* ethyl acetate and methanol leaf extract can be used as potent anti-inflammatory drug. Further, the fractions containing active compounds should be isolated from the extract and has to be examined through *in vivo* experiments. This will confirm their mechanism of action as novel therapeutic agent against inflammation. This research article also emphasizes varied pharmacological properties of *Trichosanthes lobata* in treating various disorders like cancer, neurological disorder, aging, against bacterial infections and its future prospects for improved usage in.

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AUTHORS CONTRIBUTIONS

Both the authors of the research article have sufficiently participated in the intellectual content, conception, design, analysis, interpretation of data and writing the manuscript.

CONFLICT OF INTERESTS

There remains no conflict of interest.

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