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Identification of Bioactive Phytochemicals using GC–MS in Leaf Ethanolic Extract of *Tragia Involucrata* L

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ABSTRACT

Tragia involucrata is belonging to the family Euphorbiaceae common medicinal herb in Southern India. In the present investigation, air died powdered ethanol extracts of *Tragia involucrata* leaf sample was analyzed by gas chromatography-mass spectrometry (GC–MS) to identify the important phytochemical constituents. The GC-MS analysis has shown the presence of 43 active compounds in the leaf extract. Based on area percentage, the top five major compounds present in the ethanolic extract were Palmitic acid (13.39%), (7Z,10Z,13Z)-7,10,13-Hexadecatrienal (11.44%), (3beta,24s)-Stigmast-5-En-3-One (8.10%), Pipeline (7.37%) and Friedelan-3-one (6.01%). The GC-MS analysis of selected leaf extract proved that the presence of various bioactive compounds. These bioactive compounds justify, the use of this plant to treat various diseases by traditional practitioners.

KEYWORDS: *Bioactive compounds, GC-MS analysis, Tragia involucrata, phytochemical screening, leaf ethanol extract*

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INTRODUCTION

Euphorbiaceae is a complex heterogeneous family consisting of about 322 genera and 8900 species in the world (1). In India, this family is represented by 73 genera and 410 species (2). The family is essentially tropical and occurs in diverse habitats from arid regions to humid tropics. Many plants of this family have been used in traditional systems of medicine. Still, several potent plants of *Euphorbiaceae* particularly from the rural areas are unexplored which deserve attention and research. *Tragia,* a genus of perennial, usually climbing or twining herbs, with stinging hairs, found in the tropical and sub-tropical parts of the world. The present study aimed to investigate the bioactive compounds in ethanolic extracts dried leaf powders of *Tragia involucrata* L. by Gas Chromatography and Spectroscopic analysis method.

Materials and Methods

Plant sample

Mature and healthy leaves of of the *Tragia involucrata* were collected from Southern Western Ghats in the district of Tirunelveli, South India. The specimens were identified, comparing the characteristics of floral and vegetative characters in the '*Flora of the Presidency of Madras*' (3). The taxonomic features collected from the species have been confirmed with the '*Flora of Tamilnadu Carnatic*' (4). Voucher specimens are documented in the herbarium of St. Xavier's College (XCH), Palayamkottai, Tamilnadu, India.

Preparation of Extract

The shade dried leaf powder of *Tragia involucrata* was used for the extraction process. 100 g of the powdered samples was extracted with ethanol in the soxhlet apparatus. The solvent was evaporated at temperature below its respective boiling point in an oven and all the extracts were kept in air tight containers and stored at 4^0C for further analysis.

The GC–MS analysis

GC-MS analysis on ethanolic extracts of *Tragia involucrata* was carried out in (Instrument Model: GC-MS SHIMADZU QP2010 Software: GC-MS solution ver. 2.53) Sargam laboratory Pvt. Ltd. 2 Ramavaram road, Manapakkam, Chennai- 600089. The chemical components were identified by matching their mass spectra with those recorded in the mass spectral library. GC-MS analysis was performed by using Agilent 7890A GC system, Agilent 5975C series VL MSD, capilarry column type, flow rate of 1 mL/min, carrrier gas was helium, constant flow model, injector temperature was 250°C, injection volume was 2 uL, split injection technique, oven temperature was programmed from 40°C for 2 min, with temperature increment rate of 10°C/min, and final temperature of 280°C for 2 min. Total running time was 30 min.

Results and Discussion

The bioactive compounds present in the dried leaf powder ethanolic extract of *Tragia involucrata* are shown in Table 1.

Based on area percentage, the top five major compounds present in the ethanolic extract were Palmitic acid (13.39%), (7Z,10Z,13Z)-7,10,13-Hexadecatrienal (11.44%), (3beta,24s)-Stigmast-5-En-3-One (8.10%), Pipeline (7.37%) and Friedelan-3-one (6.01%). In total 43 active compounds were identified in the leaf extract. Saturated fatty acids such as Lauric acid, Myristic acid, Linoleic acid, Adipic acid and Palmitic acid were recorded in the present study. Fatty acids had been reported to inhibit bacterial growth by disruption of bacterial membranes or inhibition of fatty acid synthesis. Zheng *et al*. (5) reported that long chain unsaturated fatty acids such as oleic acid, linoleic acid, palmitoleic acid and arachidonic acid inhibited bacterial growth (*S. aureus*) by inhibiting the bacterial enoyl-acyl carrier protein reductase (FabI), which is an essential components of bacterial fatty acid synthesis. Long chain unsaturated fatty acids, including linoleic acid are well known to inhibit Gram negative bacteria such as *Escherichia coli 6,7,8*.

Thymol, chemically known as 2-isopropyl-5-methylphenol is a colorless crystalline monoterpene phenol derivative of p-Cymene, $C_{10}H_{14}O$. Thymol possesses antibacterial9, antifungal 10, anti-inflammatory (11), antioxidant (12), anti-mutagenic (13), larvicidal (14), analgesic (15), anti-microbial (16), acaricidal (17), anticonvulsant, antiepileptogenic (18), wound healing (19), anti-hemolytic (20), antiphlogistic (21), antileishmanial (22) and radioprotective (23) properties. Thymol improves digestion by relaxing smooth muscles, prevents menstrual cramps, attenuates respiratory problems and is an active ingredient used in food flavorings, topical ointments, various soaps, toothpastes, shampoos, deodorants and mouthwashes (24, 25). Due to its potent antimicrobial properties, thymol is frequently used in dentistry for the treatment of oral cavity infections (26, 27, 28, 29).

Eugenol is a volatile phenolic constituent. eugenol show antioxidant capacity and free radical scavenging activity (30). Anti-inflammatory effects of eugenol, have suggested that this compound is able to suppress the expressions of cyclooxygenase II enzyme (31). Eugenol has been found to exert antipyretic activity in rabbits when given intravenously and intragastrically and may reduce fever through a similar central action to allopathic antipyretic drugs such as acetaminophen (32). eugenol may have recovery effects on arthritis and can be useful as a beneficial supplement in the treatment of arthritis (33)

Neophytadiene belongs to the class of organic compounds known as sesquiterpenoids. Neophytadiene is a good analgesic, antipyretic, anti-inflammatory, antimicrobial, and antioxidant compound (34), carminative antiulcerative antiparasitic and antiprotozoal (35). Carminative, Gastrin inhibitor, Histamine release inhibitor, Antiprotozoal (Leishmania), Antiparasitic and Antiulcerative

Ethyl palmitate is an organic compound with the chemical formula $C_{18}H_{36}O_2$. It is a long-chain fatty acid ethyl ester resulting from the formal condensation of the carboxy group of palmitic acid with the hydroxy group of ethanol. Ethyl palmitate (EP) are naturally occurring fatty acid esters reported as inflammatory cell inhibitors (36).

Phytol is an acyclic diterpene alcohol (3,7,11,15-tetramethylhexadec-2-en-1-ol) that can be used as a precursor for the manufacture of synthetic forms of vitamin E and vitamin K. PYT is a valuable essential oil (EO) used as a fragrance and a potential candidate for a broad range of applications in the pharmaceutical and biotechnological industry. PYT demonstrated anxiolytic, metabolism-modulating, cytotoxic, antioxidant, autophagy- and apoptosis-inducing, antinociceptive, anti-inflammatory, immune-modulating, and antimicrobial effects (37). Phytol is known to inhibit the growth of *Staphylococcus aureus* (38) and to block the teratogenic effects of retinol (39). Phytol show the pronounced antinociceptive effects and antioxidant properties/. It has significant antimicrobial activity against Escherichia coli, *Candida albicans* and *Aspergillus niger* (40). Phytol has potential as a safe and cost-effective addition to antischistosomal therapy. Schistosomiasis is an infectious parasitic disease caused by helminths from the genus *Schistosoma* (41).

Methyl linoleate is a fatty acid methyl ester of linoleic acid. methyl linoleate showed antifungal activity against *Paracoccidioides brasiliensis* (42). Methyl linoleate is used in the biosynthesis of prostaglandins and cell membranes. Lipid metabolism regulator, Antisecretoric, Anti-inflammatory and Antihelmintic (Nematodes)

Ethyl octadecanoate (ethyl stearate) is an octadecanoate ester obtained by formal condensation between the carboxy group of octadecanoic (stearic) acid and the hydroxy group of ethanol. Perturbs the cell cycle and induces apoptosis in Hep-G2 cells (43). A fatty acid ester component of volatile oil from Rhododendron anthopogonoides; shows antimicrobial activity.

Squalene is a triterpene with nutritional and medicinal values with broad expectations for pharmaceutical application. Cardioprotector, Antioxidant, Antibacterial and antifungal, Anticancer, Detoxifying. Several studies have confirmed the health benefits of SQ in nutritional, medicinal, and pharmaceutical aspects. It is considered a potent chemopreventive and chemotherapeutic agent, which inhibits the tumor growth in the colon, skin, lung, and breast, and it stimulates the immune system for the application of drugs in the treatment of diseases such as HIV, H1N1, leukemia, papilloma, and herpes, among others (44, 45, 46, 47).

Squalene and also its biocompatibility with skin, makes squalene an important component in cosmetical formulations (moisturizing creams, makeup, lipstick, nail and hair products) (48). Squalene was used as an adjuvant in vaccines, stimulating the immune response and increasing the patient's response to vaccine. It is added to lipid emulsions as drug carrier in vaccine applications (49). Squalene is a highly unsaturated isoprenoid hydrocarbon, containing 6 double bonds. Due to this double bond structure this isoprenoid action as a strong anti-oxidant and natural antibiotic. Squalene already proved to be effective as an antioxidant (50).

Solanesol is a non-cyclic terpene alcohol that consists of nine isoprene units. solanesol possesses antioxidant (50, 51), anti-inflammatory (52), neuroprotective (53), and antimicrobial (54) activities. Solanesol possesses antibacterial, antifungal, antiviral, anticancer, antiinflammatory and anti-ulcer activities, Solanesol derivatives can also be used for the treatment of cardiovascular disease, osteoporosis, acquired immune deficiency syndrome, and wound healing (55,50)

Γ-Tocopherol is one of the chemical compounds that is considered vitamin E. α-tocopherol shows anti-inflammatory

activity and modulates expression of proteins involved in the uptake, transport, and degradation of atherogenic lipids. Though α-tocopherol exhibits important antioxidant, anti-inflammatory, and antiatherogenic features Atherosclerosis and Related Cardiovascular Disease (56).

Friedelin is a triterpenoid chemical compound. Friedelin isolated from *Azima tetracantha* Lam. was previously investigated in murine models for its anti-inflammatory, antipyretic, and analgesic effects. In adult Wistar albino rats, friedelin showed potent anti-inflammatory activity in numerous *in vivo* tests: (1) friedelin markedly reduced carrageenan-induced hind paw edema, persisting for 6 h; effects of friedelin at 40 mg/kg dose were comparable with indomethacin 10 mg/kg, (2) friedelin at doses of 2 or 4 mg markedly reduced ear edema after croton oil administration, (3) friedelin inhibited peritoneal capillary permeability after acetic acid administration in a dose-related manner, (4) friedelin inhibited granuloma formation after placement of cotton pellets subcutaneously in the axilla, and (5) friedelin significantly (p<0.05) inhibited paw swelling after Freund's adjuvant injection. Friedelin may also help with fever: friedelin administered orally showed significant reduction in rectal temperature (p<0.05) after yeast injection in adult Wistar albino rats. Results were comparable with the antipyretic effect of paracetamol (acetaminophen). Friedelin significantly (p<0.05) reduced abdominal constrictions and stretching after acetic acid injection in adult Wistar albino rats. The effect was less on first phase (0–5 min) neurogenic pain than on second phase (20–30 min) inflammatory pain. However, friedelin showed no significant effect versus control on pain threshold in the hot plate test in adult Wistar albino rats (57)

Adipic acid or hexanedioic acid is the organic compound with the formula $(CH_2)_4(COOH)_2$. hexanedioic acid has antibacterial properties that effectively inhibit the growth/proliferation of pathogenic bacteria *Staphylococcus aureus*, *Klebsiella pneumonia*, and *Shigella dysenteriae* (58).

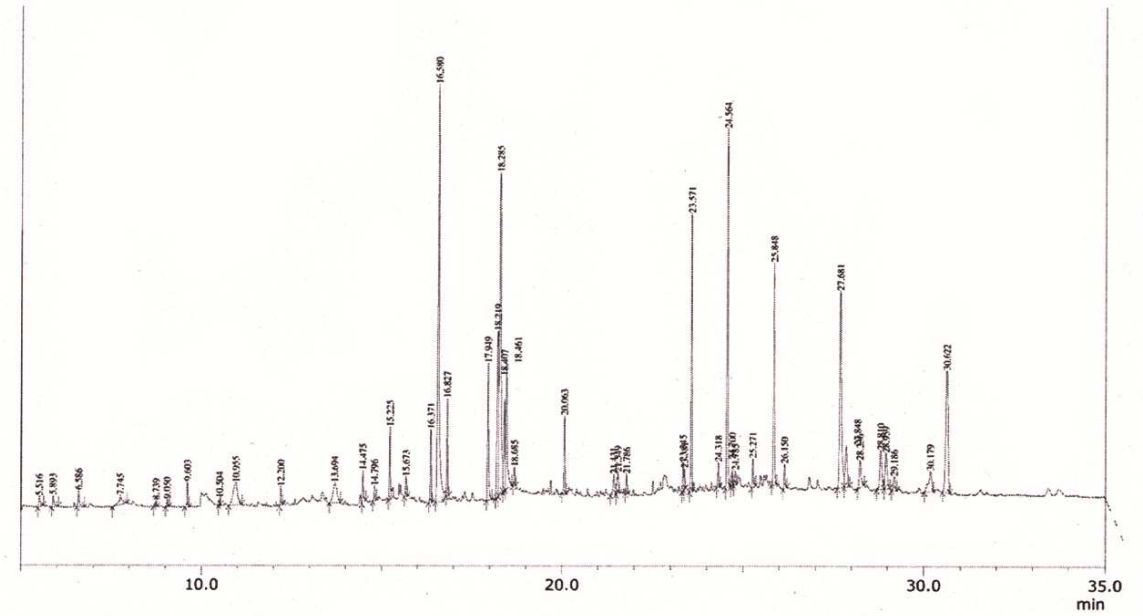
Conclusion

The GC-MS analysis of selected leaf extract proved that the presence of various bioactive compounds. These bioactive compounds justify, the use of this plant to treat various diseases by traditional practitioners. Further research warranted to isolated individual phytochemical compounds and will be analysed for biological activity in animal models.

GC-MS of *Tragia involucrata*

Peak	Retention Time	Area	Area%	Name
1	5.516	259353	0.32	Melamine
2	5.893	300200	0.37	Isoamyl acetate
3	6.586	375409	0.46	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxyl-6-methyl-
4	7.745	566554	0.70	2-(1-Piperidinylcarbonyl)aniline
5	8.739	99848	0.12	Thymol
6	9.050	121006	0.15	4-Vinyl-2-Methoxy-Phenol
7	9.603	375474	0.46	Eugenol
8	10.504	88748	0.11	4,11,1l-Trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene
9	10.955	1680331	2.07	Guanosine
10	12.200	274729	0.34	Lauric acid
11	13.694	1370888	1.69	Mome Inositol
12	14.475	534658	0.66	Myristic acid
13	14.796	304636	0.38	Ethyl Heptadecanoate
14	15.225	1127725	1.39	Neophytadiene
15	15.673	313610	0.39	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
16	16.371	1196970	1.47	1,1-Dimethyldecahydronaphthalene
17	16.580	10863459	13.39	Palmitic acid
18	16.827	1311494	1.62	Ethyl Palmitate
19	17.949	2520107	3.11	Phytol
20	18.219	4305269	5.31	Linoleic acid
21	18.285	9284443	11.44	(7Z,10Z,13Z)-7,10,13-Hexadecatrienal
22	18.407	1842601	2.27	Methyl linoleate
23	18.461	3023953	3.73	9,9-Dimethoxybicyclo[3.3.1]nonane-2,4-dione _____
24	18.685	269586	0.33	Ethyl Octadecanoate
25	20.063	1255567	1.55	n-Propyl cinnamate
26	21.431	571272	0.70	2-Hexadecanoyl glycerol
27	21.549	571272	0.70	Diisooctyl phthalate
28	21.786	295881	0.36	Ethyl (13z)-13-docosenoate
29	23.381	282721	0.59	4-Caranone,(ls,3s,6r)-(-)-
30	23.571	4477621	0.35	l-[(2e,4e)-5-(l,3-Benzodioxol-5-Yl)-2,4-Pentadienoyl]Piperidine
31	24.318	462202	5.52	Squalene
32	24.564	5978586	0.57	Solanesol
33	24.700	204284	7.37	Pipeline
34	24.785	284597	0.25	Bicyclo[4.1.0]Heptane-3-One,4,7,7-Trimethyl-,[lr(l.Alpha.,4.Beta.,6.Alpha.)]-
35	25.271	600890	0.35	6-Methyl-3 -Methyleneoctahydro-1-Benzofuran
36	25.848	4432978	0.74	Gamma. - Tocopherol

37	25.848	4432978	5.46	Vitamin E
38	26.150	524325	0.65	Adipic acid, monopiperidide, ethyl ester
39	27.681	6573141	8.10	(3beta,24s)-Stigmast-5-En-3-One
40	27.848	1459664	1.80	D:B-Friedo-B':A'-Neogammacer-5-En-3-One
41	28.236	938171	1.16	Norolean-12-Ene
42	28.810	1521143	1.87	Methyl Commate A
43	28.959	1322465	1.63	D:B-Friedo-B':A'-Neogammacer-5-En-3-One
44	29.186	440871	0.54	Cholest-4-en-3-one
45	30.179	1185430	1.46	(2E)-3,7.11,15-Tetramethyl-2-hexadecen-l-ol
46	30.622	4877126	6.01	Friedelan-3-one



GC-MS of *Tragia involucrata* L.

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