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GC-MS ANALYSIS OF BIOACTIVE COMPOUNDS FROM ETHANOLIC LEAF EXTRACT OF *WALTHERIA INDICA* LINN. AND THEIR PHARMACOLOGICAL ACTIVITIES

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Keywords:

Bioactive compounds, Ethanolic leaf extract, GC-MS, Pharmacological activity, Sterculiaceae, *Waltheria indica* L.

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ABSTRACT: Bioactive compounds are the frontline potent agents in both nutraceutical and pharmaceutical industries. Recently, the bioactive compounds are gaining much importance for their ability in enhancing resistance to various diseases and to improve the health of people both by traditional and modern ways of administrations. Therefore, in the present investigation was undertaken to identify the compounds present in the leaf of *Waltheria indica* Linn. by Gas Chromatography-Mass Spectrometry (GC-MS) analysis by ethanol extract. The GC-MS analysis showed 27 bioactive compounds. The major compounds identified are 2,3-Dihydro-3,5-Dihydroxy-6-methyl-4h-pyran-4-one, tetradecane, nonadecane, tetracosane, phytol and squalene. In a mass spectrum, each compounds were identified based on their retention time and peak area. Pharmacological activities of these compounds indicated that the compounds present in the leaf of said plant can be used as a crude drug and can also be used to develop a novel drug.

INTRODUCTION: The pharmaceutical industries are using bioactive compounds as potent agents in treatment of many diseases. In the recent days bioactive compounds are gaining importance for their ability to cure many diseases. India has a rich heritage of medicinal plants [1]. Plants have the capacity of synthesizing the organic compounds and are called as secondary metabolites, they have unique and complex structures. The secondary metabolites are used in the treating of chronic as well as infectious diseases [2]. *Waltheria indica* L. is a highly valued medicinal plant used to treat the cancer [3, 4], malaria [5] and viral [6] diseases. It also possesses the antimicrobial [7], antioxidant [8], anti-inflammatory [8] and analgesic [8] actions.

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Traditionally it is also used to treat wound healing[9], sleeping sickness [10] and cough [9]. Overall the plant is considered as febrifugal, purgative and emollient [9]. In the last few years, the GC-MS has become the key tool for secondary metabolite profiling in plants. However, there are few reports are available on the pharmacological properties of the plant. Keeping this in view, the present study is undertaken to establish the GC-MS profile of ethanolic leaf extract of *Waltheria indica* Linn. and their pharmacological activities.

MATERIALS AND METHODS:

Material: *Waltheria indica* Linn. belongs to the family Sterculiaceae[11]. It is an under shrub growing in sub tropical and tropical regions including in India. It is commonly known as velvet leaf, marsh mallow and monkey bush [12]. The *Waltheria indica* Linn. (Voucher specimen no: PB/MJ/01) leaves were collected from the plants grown in Karnatak University Campus, Dharwad, Karnataka. The leaves were washed with running tap water and later dried at room temperature.

The dried leaves were powdered using an electric blender. The powder obtained was sieved and stored in an air tight container at room temperature till further analysis [13].

Leaf Extract: About 10gms of dried and coarsely powdered leaves were subjected to extraction with ethanol (250 ml) using Soxhlet apparatus. The extract was subjected to GC-MS analysis [13].

GC - MS Analysis: The analysis was performed using a GC-MS (Shimadzu, Kyoto): QP2010S fitted with a 1.4 µm column Rxi-5silMS 30 meter length, 0.25 mm inner diameter and 0.25 µm film thickness. Carrier gas helium with a flow rate of 0.98 ml/min; column temperature 80 °C; initial temperature 70 °C, injector temperature 260 °C and detector temperature 300 °C, followed by a linear programmed temperature from 70 - 280 °C at a rate of 10 °C/min, operating in electron impact mode.

The samples were injected in splitless mode. Interface temperature was kept at 280 ºC. Oven temperature programming was done from 80 °C - 260 ºC at 10 ºC /min. The pressure of the carrier gas was kept at 63.6 kPa. The constituents were identified based on the RT values using the NIST 11 and WILEY 8 library [14].

Identification of Components: Bioactive compounds are identified based on interpretation on mass spectrum of GC - MS using the database of National Institute Standard and Technology (NIST11) and WILEY 8. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST 11 and WILEY 8 library [15]. The name, molecular weight and molecular formula of the components of the test materials were tabulated.

RESULTS: The GC - MS chromatogram of the ethanolic leaf extract of *Waltheria indica* Linn. showed 27 peaks indicating the presence of twenty seven compounds (**Fig. 1**).

The active principles with their peak, retention time (RT), area (%), height (%), molecular formula and molecular weight are presented in the **Table 1**.

The tetradecane, hexadecane, squalene, 2,3-Dihydro-3,5-Dihydroxy-6-methyl-4H-pyran-4-one showed maximum per-centage. The 2-propenoic acid, 10- Heneicosene (c.t), Nonadecane, 3-Eicosene, (E)-, 1,1 Bicyclo propyl-2-octanoic acid, megasti-gmatrinone-4 showed a moderate percentage. The minimum percentage compounds are 1-docosanol, 3',5'-Di-methoxyacetophenone, 2-Hexadecen-1-Ol, 3,7,11, 15-Tetramethyl-, [R-[R*,R*-(E)]]-, Z,Z-8,10-Hexa-decadien-1-ol, phytol, tetracosane, 2-bromodecane, 5,5, Diethyl hepta-decane.

The GC - MS identified compounds shows various pharmacological activities and are ascertained as in the **Table 2**.

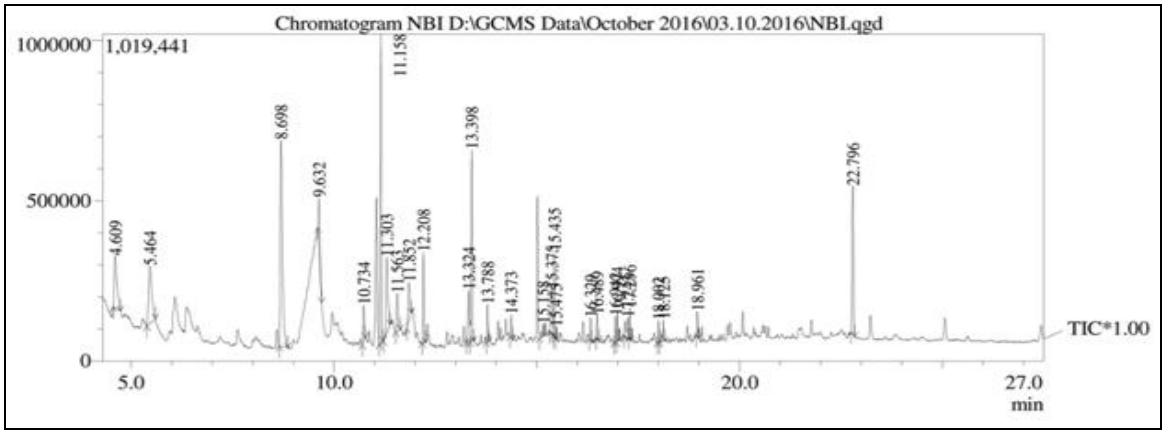

FIG. 1: CHROMATOGRAM OF GC - MS ETHANOLIC LEAF EXTRACT

TABLE 1: GC-MS ANALYSIS OF BIOACTIVE COMPOUNDS IN ETHANOL LEAF EXTRACT OF *WALTHERIA INDICA* LINN.

Peak	R. Time	Area %	Height %	Molecular formula	Molecular weight (g/mol)	Name of the compound
1	4.609	5.65	2.89	$C_3H_6N_6$	126.2	1,3,5-Triazine-2,4,6-Triamine
2	5.464	7.67	3.21	$C_6H_8O_4$	144.12	2,3-Dihydro-3,5-Dihydroxy-6-methyl-4H-pyran-4-one

3	8.698	18.21	11.55	$C_{14}H_{30}$	198.39	Tetradecane
4	9.632	2.58	2.77	$C_{17}H_{36}O$	256.46	1-Hexadecanol, 2-Methyl-
5	10.734	1.67	2.05	$C_{10}H_{12}O_3$	180.20	3',5'-Dimethoxyacetophenone
6	11.158	11.91	17.28	$C_{16}H_{34}$	226.44	Hexadecane
7	11.563	2.23	2.10	$C_{13}H_{18}O$	190.28	Megastigmatrienone 4
8	11.852	2.79	2.16	$C_{21}H_{38}O_2$	322.53	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester
9	12.208	3.79	5.09	$C_{16}H_{30}O_2$	254.41	2-Propenoic acid, tridecyl ester
10	13.324	2.92	2.89	$C_{20}H_{40}$	280.54	3-Eicosene, (E)-
11	13.398	7.53	10.75	$C_{18}H_{38}$	254.50	OCTADECANE
12	13.788	1.39	2.11	$C_{20}H_{40}O$	296.53	2-Hexadecen-1-ol, 3,7,11,15-Tetramethyl-, [R-[R*,R*-(E)]]-
13	14.373	0.79	1.29	$C_{19}H_{38}$	266.51	1-Nonadecane
14	15.158	0.60	0.70	$C_{12}H_{25}Br$	249.23	2-Bromo dodecane
15	15.375	3.11	2.53	$C_{21}H_{42}$	294.55	10-Heneicosene (c,t)
16	15.435	3.10	4.77	$C_{19}H_{40}$	268.52	Nonadecane
17	15.475	0.34	0.63	$C_{21}H_{44}$	296.57	5,5-Diethylheptadecane
18	16.329	0.84	1.29	$C_{36}H_{75}O_3P$	586.95	Phosphonic acid, Dioctadecyl ester
19	16.489	1.02	1.37	$C_{20}H_{40}O$	296.53	Phytol
20	16.942	1.06	1.46	$C_{14}H_{23}F_3O_2$	280.32	trans-2-Dodecen-1-ol, trifluoroacetate
21	16.994	1.18	1.76	$C_{16}H_{30}O$	238.41	Z,Z-8,10-Hexadecadien-1-ol
22	17.243	1.69	1.20	$C_{22}H_{46}O$	326.60	1-Docosanol
23	17.296	1.01	1.72	$C_{24}H_{50}$	338.66	Tetracosane
24	18.002	0.83	1.22	$C_{26}H_{52}$	364.70	Cyclopentane, Heneicosyl-
25	18.125	1.08	1.22	$C_{21}H_{35}F_7O_2$	452.49	Heptadecyl heptafluorobutyrate
26	18.961	0.84	1.42	$C_{26}H_{52}$	364.69	Cyclohexane, Eicosyl-
27	22.796	8.48	8.30	$C_{30}H_{50}$	410.71	Squalene

TABLE 2: PHARMACOLOGICAL ACTIVITY OF PHYTOCOMPONENTS IN THE ETHANOLIC EXTRACT OF *WALTHERIA INDICA* LINN. LEAF

Name of the compound	Pharmacological activity
1,3,5-Triazine-2,4,6-Triamine	Antagonists [16], Cytotoxicity against leukemia and adenocarcinoma [17] Inhibites glucocerobrosidese [18], Antileismenial activity [19], Anti-HIV [20], Antimalarial [21] Antioxidant, Cancer preventive [21] Antimicrobial [22]
2,3-Dihydro-3,5-Dihydroxy-6-Methyl-4H-Pyran-4-One	Antioxidant [1], Antimicrobial [1], Automatic nerve activity, Anti-inflammatory [23], Antiproliferative [24, 25], Anti-arthritic [26], Anticancer, Antidiabetic [1], Pro-apoptic effects [24]
Tetradecane	Antimicrobial [27], Cytotoxicity [28], Antipyretic, Anthelmentic, Tumour, Bronchitis, Asthma, Tuberculosis, Dyspepsia, Constipation, Anemia, Throat diseases, Elephantiasis, Antidiabatic, Anti-inflammatory, Antidiarrhoeal [29]
1-Hexadecanol, 2-Methyl-	Antimicrobial [30]
3',5'-Dimethoxyacetophenone	No activity reported
Hexadecane	Cytotoxicity [28], Antimicrobial, Antioxidant [31], Antipyretic, Anthelmentic, Tumour, Bronchitis, Asthma, Tuberculosis, Dyspepsia, Constipation, Anemia, Throat diseases, Elephantiasis, Antidiabetic, Anti-inflammatory, Antidiarrhoeal [32]
Megastigmatrienone - 4	Aroma Nature [33], Cytotoxic Activity [34]
[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	Antidiabatic, Anticancer [35], Haemolytic, Pesticide, Skin irritant, Hypocholesterolemic [32]
2-Propenoic acid, tridecyl ester	No activity reported
3-Eicosene, (E)-	Antimicrobial, Antihyperglycemic [36], Cytotoxic Activity [36, 28], Antioxidant, Insecticidal activity [37]
Octadecane	Antioxidant, Anti-inflammatory [38] Cough, lung diseases, Cold and fever detoxification [39], Anticorrosion agent [23], Antisepsis [40]
2-Hexadecen-1-ol, 3,7,11,15-Tetramethyl-, [R-[R*,R*-(E)]]-	Anti-inflammatory, Antimicrobial, Cancer-preventive [41] Antitumor, Analgesic, Antibacterial, Anti-inflammatory, Fungicide [42]
1-Nonadecane	Antimicrobial, Antioxidant, Anticancer [43]

2-Bromo dodecane	Antibacterial activity [44]
10-Heneicosene (c,t)	No activity reported
Nonadecane	Anti HIV, Antioxidant, Antibacterial [45], Antimicrobial [27], Cytotoxic effect, Antimicrobial [46, 27] Antimalarial [47], Unini uses like weakness of the principal organs like heart, Brain, liver, General weakness, Haemoptysis, Palpitation, Conjunctivitis, Earache, Stomatitis [48]
5,5-Diethylheptadecane	No activity reported
Phosphonic acid, Dioctadecyl ester	No activity reported
Phytol	Antimicrobial [32], Anti inflammatory [32], Anticancer [32], Diuretic, Antifungal against *S. typhi*, Resistant gonorrhea, Joint dislocation, Headache, Hernia, Stimulant and antimalarial [49]
trans-2-Dodecen-1-ol, trifluoroacetate	No activity reported
Z,Z-8,10-Hexadecadien-1-ol	No activity reported
1-Docosanol	Antimicrobial [50], Emollient, Emulsifier, Thickener in Cosmetics [51] Antiviral [52], Antitumor activity [53]
Tetracosane	Cytotoxicity against cancerous cells [54], Antidiarrheal [55], Antibacterial [35], Cardiotonic, laxative, Anthelemntic and removes fatigue, Anti-inflammatory, used in peptic ulcer treatment [56] Anticorrosive, Antioxidant [49], Antitrichomonas [57]
Cyclopentane, Heneicosyl-	No activity reported
Heptadecyl heptafluorobutyrate	No activity reported
Cyclohexane, Eicosyl-	No activity reported
Squalene	(Antibacterial, Antioxidant, Antitumor, Cancer preventive, Immunostimulant, Chemo preventive, Lipoxygenase-inhibitor, Pesticide) [58, 41]

DISCUSSION: The GC-MS analysis revealed the 27 chemical compounds. The tetradecane (18.21%) is the highest chemical compound and 5,5 Di-ethylheptadecane (0.34%) as the lowest chemical compound. The compound 2,3-Dihydro-3,5-Di-hydroxy-6-methyl-4H-pyron-4-one, tetradecane, Hexadecane, 2-Hexadecen-1-ol, 3,7,11,15-Tetra-methyl-, [R-[R*,R*-(E)]]-, showed pharmacological activity as reported in the plants of *Hibiscus tiliaceus* Linn.[1], *Marsilea quadrifolia* Linn.[25] and *Gymnema sylvestre* (Retz) Schult [35]. Similarly, phytol and squalene also showed the various biological activities as reported for *Coldenia procumbens* Linn.[2] However, isolation and characterization of individual phytochemical constituents may proceed to discover the novel drugs and their pharmacological activities.

CONCLUSION: The ethanolic leaf extract of *Waltheria indica* Linn. has 27 different chemical compounds and they have different pharmacological activities. Each chemical compound can be extracted individually and can be used in clinical trials to check efficacy, and to develop a novel drug from a crude drug. The GC-MS analysis of leaf extract will also be a part of database of bioactive products of natural drugs.

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CONFLICT OF INTEREST: We have no conflict of interest.

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