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GC-MS analysis of bioactive compounds from the methanolic leaf extract of *Tephrosia villosa* (Linn.) pers. an important medicinal plant of Indian Thar desert

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Abstract
The aim of the present study was to analyse, identify and find out the biological activity of bioactive compounds from methanolic leaf extract of *Tephrosia vollosa* of India Thar Desert by Gas Chromatography Mas Spectrometry analysis. Fresh disease free leaves were collected from the field, shade dried and powdered for extraction with methanol. Preliminary phytochemical screening of the extract was carried out following the standard methods to determine the presence of different phytochemical further the extract was analysed by GC-MS for identification of bioactive compounds present in the methanolic extract of *T. villosa* leaves. Phytochemical screening of crude extract showed the presence of alkaloids, phenols, flavonoids, terpenoids, sterols, and saponins. GC-MS analysis revealed 72 phytochemical compounds, out of which 61 were identified. Terpenes and their derivatives were found in good quantity. Some major biologically important and medicinally important phytochemical identified in *T.villosa* leaf extract were naphthaline, 3-carene, neophytadiene, phytol, octacosanol, ergot-5-en-3-ol, lupeol and beta-sitosterol.

Keywords: GC-MS analysis, bioactive compounds, *Tephrosia villosa*, methanolic extract Indian Thar desert

Introduction

Plants have been used for medicinal purposes for over 4000 years. About 80 per cent of people of the world rely on herbal medicine. Almost 21000 species of plants have the potential to cure diseases as medicinal plants. The medicinal properties of any plant are determined by the bioactive compounds present in different plant parts. These compounds are mostly secondary metabolites such as phenols, flavonoids, sterols, terpenoids, saponin, glycosides etc. Primarily, plants formed these compounds for the defense and pollination purpose but these are also valuable for humans in the treatment of various diseases.

The western part of Rajasthan is largely occupied by the great Indian desert which is commonly known as the thar desert. It is situated between 27°N and 70°E. This desert area is well known for its biodiversity among all the desert of the world. This desert is characterized by high wind velocity, extremes of temperature and scanty rainfall. Vegetation of Thar Desert can distinctly be divided into ephemerals and perennials [1]. Genus *Tephrosia* is a frequently occurring perennial plant in thar region which is commonly known as "Biyani". This genus belongs to family Fabaceae and comprises of 300 to 400 species worldwide [2, 3]. This genus is mainly distributed in tropical and sub-tropical regions of world [4]. *Tephrosia* is a well-known medicinal herb and has traditionally used in folk medicine for long time. The richness of bioactive compounds makes genus *Tephrosia* very useful to cure various diseases. It contains anti-viral, anti-ulcer, anti-microbial, anti-inflammatory and anti-oxidant properties [5].

T. villosa is an annual bushy herb or small shrub with profusely branched stem (Photo plate 1, A). The young stem is usually hairy while mature stem is striped and leaves always imparipinnate compound. Flowers are arranged in a terminal spicate raceme. Calyx is densely hairy with long hairy teeth. Standard petal is densely hairy outside with glabrous keel. Style is glabrous with penicillate stigma. Plants are easily distinguishable in field due to densely arranged velvety tomentose deflexed falcate pods which contain 6-12 seeds per pod. Seeds are slightly irregular in shape with blisterous marking [1]. (Photo plate 1, B).

Plate 1: (A) *T. villosa* in field (B) Flower and fruits

T. villosa is a medicinally valuable herb and has been traditionally used in folk medicine in India and various parts of world. Leaves of *T. villosa* are used to cure skin diseases [6].Root powder of this plant is used in dental pain, bleeding [7], in stomach ache, stomach disorders [8] and respiratory track problems [9]. Plant used in cold cough, asthma, constipation, calculus, dropsy etc. [10] and alsoas potential bio in secticide [11, 12].

Gas chromatography-mass spectroscopy is recognized as key technological platform for secondary metabolite profiling for plant as well as non-plant species from previous few years [13]. GC-MS based metabolite profiling offers a virtuous balance of sensitivity and reliability as compare to NMR or liquid chromatography linked mass spectroscopy [14]. Present study was carried out to investigate

the probable bioactive compounds of methanolic leaf extract of *T. villosa* by subjecting it to GC-MS analysis.

Materials and Methods

Plant sample collection

The fully matured fresh leaves of *T.villosa* were collected from the healthy plants in month of August. Identification of plant specimens was done by taxonomist of BSI, Jodhpur. A herbarium sheet of plant material was also prepared and deposited to the herbarium of Department of Botany, JNV University. Collected leaves were first washed under tap water to remove dust and dirt and then washed with distilled water. Leaves dried under shade at room temperature for 15 days. Dried leaves were pulverized to a fine powder in a mechanical grinder.

Extract preparation

Five gram of dried leaves powder was weighed by electric balance and dissolved in 50 ml of methanol and left for 48 hrs with frequent shaking. This extract was first filtered by a muslin cloth and then re-filtered by Whatman No.1 filter paper. The filtrate was centrifuged for 15 minutes at 2500 rpm. After centrifugation extract was again filtered by Whatman paper no.1 and left to evaporate at room temperature until the crude extract was obtained. This crude extract was transferred into sterile airtight containers and stored in the refrigerator on 4℃ for further use.

Preliminary phytochemical screening of leaf extract

To confirm the presence and absence of phytochemicals, preliminary phytochemical screening of methanolic leaf extract was carried out by the standard methods [15, 16, 17]. Further, the methanolic extract was subjected to GCMS analysis to identify the bioactive compound present in leaves.

GC-MS analysis of leaf extract

The gas chromatography-mass spectroscopy (GC-MS) analysis of leaf extract was performed on Shimadzu QP-2010 plus system. Helium gas (99.99%) was used as carrier gas at a constant flow rate 1 ml/min. and an injection volume of 1 µl of the sample was injected into the column. Injector temperature andion-source temperature was set at 260° and 230°C respectively. The temperature of the column oven was adjusted at 80°C, pressure was kept at 81.9 k Pa. The total running time for GC-MS was 60 minutes.

Interpretation of mass spectrum was done by NIST database and Wiley 8 library by comparing the unknown spectrum of compounds with the known compounds: NIST library has more than 62000 patterns. The name, molecular structure and molecular weight were determined.

Results and Discussion

Preliminary phytochemicals screening confirmed the presence of protein, carbohydrates, alkaloids, phenols, flavonoids, terpenoids, glycosides, saponins, gum and mucilage. Results of phytochemical tests performed are presented in table 1.

The bioactive phytoconstituents of leaves of *T. villosa*, obtained by GC-MS analysis, are presented in table 2. Chromatogram of methanolic leaf extract is presented in Figure 1. Total seventy-two compounds were obtained GC-MS analysis and the interpretation of unknown phytochemical compounds was done by comparing the fragmentation patterns of the mass spectra with the known and standard compounds. The compounds were identified by their GC retention time. Molecular formula and molecular weight of each detected compound were determined. Total 61 compounds were identified out of 72 phytochemicals of *T. villosa*. Methanolic leaf extract of *T. villosa* was found rich in terpenoids followed by flavonoids. Molecular formula, molecular weight, retention time and per cent peak area is given in Table 2. The major compound with 16.28% pear area was 9, 12, 15-Octadecatrienoic acid, (z,z,z)- followed by n-Hexadecanoic acid with 15.37 % and Naphthalene with 11.77%. The lowest peak area was occupied by Carbamic acid,2-(dimethylamino) ethyl ester:3-Cyclopentyl propionic acid each and 2-dimethylaminoethyl ester each with 0.06% followed by Bicyclo [4.4.1] undeca-1,3,5,7,9-pentaene with 0.07% and then 9,10secoergosta-5,7,10 (19), 22-Tetraene-3, 25-Diol, (3. beta.,5Z, 7E,22E)- with 0.08%.

Phytochemical screening revealed the presence of various bioactive phytoconstituents that are responsible for the therapeutic ability of plant. GC-MS analysis is the most widely used technique for the determination of these compounds [18]. GC-MS analysis leaf extract of *T.villosa* is revealed 72 major and minor peaks indicates the presence of various phytoconstituents. Benzo[b]thiophene and Naphthalene are aromatic hydrocarbons reported in leaf extract of *T.villosa*.Benzo[b]thiophene exhibits anti-cancer [19], anti-convulsent [20], anti-depressent [21], anti-tubercular [22] and anti-malarial [23]. Two nitrogen containing compounds Carbamic acid, 2-(dimethylamino) ethyl ester and 3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester were reported in methanolic extract. Methanolic leaf extract of *T.villosa* was found rich in terpene compounds. Various terpens like Neophytadiene, Lupeol (pentacyclic triterpene): Lup-20(29)-en-3-one (triterpenoid): Limonene dioxide (monoterpene): 3-Carene (bicylic monoterpene): 2-Pentadecanone, 6,10,14-trimethyl-(sesquiterpene): 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (diterpene alcohol): Squalene and Phytol. 3-Carene exhibits anti-oxidant and antimicrobial [24], anti- cancer [25], fumigant properties [26] and antifungalproperties [27]. Lup-20(29)-en-3-one possess anti-inflammatory, anticancer, antivirus and anti-diabetes activities and also found effective against Chagas disease [28]. Stigmasta-5, 22-dien-3-ol and beta - Sitosterol are the common phytosterols. Beta-sitosterol found effective potential against pathological targets of Alzheimer disease especially its dual efficiency i.e enzyme inhibition and free radicals scavenging capacity is of potential benefits [29]. Another fatty alcohol- Octacosanol is chemically similar to vitamin E studied for antifeedant activity [30, 31]. A long chain fatty aldehyde, Heptadecanal present in leaf extract shows antibacterial activity against *Staphylococcus aureus* and *Salmonella typhimurium* [32]. Ergost-5-en-3-ol, (3.beta.)-or Campesterol is a steroid derivative that shows bioactivities like anti-cancer, antioxidant and hypocholesterolemic (cholesterol absorption reducing agent) [33]. Leaf extract of *T. villosa* was used to minimize the corrosion rate of mild steel in acidic medium and reported the leaf extract as an outstanding eco-friendly green corrosion inhibitor [34]. In earlier studies flavens and isoflavones were also reported in *T.villosa* [35] which verifies the pharmacological significance of plant. Phytol is studied for anti-oxidant and antinociceptive activities [36, 37]. Phytol

is the precursor of synthetic vitamin E and vitamin K and shows ovicide and larvicide activities [38] and cytotoxic effect against breast cancer cell lines [39, 40]. Two geranyl flavanones namely (2*S*)-5,4′-dihydroxy-7-*O*-[(*E*)-3,7-dimethyl-2,6-octadienyl] and (2*S*)-5,4′-dihydroxy-7-*O*-[(*E*)-3,7-dimethyl-2,6-octa-dienyl]-8-*C*-[(*E*)-3,7-dimethyl-2,6-octadienyl] were earlier isolated from the roots of *Tephrosia villosa* [41]. Four compounds namely Rotenone, Dihydro rotenone, Lupeol and Stigmasterol reported from root extracts showed the moderate antifungal and antibacterial activity [42]. Earlier, a rotenoid 12a-dehydro-6 hydroysumatrol and lupenone were isolated from the whole plant of *Tephrosia villosa* [43]. Two minor prenylated rotenoid namely 12a-dehydro-2,3,6--trimethoxy-8-(3',3'-dimethylallyl)-9,11-dihydroxyrotenone and 12a-dehydro-6-hydroxy sumatrol were isolated from the seeds of *Tephrosia villosa*along with lupeol [44]. One Flavan namely tephrinone were also reported from *Tephrosia villosa* [45].Earlier few C-6 oxygenated rotenoids were reported from *Tephrosia villosa* namely villol, villosone, villosin and villinol [46].

Conclusion

Presently, the herbal medicines are become popular due to their availability, accessibility, affordability and the promising efficacy as compare to the expensive synthetic drugs which have their own side effects. Present study revealed the many bioactive compounds which establishes the medicinal importance of *Tephrosia villosa*. Further investigations on the medicinal properties of *Tephrosia villosa*can help in the formulation of novel pharmaceutical drugs to treat various disease.

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Table 1: Preliminary Phytochemical screening of methanolic leaf extract of *T. villosa*

S.no	Phytochemical constituents	Phytochemical test	Result
1.	Carbohydrates	Fehling's Test Molisch's Test	+ +
2.	Amino acids	Ninhydrin test Xanthoproteic Test	+ +
3.	Alkaloids	Dragendorff's Test Wagner's Test	+ +
4.	Phenols	Ferric chloride Test, Lead acetate Test	+ +
5.	Flavonoids	Shinoda Test, Alkaline reagent Test	+ +
6.	Phytosterols and Terpenoids	Libermann burchard's Test, Salkowski Test	+ +
7.	Glycosides	Keller Killani Test Glycosides Test	+ +
8.	Saponin	Foam Test, Olive oil Test	+ +
9.	Gumand Mucilage	Alcohol Test Ruthenium Red Test	+ +

Table 2: Phytochemicals reported in GC-MS analysis of methanolic extract of *T.villosa*

Peak#	R.Time	Area%	Compound name	Molecular Weight	Molecular Formula
1	10.318	0.16	4-METHYL-3-ISOPROPENYL-4-VINYL-1-CYCLOHEXENE	162	$C_{12}H_{18}$
2	11.100	11.77	NAPHTHALENE	128	$C_{10}H_8$
3	11.179	0.14	BENZO[B]THIOPHENE	134	C_8H_6S
4	11.578	1.32	2,3-DIHYDRO-BENZOFURAN	120	C_8H_8O
5	12.708	0.07	Bicyclo[4.4.1]undeca-1,3,5,7,9-pentaene	142	$C_{11}H_{10}$
6	12.876	1.14	2-Methoxy-4-vinylphenol	150	$C_9H_{10}O_2$
7	14.600	0.73	ISOBENZOFURAN,4-ETHENYL-1,3,3A,4,5,7A-HEXAHYDRO-,(3A.ALPHA.,4.ALPHA	150	$C_{10}H_{14}O$
8	14.936	0.09	Bicyclo[5.2.0]nonane, 4-methylene-2,8,8-trimethyl-2-vinyl-	204	$C_{15}H_{24}$
9	15.118	0.37	Bergamotol, Z-.alpha.-trans-	220	$C_{15}H_{24}O$
10	15.301	0.11	exo-2,7,7-trimethylbicyclo[2.2.1]heptan-2-ol	154	$C_{10}H_{18}O$
11	15.409	0.20	Z,Z-8,10-Hexadecadien-1-ol	238	$C_{16}H_{30}O$
12	15.514	0.16	3-Carene	136	$C_{10}H_{16}$
13	15.854	0.22	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	180	$C_{11}H_{16}O_2$
14	16.006	0.32	Cyclohexanemethanol,4-ethenyl-.alpha.,.alpha.,4-trimethyl-3-(1-methylethenyl)-, [1R-(1.alp	222	$C_{15}H_{26}O$
15	16.584	8.14	1-Methyl-6-(3-methylbuta-1,3-dienyl)-7-oxabicyclo[4.1.0]heptane	178	$C_{12}H_{18}O$
16	16.716	0.58	BICYCLO[3.1.0]HEXANE, 4-METHYLENE-1-(1-METHYLETHYL)-	136	$C_{10}H_{16}$
17	17.092	0.12	2,4,6-OCTATRIENE, 2,6-DIMETHYL-	136	C10H16
18	17.267	0.17	2-CYCLOHEXEN-1-OL,2,4,4-TRIMETHYL-3-(3-METHYL-1,3-BUTADIENYL)-, ACET	248	$C_{16}H_{24}O_2$
19	17.459	0.30	6-Methyl-2-(4-methylcyclohex-3-en-1-yl)hepta-1,5-dien-4-ol	220	$C_{15}H_{24}O$
20	17.640	0.56	LIMONENE DIOXIDE 1	168	$C_{10}H_{16}O_2$
21	17.905	2.15	Cyclohexanemethanol,4-ethenyl-.alpha.,.alpha.,4-trimethyl-3-(1-methylethenyl)-, [1R-(1.alp	222	$C_{15}H_{26}O$
22	18.025	0.27	Cyclohexanemethanol,4-ethenyl-.alpha.,.alpha.,4-trimethyl-3-(1-methylethenyl)-, [1R-(1.alp	222	$C_{15}H_{26}O$
23	18.524	3.14	Cyclohexanemethanol,4-ethenyl-.alpha.,.alpha.,4-	222	$C_{15}H_{26}O$

			trimethyl-3-(1-methylethenyl)-, [1R-(1.alp		
24	18.679	0.31	7-Oxabicyclo[4.1.0]heptane, 1-methyl-4-(2-methyloxiranyl)-	168	$C_{10}H_{16}O_2$
25	19.140	1.65	Neophytadiene	278	$C_{20}H_{38}$
26	19.199	0.13	2-Pentadecanone, 6,10,14-trimethyl-	268	$C_{18}H_{36}O$
27	19.386	0.34	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	296	$C_{20}H_{40}O$
28	19.582	0.48	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	296	$C_{20}H_{40}O$
29	19.708	0.16	Tricyclo[4.4.0.0(2,7)]dec-8-ene-3-methanol,.alpha.,.alpha.,6,8-tetramethyl-, stereoisomer	220	$C_{15}H_{24}O$
30	19.989	0.33	7-Hexadecenoic acid, methyl ester, (Z)-	268	$C_{17}H_{32}O_2$
31	20.054	4.17	Pentadecanoic acid, 14-methyl-, methyl ester	270	$C_{17}H_{34}O_2$
32	20.630	15.37	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$
33	20.981	0.07	Bicyclo[4.4.0]dec-2-ene-4-ol, 2-methyl-9-(prop-1-en-3-ol-2-yl)-	236	$C_{15}H_{24}O_2$
34	21.688	0.50	9,12-Octadecadienoic acid, methyl ester	294	$C_{19}H_{34}O_2$
35	21.755	2.10	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	292	$C_{19}H_{32}O_2$
36	21.868	3.70	Phytol	296	$C_{20}H_{40}O$
37	21.982	0.53	Methyl stearate	298	$C_{19}H_{38}O_2$
38	22.360	16.28	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	278	$C_{18}H_{30}O_2$
39	22.483	2.17	Octadecanoic acid	284	$C_{18}H_{36}O_2$
40	23.177	0.08	9,10-SECOERGOSTA-5,7,10(19),22-TETRAENE-3,25-DIOL, (3.BETA.,5Z,7E,22E)-	412	$C_{28}H_{44}O_2$
41	23.409	0.06	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	213	$C_{12}H_{23}NO_2$
42	23.513	0.08	1,2-15,16-Diepoxyhexadecane	254	$C_{16}H_{30}O_2$
43	24.867	0.06	Carbamic acid, 2-(dimethylamino)ethyl ester	132	$C_5H_{12}N_2O_2$
44	25.158	0.33	Heptadecanal	254	$C_{17}H_{34}O$
45	25.321	3.97	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	330	$C_{19}H_{38}O_4$
46	25.451	1.36	1,2-BENZENEDICARBOXYLIC ACID	390	$C_{24}H_{38}O_4$
47	26.717	0.13	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	354	$C_{21}H_{38}O_4$
48	26.788	0.42	Methyl 2-hydroxy-octadeca-9,12,15-trienoate	308	$C_{19}H_{32}O_3$
49	26.965	2.09	Octadecanoic acid, 2,3-dihydroxypropyl ester	358	$C_{21}H_{42}O_4$
50	27.810	2.35	Squalene	410	$C_{30}H_{50}$
51	28.737	0.10	Octacosanol	410	$C_{28}H_{58}O$
52	28.976	0.25	1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hexamethyl-, (all-E)-	426	$C_{30}H_{50}O$
53	29.849	0.07	1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hexamethyl-, (all-E)-(.+/-.)-	426	$C_{30}H_{50}O$
54	30.100	0.07	1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hexamethyl-, (all-E)-(.+/-.)-	426	$C_{30}H_{50}O$
55	32.117	0.35	Vitamin E	430	$C_{29}H_{50}O_2$
56	34.122	0.21	Ergost-5-en-3-ol, (3.beta.)-	400	$C_{28}H_{48}O$
57	34.724	1.11	STIGMASTA-5,22-DIEN-3-OL	412	$C_{29}H_{48}O$
58	36.218	1.82	.beta.-Sitosterol	414	$C_{29}H_{50}O$
59	37.712	0.40	Lup-20(29)-en-3-one	424	$C_{30}H_{48}O$
60	38.534	0.42	Lupeol	426	$C_{30}H_{50}O$
61	44.637	0.37	Phytyl tetradecanoate	506	$C_{34}H_{66}O_2$
		100.00			

Table 3: Biological activities of some chemical compounds obtained in GC-MS analysis of *T.villosa* leaves

S. No	Compound	Nature of compound	Biological activity of compound
1.	Benzo[b]thiophene	Heterocyclic compounds	Anti-cancer [29], anti-convulsant [20],
2.	Naphthalene	Aromatic compound	Anti- viral [47], anti-tubercular [48]), antidiabetic [49], anti- cancer [50],anti- anti-hypertensive [51]
3.	3-Carene	Bicyclic monoterpene	Anti -oxidant and antimicrobial [24], Anti- cancer [25], Fumigant properties [26], Anti-fungal [27]
4.	Neophytadiene	Sesquiterpene	Antipyretic, anti-microbial, analgesic, anti- oxidant [52]
5.	Phytol	Diterpene	Anti-cancer, antimicrobial and anti –oxidant properties [53]
6.	Octacosanol	Long chain aliphatic alcohol	Antinociceptive effect and anti –inflammatory activity [54]
7.	Squalene	Triterpene	Anti-oxidant [55],
8.	Ergost-5-en-3-ol, (3.beta.)-	Phytosterol	Anti- cancer [56]
9.	STIGMASTA-5,22-DIEN-3-OL	Phytosterol	Anti-microbial [57]
10	Lupeol	Pentacyclic triterpene	Anti-inflammatory [58]
11.	beta-Sitosterol	Phytosterol	Cholesterol lowering activity [59],hepato protective [60]

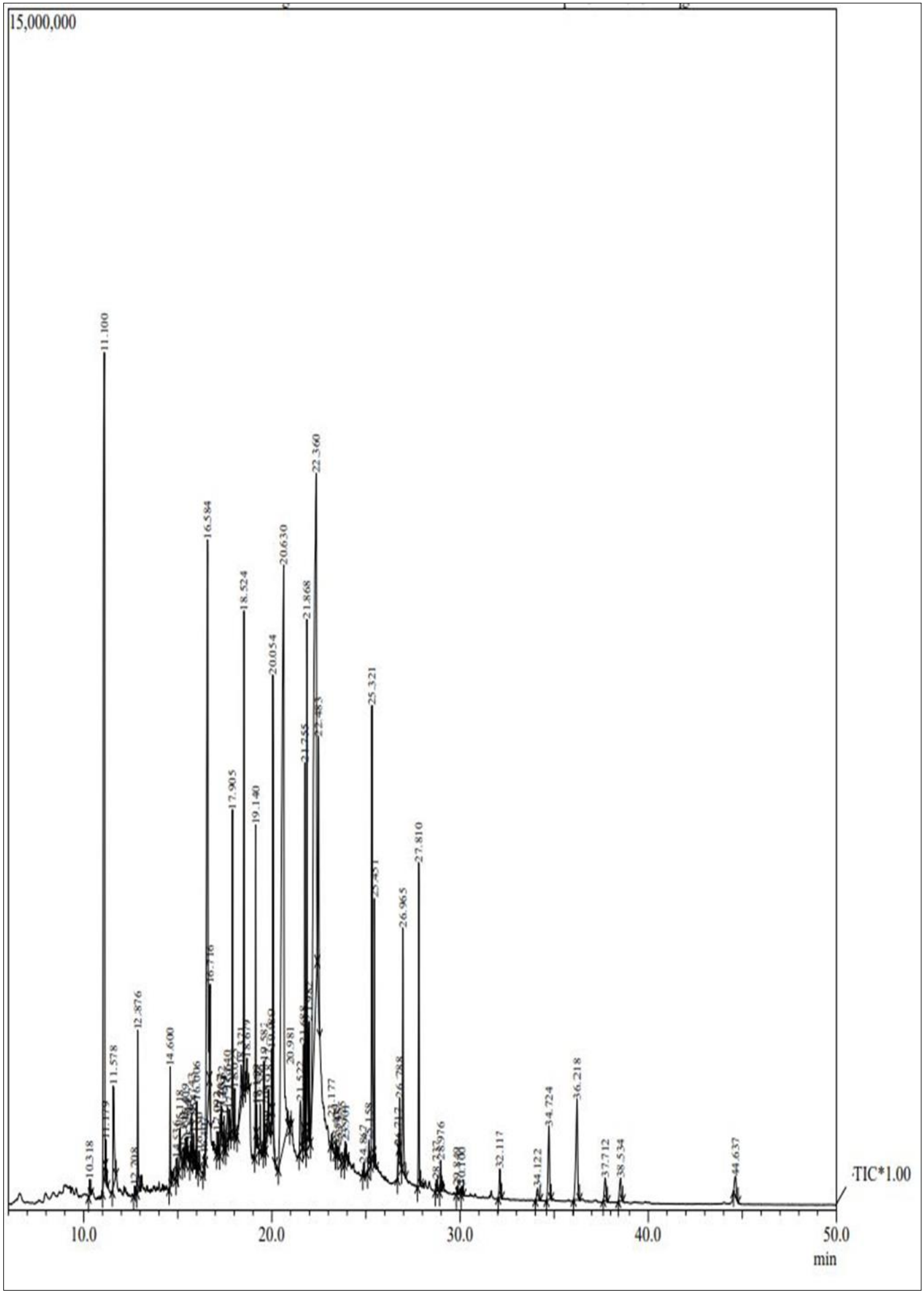

Fig 1: Chromatogram of methanolic leaf extract of *T. villosa*

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