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Research Article

A GC-MS STUDY FOR THE IDENTIFICATION OF THE BIOACTIVE COMPONENTS PRESENT IN THE LEAVES OF *TECOMELLA UNDULATA*

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ABSTRACT

Plants are a great repository for enormous amount of products. Crude extracts obtained from plants includes various phytochemicals like alkaloids, flavanoids, phenolics etc. Various methods are employed for the identification of possible bioactive compounds present in the plant crude extract. GC-MS (Gas chromatography-Mass spectrometry) is one such method. The present study was intended for the identification of the various components present in the crude extract obtained from the selected plant i.e. *Tecomella undulata*. The solvents methanol, petroleum ether and acetone were used for the biocrude extraction from the leaves. Comparative study was also carried out after the identification of components. Extract obtained with petroleum ether was found to contain maximum phytochemicals followed by acetone and methanol. The amount of the components also differed significantly with different solvents. These findings were further used to illustrate and support the usage of *Tecomella undulata* in various biological activities.

INTRODUCTION

Tecomella undulata (Smith) Seem belongs to family Bignoniaceae and commonly known as Desert Teak and Marwar Teak. It is locally known as "Rohida". This tree is basically found in drier regions like Arabia, Southern Pakistan and Northwest India (Sharma *et al*., 2013). It has the honour of being called upon as "State flower of Rajasthan" and "Marwar Teak". Marwar is the dry desert part of Rajasthan State of India, where it is found and it has a very good quality wood. ***T. undulata*** is a small deciduous tree that grows in area of scanty rainfall (150-500 mm annually) and high temperature ranging from 35° C- 45°C (Arya *et al*., 1992). It is considered among the few core species that constitutes desert afforestation.

Pharmacognostical and phytochemical studies from the stem, bark and leaves of this tree showed the presence of phytoconstituents like saponin glycosides, anthraquinones, flavanoids, phenolics, sterols and triterpenoids (Thanawala *et al*., 1993; Rohilla *et al*., 2014); that are responsible for its medicinal properties. Review of literature shows that its bark is used for syphilis treatment. It is also used as a cure for urinary disorders, enlargement of spleen, gonorrhoea, leucoderma and liver diseases (Chal *et al*., 2011). Similarly other parts are also rich in various secondary metabolites that are associated with numerous uses. This study was carried out for the identification of various compounds present in the leaves that may help in proceeding to find a novel drug.

MATERIAL AND METHODS

Dry powder preparation

Plants were identified from the field area of Department of Botany, University of Rajasthan, Jaipur. Leaves from the plant were taken and rinsed with water several times. Then they were shade dried and coarsely crushed in a mixer grinder to make a fine powder.

Preparation of Extract

About 10 gm powder of leaves was weighed and extracted with 100 ml of acetone, petroleum ether and methanol separately at 45-50°C, by hot continuous percolation method in soxhlet apparatus for 42 hours. The extracts were taken and filtered through Whatmann filter paper no 1. Then, the filtrates were concentrated by rotary evaporator to obtain crude extracts in different solvents.

GC-MS analysis

The GC-Ms analysis of acetone, methanol and petroleum ether extract of leaves of *Tecomella undulata* was carried out on Shimadzu QP-2010 plus with thermal desorption system TD

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20. It includes auto sampler and a gas chromatograph that is coupled to a mass spectrophotometer. The column size of this system is 30m × 0.25mm 1D × 0.26µ Mdf with a film thickness of 0.26mm, composed of 5MS (5% diphenyl/ 95% dimethyl poly siloxane). Helium gas (99.999%) was used as carrier gas at constant flow rate of 1ml/min. The 2µl injection volume of sample was utilized with split ratio of 10:1. The injector temperature was programmed initially at 280 °C, the ion-source temperature was 200 °C, the oven temperature was programmed from 110 °C (for 4 min), with an increase of 10 °C/min to 200°C, then 5 °C/min to 280°C, ending with a 9 min isothermal at 280 °C. Mass spectra were analyzed using electron impact ionization at 70 eV. The total running time for each sample was 60 min.

Identification of phytochemicals

Interpretation of phytochemicals present in the sample was conducted using National Institute of Standard and technology (NIST), having more than 62,000 patterns and Wiley8 Library. The comparison of unknown spectrum with known spectrum of various components was done by stored spectrum of NIST library and Wiley8 Library. The name, molecular weight and structure of the components were ascertained.

RESULTS AND DISCUSSION

GC-MS is a combined technique of Gas Chromatography and Mass Spectrophotometry. GC-MS is one of the best techniques to identify the constituents of volatile compounds. The GC-MS analysis of various extracts of leaves of *Tecomella undulata* showed the presence of several phytochemicals. The identification of the phytochemical compounds was confirmed based on the peak area, retention time and molecular formula. The active principles with their retention time (RT), area % and name of the compounds present in the extracts with acetone, petroleum ether and methanol of *Tecomella undulata* leaves are presented in Figures 1, 2 and 3 and Tables 1, 2 and 3 respectively.

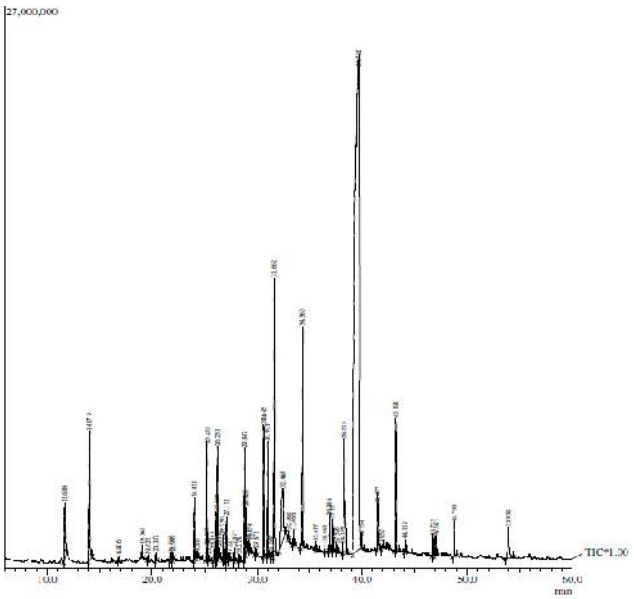

Fig 1 GC-MS Chromatogram of acetonic extract of leaves of *Tecomella undulata.*

Table 1 List of few bioactive components present in acetone extract of leaves of *Tecomella undulata*.

Peak	R.Time(min.)	Area %	Compound
1.	11.684	1.71	2,3-Dihydro-benzofuran
2.	14.079	2.98	2-Methoxy-4-vinylphenol
3.	25.255	1.42	Phenol,2-(1,1-dimethylethyl)-4-(1,1,3,3,-tetraethylbutyl)
4.	26.250	1.38	2,6,10-Trimethyl,14-ethylene-14-Pentadecne
5.	26.934	0.11	1,2-Benzenedicarboxylic acid, bis(2methylpropyl) ester
6.	27.278	0.10	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester
7.	28.847	1.54	Dibutyl phthalate
8.	28.968	1.06	Hexadecanoic acid
9.	30.645	1.95	2,4-Dioctylphenol
10.	30.993	1.71	bis(2,4-ditert-butylphenyl) Pentanedioate
11.	31.367	0.04	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-
12.	31.662	4.73	Phytol
13.	32.468	3.14	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-
14.	34.360	4.13	1-Butyl 2-(8-methylnonyl) Phthalate
15.	38.316	3.59	Dioctyl phthalate
16.	39.720	58.49	1,2-Benzenedicarboxylic acid
17.	41.477	0.82	Celidoniol, deoxy-
18.	43.191	1.59	Squalene
19.	48.790	0.83	di-.alpha.-Tocopherol
20.	53.952	1.40	Stigmast-5-en-3-ol, (3.beta.)-

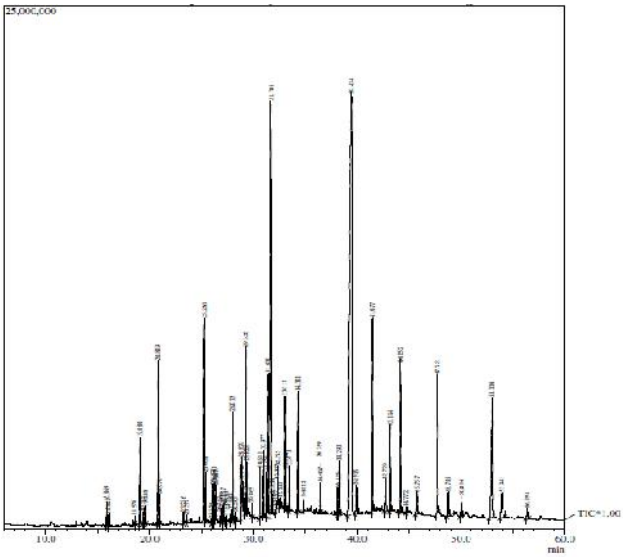

Fig 2 GC-MS Chromatogram of petroleum ether extract from the leaves of *Tecomella undulata.*

Table 2 List of few bioactive components present in petroleum ether extract from the leaves of *Tecomella undulata.*

Peak	R.Time(min.)	Area%	Compound
1.	19.080	1.36	Phenol, 2,4-bis(1,1-dimethylethyl)-
2.	20.819	2.43	9-Eicosene, (E)-
3.	25.286	4.00	1-Octadecene
4.	28.013	1.50	Hexadecanoic acid, methyl ester
5.	28.283	0.17	1,2-Benzenedicarboxylic acid, butyl octyl ester
6.	28.829	0.80	Dibutyl phthalate
7.	28.954	0.39	Hexadecanoic acid
8.	29.326	2.53	1-Heneicosanol
9.	31.254	0.63	9,12-octadecadienoic acid (z,z)-, methyl ester
10.	31.401	2.37	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-
11.	31.701	11.16	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, r-[r*r*,-(e)]]
12.	34.311	1.98	1-Butyl 2-(8-methylnonyl) phthalate
13.	38.290	1.05	Dioctyl phthalate
14.	39.434	31.60	1,2-Benzenedicarboxylic acid
15.	39.919	0.96	Hexadecanoic acid, dodecyl ester
16.	41.477	3.00	Celidoniol, deoxy-
17.	43.184	1.35	Squalene
18.	44.152	2.89	Tetratetracontane
19.	53.004	5.42	Tetracontane
20.	53.941	1.35	gamma.-Sitosterol

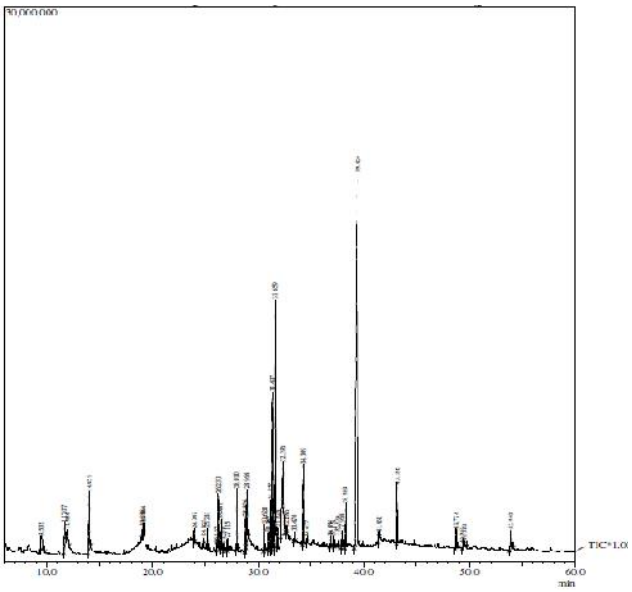

Fig 3 GC-MS Chromatogram of methanolic extract from the leaves of *Tecomella undulata*

Table 3 List of few bioactive components present in petroleum ether extract from the leaves of *Tecomella undulata*

Peak	R.Time(min.)	Area%	Compound
1.	9.532	1.43	2,3-dihydro-3,5-dihydroxy-6-methyl-4h-Pyran-4-one
2.	11.707	1.59	2,3-dihydro-Benzofuran
3.	11.954	0.68	5-hydroxymethylFurfural
4.	14.059	3.04	2-methoxy-4-Vinylphenol
5.	24.007	0.40	Benzene, ethylphenoxy-ethylphenyl phenyl ether
6.	26.230	1.74	5-methyl-1,3-Diazaadamantan-6-one
7.	28.010	1.60	Hexadecanoic acid, methyl ester
8.	28.826	0.97	Dibutyl phthalate
9.	28.964	3.51	Hexadecanoic acid
10.	31.257	1.39	9,12-Octadecadienoic acid (z,z)-, methyl ester
11.	31.407	5.48	9,12,15-Octadecatrienoic acid, methyl ester,
12.	31.659	9.15	Phytol
13.	32.396	7.96	9,12,15-Octadecatrien-1-ol
14.	34.309	2.30	1-butyl 2-(8-methylnonyl)Phthalate
15.	38.289	1.70	Dioctyl phthalate
16.	39.424	45.48	1,2-benzenedicarboxylic acid
17.	41.450	0.17	Tetracontane
18.	43.186	1.74	Squalene
19.	48.774	0.99	di-.alpha.-Tocopherol
20.	53.940	1.89	gamma.-Sitosterol

The GC-MS chromatogram of petroleum ether, acetone and methanol extract of leaf of *T. undulata* showed 64, 53 and 40 peaks (Figure 1-3) indicating the presence of 64, 53 and 40 phytochemicals respectively. Petroleum ether extract for the leaves of *Tecomella undulata* has more compounds than the acetonic and methanolic extract. First major compound that was present commonly in all the solvents was 1,2- Benzene dicarboxylic acid (commonly known as Phthalic acid) with the peak area 58.49 % in petroleum ether and in acetonic and methanolic extract it showed the peak area as 31.60 and 45.48% respectively (Tables 1-3). This aromatic dicarboxylic compound is used mainly in the form of the anhydride to produce other chemicals such as dyes, perfumes, saccharins, phthalates and many other useful products (Pubchem, NCBI). Second major compound was 9,12,15-Octadecatrienoic acid (Z,Z,Z)- methyl ester which has Anticancer, antimicrobial, antioxidant and hyperchloesteralemic activity (Akpuaka *et al.*, 2013, Praveen *et al.*, 2010) followed by hexadecanoic acid which shows varied nature of chemical properties viz Antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant, anti androgenic, hemolytic, 5-alpha Reducatase inhibitor (Gomathi *et al.*, 2015, Rajeshwari *et al.*, 2012). Other compound such as squalene, phytol, gamma-Sitosterol, 2,3-dihydro-Benzofuran, 1- Octadecene, 9-Eicosene, E-, 5-hydroxy methyl Furfuryl (HMF) ; were also found that are associated with numerous properties as listed in the Table 4. Activities exhibited by other important components have also been tabulated. They are being used in numerous ways in cure of various diseases and in appurtenance of human health and welfare. The presence of these various phyotchemicals further justifies the usage of this plant for various ailments by traditional practitioners.

Further from the results shown in Tables 1-3, it is clear that a large number of secondary byproducts are present in the extracts of different solvents. The number and amount of the compounds depend on the compatibility of reactive groups between solute and the solvent. From the Table 4, it is clear that the peak area of any compound that was identified in all the extracts, is different because of the varied solubility of the compound with the respective solvent and because of this, they show difference in the retention time in the column.

Table 4 Activity of few phytochemicals identified in all the crude extracts. S1- Solvent; acetone, S2-Solvent ; Petroleum ether and S3- Solvent 3; methanol.

S.No.	Name of the phytochemical	S1 Peak area	S2 Peak area	S3 Peak area	Biological activity
1.	2,3-dihydro Benzofuran	1.71	-	1.59	Analgesic and anti-inflammatory (Idan *et al.*, 2015; Arora *et al.*, 2017).
2.	2- methoxy- 4- Vinylphenol	2.98	-	3.04	Natural germination inhibitor (Darabi *et al.*, 2007)
3	Dibutyl phthalate	1.54	0.80	0.97	Antimicrobial, antifouling (Khatiwora *et al.*, 2012; Rameshwari *et al.*, 2012)
4.	Hexadecanoic acid	1.06	0.39	3.51	Antioxidant, Hypocholesterolemic Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic, 5-Alpha reductase inhibitor (Rajeshwari *et al.*, 2012; Gomathi *et al.*, 2015).
5.	9,12,15-Octadecatrienoic acid(Z,Z,Z)- methyl ester	0.04	2.37	5.48	Anticancer, Antimicrobial, Antioxidant and Hyperchloesteralemic Anticancer, antimicrobial, antioxidant and hyperchloesteralemic (Praveen *et al.*, 2010; Akpuaka *et al.*, 2013).
6.	Phytol	4.73	-	9.15	Anti cancer, anti inflammatory, diuretic (Rajeshwari *et al.*, 2012; Raman *et al.*, 2012), antifungal, active against *Salmonella typhi*, resistant against gonorrhea, anti malaria. (Akpuaka *et al.*, 2013) precursor of Vit-E (Byju *et al.*, 2013).
7.	Dioctyl phthalate	3.59	1.05	1.70	Used predominantly as plasticizer, environmental pollutants (Romeh., 2013).
8.	1,2- Benzene dicarboxylic acid	58.49	31.60	45.48	In form of hydrides used in the production of dyes, perfumes, saccharin. Suppresses androgen synthesis as Pthalates. (Pubchem, NCBI).
9.	Celidoniol, deoxy-	0.82	3.00	-	Chemical communication of several insects, part of essential oils (Kanimozhi *et al.*, 2012).
10.	Squalene	1.59	1.35	1.74	Neutralises different xenobiotics, anti inflammatory, anti atherosclerotic and anti neoplastic activities. Role in skin ageing and adjuvant activities (Raman *et al.*, 2012). Intermediate of sterol synthesis (Spanova *et al.*, 2011) Antioxidant (Saint-Leger *et al.*, 1986), Antitumor and Immunostimulant (Rajeshwari *et al.*, 2012; Sermakkani *et al.*, 2012; Gomathi *et al.*, 2015).
11.	di- alpha- Tocopherol	0.83	-	0.99	Antioxidant (Isaiah *et al.*, 2012), antimicrobial, Radical scavenging, antispasmodic (Raman *et al.*, 2012).
12.	9- Eicosene	-	2.43	-	Antimicrobial, Cytotoxic (Kuppuswamy *et al.*, 2013; Arora *et al.*, 2017).
13.	1-Octadecene	-	4.00	-	Antibacterial, antioxidant, anticancer (Lee *et al.*, 2007; Adeyemi *et al.*, 2017).
14.	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-,r-[r*r*,(e)]]	0.20	11.16	0.14	Antimicrobial, sedatives and anaesthetics (Arora *et al.*, 2017).
15.	gamma-Sitosterol	-	1.35	1.89	Antioxidant, antibacterial (Akpuaka *et al.*, 2013) and Anti diabetic activities (Balamurugan *et al.*, 2012).
16.	5-hydroxy methyl Furfuryl	-	-	0.68	Antioxidant, Antiproliferative activity (Zhao *et al.*, 2013; Keertiga *et al.*, 2015). Very useful as intermediate for the productionof the biofuel dimethylfuran (DMF) (Rostella *et al.*, 2011).

This is because of the interaction between the solvent and compound i.e. between the reactive groups of the solvent and the solute. Therefore according to the need of any specific compound, the selection of solvent is very important to extract maximum amount of the compounds from any plant part.

CONCLUSION

The bioactive compounds in the various extract from the leaves of *Tecomella undulata* have been screened using this GC-MS analysis which revealed good amount of bioactive compounds present in the plant. So, it could be recommended as a plant of pharmaceutical importance. Further work regarding isolation and specific activity of the identified compound will provide more insights about the role of plant and may help to find a new drug.

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Conflict of Interest

The authors declare no conflict of interest.

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