Research Paper

Volume : 4 | Issue : 6 | June 2015 • ISSN No 2277 - 8179

Phytochemical and GC-MS studies of Diospyros ferrea (Willd) Bakh. Leaf

Botany

KEYWORDS : Diospyros ferrea leaf, Hexane, Eythylacetate, Methanol extracts, phytoconstituents, GC-MS analysis.

Nitya Jeeva Prada P — Department of Zoology, Maris Stella College, Vijayawada

Vishnu Vardhan Z* — Department of Botany and Microbiology, Acharya Nagarjuna University, Guntur

Srinivasa Reddy Ch — Department of Botany, PB Siddhartha College of Arts and Science, Vijayawada-10

ABSTRACT

Diospyros ferrea is a small sized tree, belongs to family Ebenaceae. The present study was carried out to analyze the phytochemicals and to characterize the bioactive constituents present in different solvent extracts of Diospyros ferrea leaf using GC-MS. The crude extracts were scanned in the wavelength ranging from 200-1100 nm by using Perkin Elmer Spectrophotometer and the characteristic peaks were detected.10 g of sample is extracted with 30ml Hexane, ethylacetate and methanol separately for GC-MS analysis, filtered in ash less filter paper with 2 g sodium sulphate and extract is concentrated to 1 ml by bubbling nitrogen in to the solution. The results of the GC-MS analysis provide different peaks determining the presence of 40 phytochemical compounds with different therapeutic activities.

Introduction

Plants as sources of bioactive compounds continue to play a dominant role in the maintenance of human health. Reports available on green plants represent a reservoir of effective therapeutants, these are non-phytotoxic, more systemic and easily biodegradable (Vyas, 1999; Kaushik *et al*., 2002; Chaman Lal and Verma, 2006). Plants are a rich source of secondary metabolites with interesting biological activities. In general, these secondary metabolites are an important source with a variety of structural arrangements and properties (de-Fatima *et al*., 2006). *Diospyros* is a genus of over 700 species of deciduous and evergreen trees, shrubs and small bushes. The majority are native to the tropics, with only a few species extending into temperate regions. *Diospyros ferrea*, known as Black ebony, is a tree in the Ebony family, Ebenaceae distributed in Burma (Myanmar), Cambodia, India, Indonesia, Malay Peninsula, Philippines, Sri Lanka, Thailand and Laos. The objective of the present study was to investigate phytochemical screening of the leaf extracts of *Diospyros ferrea* and GC-MS analysis for bioactive compounds.

Botanical Description

Evergreen small tree; branch lets glabrous, bark grey to black. Leaves obovate-spathulate or elliptic, entire, obtuse or emarginated, base acute to attenuate, glabrous, coreaceous. Flowers white,trimerous, male flowers in short cymes; female flowers 10-13, together. Sepals and petals 3 each. Stamens-6. Ovary trolocular. Berries globase, orange when ripe.

Systematic position

Kingdom	:	Plantae
Phylum	:	Magnoliophyta
Class	:	Magnoliopsida
Family	:	Ebenaceae
Genus	:	*Diospyros*
Species	:	*ferrea*

2. Materials and methods

Collection of Plant Materials

The Leaf of *Diospyros ferrea* (Willd) Bakh. of family Ebenaceae, collected from hill slopes of Tirumala, Andhra Pradesh, India and authenticated by Dr.A.Ravi Kiran, BSI, Coimbatore. A voucher specimen is deposited in Department of Botany, Acharya Nagarjuna University, Guntur and the specimen numbers is ANU Y9B0R028. Collected leaf dried till the moisture content is evaporated and finally pulverized in to small pieces.

Soxhlet Extraction

Crude plant extract was prepared by Soxhlet extraction method. Plant Leaf pieces were uniformly packed into 3/4th volume of the thimble and extracted with 300ml of different solvents separately, Polarity based solvents used were Hexane, Ethylacetate and Methanol. The process of extraction continues till the solvent in siphon tube of an extractor become colorless. The extract was taken in a beaker and kept it for air dry till the solvent got evaporated. Dried extract was kept in refrigerator at 4^{0}C for their future use in phytochemical analysis.

GC-MS Analysis

Phytochemicals were analyzed by GC-MS (SHIMADZU QP 2010) employing the electron impact (EI) mode at an ionizing potential of 70 e v with a 30m × 0.32 mm, film thickness and 1.8 µm capillary column (Restech-624 MS) packed with 5% phenyl dim ethyl silicone at an ion source temperature of 200^{o}C. For further analysis, GC/MS settings were as follows: the column temperature was set at 45^{o}C and held for 4min; the temperature was raised to 50^o C and then increased up to 175^{o}C at a rate of 100C/min for 2 minutes, and then finally programmed to 240^{o}C at a rate of 25^{o}C/min, and kept isothermal for 2 minutes. Helium was used as carrier gas with a flow rate of 1.491 ml/min with a split ratio of 1:10. During sample analysis the column oven temperature was maintained 280^{0}C (Zhang *et al.*,2011).

Identification of phytocomponents

Interpretation on mass-spectrum GC-MS conducted using the database of National Institute Standard and Technology (NIST). The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. The name of the compound, molecular weight and molecular formula of the test materials were ascertained.

Qualitative Phytochemical Screening (Evans WC, 1989; Gokhale *et al.*,1993; Harborne JB, 1973; Shanmugam *et al.,* 2010; Trease *et al.,* 1996)

I.Detection of Alkaloids

Mayer's Test: One or two drops of Mayer's reagent was added to plant extract by the sides of the test tube. White precipitate indicates the presence of alkaloids.

Wagner's test: One or two drops of Wagner's reagent was added to plant extract by the sides of the test tube. Reddish brown precipitate indicates the presence of alkaloids.

Hager's Test: One or two drops of Hager's reagent was added to plant extract. Prominent yellow precipitate indicates the presence of alkaloids.

II.Detection of Phlobatannins

To 0.5ml plant extract few drops of 10% ammonia solution was added. Pink color precipitate indicates the presence of Phlobatannins.

III. Detection of Coumarins

1ml of plant extract is added with 10% sodium hydroxide.Yellow colour indicates the presence of coumarins.

IV.Detection of Anthraquinones

0.5ml of plant extract was treated with few drops of 2% HCL.Red color precipitate indicates the presence of anthraquinones.

V.Detection of Tannins

Ferric chloride test: 5mg of plant extract was dissolved in 5 ml of distilled water and few drops of neutral 5% ferric chloride solution were added. The formation of blue green color indicates the presence of tannins.

Gelatin test: Few ml of plant extract was treated with gelatin solution. Formation of white precipitate indicates the presence of tannins.

VI.Detection of Glycosides

Legalstest: 2ml of plant extract was treated with 3ml of chloroform and10% ammonia solution. Formation of pink colour indicates the presence of Glycosides.

Liebermann's test: Few ml of plant crude extract was mixed with 2ml of chloroform and 2ml of acetic acid. The mixture was cooled in ice. Conc.$H_2SO_4$was added carefully. A colour change from violet to blue to green indicates the presence of glycosides.

Keller-kilani test:

Few ml of plant crude extract was treated with 2ml of glacial acetic acid containing 1-2 drops of 2% solution of $FeCl_3$. The mixture was then poured into a test tube containing 2ml of concentrated H_2SO_4. Formation of brown ring at the interphase indicated the presence of cardiac glycosides.

VII. Detection of Phytosterols

Salkowski reaction test:

0.5 ml chloroform extract is treated with 1 ml of concentrated H_2SO_4 from the sides of the test tube. Formation of reddish brown colour in chloroform layer indicates the presence of phytosterols.

VIII.Detection of flavonoids

Watery plant extract was treated with ammonium hydroxide solution. The yellow fluorescence indicated the presence of flavonoids.

Alkaline reagent Test: When plant extract was treated with sodium hydroxide solution, shows increase in the intensity of yellow color which would become colorless on addition of few drops of dilute Hydrochloric acid, indicates the presence of flavonoids.

Lead acetate solution Test: Plant extract treated with few drops of 10% lead acetate solution. Formation of yellow precipitate indicates the presence of flavonoids.

IX.Detection of Phenols

Lead acetate test

5 mg of plant extract was dissolved in distilled water and 3 ml of 10% lead acetate solution was added. Formation of a bulky white precipitate indicates the presence of phenols.

Ferric chloride test: Plant extract was treated with 5% ferric chloride. Formation of intense colour indicates the presence of phenols.

X. Detection of Saponins

The plant extract was mixed with 5ml of distilled water and was shaken vigorously. Formation ofstable foam indicates the presence of saponins.

XI. Detection of Terpenoids

0.5ml of plant extract was mixed with 2ml of chloroform and concentrated H_2SO_4 is added carefully. Formation of red brown color at the interface indicates the presence of terpenoids.

3. Results

The medicinal properties of a plant lies with the presence of its secondary metabolites such as alkaloids, tannins, glycosides, phytosterols, flavanoids, phenols, Phytosterols. The three different extracts from leaf found to contain Alkaloids, Coumarins, Tannins, Glycosides, Phytosterols, Flavonoids, Phenols and saponins. From the analysis hexane extract contains more phytoconstituents followed by ethylacetate and methanol respectively. The results of preliminary phytochemical analysis are shown in **Table No.1**

Table No. 1: Phytochemical and GC-MS studies of *Diospyros ferrea* (Willd) Bakh. Leaf

S.No	Name of the Test	Hexane Extract	Ethyl Acetate Extract	Methanol Extract
1	**Alkaloid test**			
	Mayer's test	+	-	-
	Wagner's test	+	+	-
	Hagers' test	+	-	-
2	**Phlobatanins**	+	-	-
3	**Coumarins**	-	-	-
4	**Anthraquinones**	-	-	-
5	**Tannins**			
	$Fecl_3$ test	-	-	-
	Gelatin test	-	-	+
6	**Glycosides**			
	Legals's test	-	-	-
	Liebermann'stest	+	-	-
	Keller-Kilani test	+	+	+
7	**Phytosterols**			
	Salkowski test	-	-	-
8	**Flavonoids**			
	Ammonium hydroxide test	+	-	-
	Alkaline reagent test	-	-	-
	Lead acetate test	-	+	-
9	**Phenols**			
	Lead acetate test	+	-	-
	$Fecl_3$ test	+	-	+
10	**Saponins**	+	-	+
11	**Terpenoids**	+	+	-

The study on active principles of hexane, ethyl acetate and methanol leaf extract of *Diospyros ferrea* by GC-MS showed the presence of forty compounds. The major and minor compounds in hexane, ethyl acetate and methanol extracts with their retention times in minutes (RT), molecular formulae weight (MW) are listed in this study. The major constituents in hexane extract were found to be seven (Table:2 and Fig:1). The major constituents in ethylacetate extract were found to be sixteen (Table :3 and Fig:2). The major constituents in methanol extract were found to be seveteen (Table:4 and Fig:3).

Fig:1 GC-MS Chromatogram of Hexane extract of *Diospyros ferrea leaf*

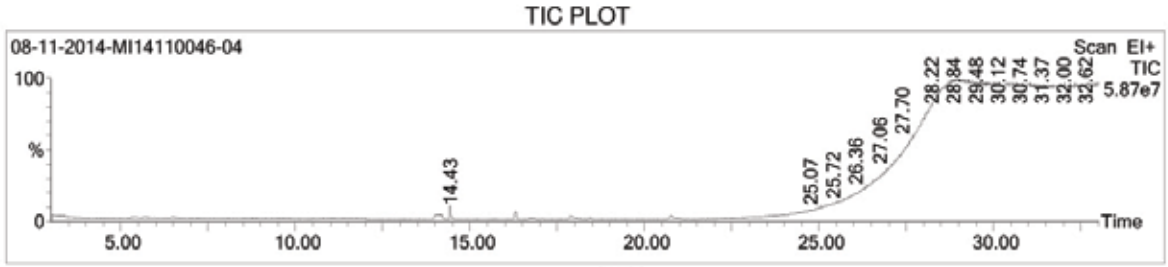

Table:2 Phytocomponents in Hexane extract of *Diospyros ferrea leaf* **by GC-MS**

S.No.	RT	Name of the Compound	MW	MF	Area%
1.	14.171	Hexamethyl -3, 5, 5-tris	590	$C19H54O_7S_{17}$	8.302
2.	14.431	Phenol, 3,5-bis(1,1- dimethyle)-	206	$C_{14}H_{22}O$	100.00
3.	16.301	Saline, trimethyl silylacy	458	$C_2H_{42}O_4SO_4$	12.128
4.	16.7511	Benzoic acid, 2,4 bis, trimethylsily (ester)	370	$C_{16}H_{30}O_4Si_3$	4.623
5.	16.811	Undecane – 3, methyl	170	$C_{12}H_{26}$	5.371
6.	17.871	1 octane, 6-methyl	126	C_9H_{18}	13.522
7.	20.752	Oxalic acid, allyl Pentadecyl ester	340	$C_{20}H_{36}O_4$	12.460

Fig:2 GC-MS Chromatogram of Ethyl acetate extract of *Diospyros ferrea leaf*

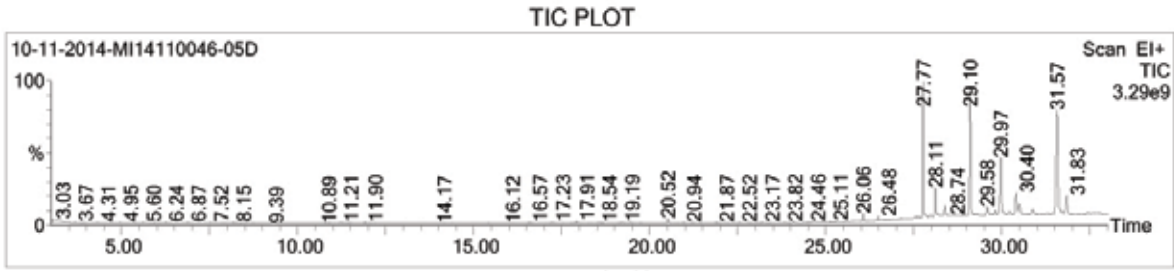

Table:3 Phytocomponents in Ethyl acetate extract of *Diospyros ferrea leaf* **by GC-MS**

S.No.	RT	Name of the Compound	MW	MF	Area%
1.	20.517	Phytol	296.0	C20H40O	0.794
2.	26.058	Trans-Geranylgeraniol	290.0	C20H340	0.796
3.	27.766	Nonadecane, 2-methyl-	282.0	C20H42	14.573
4.	28.112	dl-aTocopherol	430.0	C29H50O2	3.424
5.	28.379	Heneicosane	296.0	C2H44	1.201
6.	29.099	Heptadecane, 2,3-dimethyl-	268.0	C19H40	17.536
7.	29.579	Cholest-5-en 3-ol(3a)-tetradecanoate	596.0	C41H72O2	1.443
8.	29.799	c-HIMACHALENE	204.0	C15H24	0.628
9.	29.966	a-Amyrin	426.0	C30H50O	10.618
10.	30.213	Betulin	43432.0	C30H50O2	0.736
11.	30.400	á-Amyrin,trimethylsilyl ether	498.0	C33H58OSi	4.272
12.	30.493	Androstan-6-one, (5á)	274.0	C19H30O	2.157
13.	30.873	Geranyl isovalerate	238.0	C15H26O2	1.201
14.	31.567	Friedelan-3-one	426.0	C30H50O	23.626
15.	31.833	Tetracosapentaene, 2,6,10,15,19,23-hexamethyl-	412.0	C30H52	3.682
16.	32.620	Ethyl iso-allocholate	436.0	C326H44O5	0.922

Fig:3 GC-MS Chromatogram of Methanol extract of *Diospyros ferrea leaf*

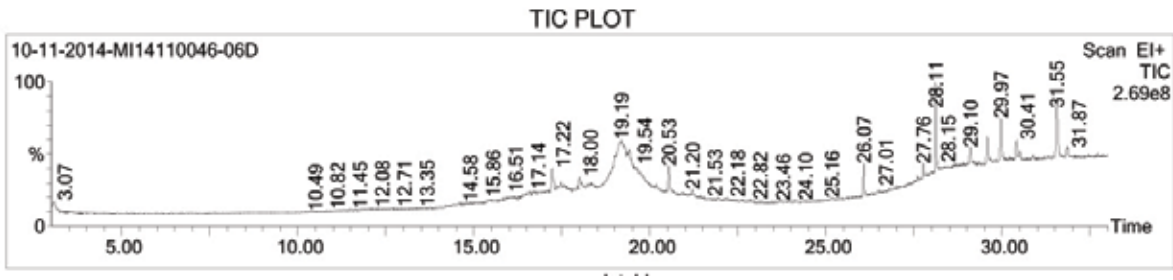

Table:4 Phytocomponents in Methanol extract of *Diospyros ferrea leaf* by GC-MS

S.No.	RT	Name of the Compound	MW	MF	Area%
1.	17.216	E-9-Methyl-8-tridecen-2-ol, acetate	254.0	C16H30O2	1.900
2.	17.996	Trans-Geranylgeraniol	196.0	C 13H240	1.116
3.	18.063	1-Dimethydodecylsiyloxpent-2-en-4-yne	308.0	C19H360Si	1.067
4.	18.310	1,4-Di-O-acetyl-2,3,5-tri-O-methylribitol	278.0	C12H2207	2.356
5.	19.190	Galactitlol	182.0	C6H1406	29.940
6.	19.417	Cetene	224.0	C16H32	20.007
7.	20.170	Decane,2-cyclohexyl-	224.0	C16H32	2.265
8.	20.530	Cintronellol	156.0	C10H200	3.366
9.	21.204	Eicosyl acetate	340.0	C22H4402	0.841
10.	26.072	Farnesol isomer a	222.0	C15H260	1.090
11.	28.112	a-Tocopheryl acette	472.0	C31H5203	3.122
12.	29.099	Stigmastan – 6,22-dien,3,5-dedihydro-	394.0	C29H46	1.390
13.	29.579	Pregnenolone	316.0	C21H3202	1.712
14.	29.966	Olen-12-ene	410.0	C30H50	2.671
15.	30.406	Urs-12-en-24-oicacid,3-oxo-,methylester,(+)-	468.0	C31H4803	1.287
16.	31.553	Thunbergol	290.0	C20H340	4.381
17.	31.840	D.A-Fridooleann-28-l,3-oxo-	428.0	C30H4802	0.817

Modern medicine has evolved from folk medicine and traditional system only after through chemical and pharmaceutical screening (Boopathi and Sivakumar, 2011). Phytochemical constituents are the basic source for the establishment of several pharmaceutical industries. The constituents are playing a significant role in the identification of crude drugs (Savithramma *et al.* 2011).

Conclusion

In this study forty bioactive compounds from hexane, ethylacetate extract and methanol extract of *Diospyros ferrea leaf* by GC-MS method were identified and is recommended as plant of phytopharmaceutical importance. This study has revealed the presence of many secondary metabolites in the leaves of *Diospyros ferrea*. It has the further confirmed that the plant extracts could be used for the treatment of various ailments.

REFERENCE

Boopathi AC and Sivakumar R(2011). Phytochemical screening studies on the leaves and stem of Andrographis neesiana wight – An endemic medicinal plant from India. World App Sci J., 12(3): 307-311. | | Chaman Lal and Verma LR(2006). Use of certain bio-products for insect-pest control. Indian Journal of Traditional Knowledge. 5(1):79-82. | | de-Fatima A, Modolo LV, Conegero LS, Pilli RA, Ferreira CV, Kohn LK, de-Carvalho JE | (2006). Lactones and their derivatives: biological activities, mechanisms of action and | potential leads for drug design. Curr Med Chem, 13: 3371-3384. | | | Evans WC(1989)Trease and Evans.Pharmacognosy13th ed., BailliereTindall, London. | | Gokhale, S.B., Kokate, C.K, Purohit, A.P.(1993).A text book of Pharmacognosy, Nirali Prakshan, Pune, India, pp. 1–50. | | Harborne JB(1973). Phytochemical methods, Chapman and Hall Ltd., London.pp.49-188. | | Kaushik JC, Arya Sanjay, Tripathi NN, Arya S (2002). Antifungal properties of some plant extracts against the damping off fungi of forest nurseries. Indian Journal of Forestry., 25: 359-361. | | Savithramma N, Linga Rao M and Beenaprabha(2011). Phytochemical studies of Dysophylla myosuroides (Roth.) Benth. In. wall. and Talinum cuneifolium (Vahl.) Willd. Res J Phyto., 5(3): 163-169. | | Shanmugam, S., Kumar, T.S and Selvam K.P.(2010)Laboratory hand book on biochemistry, PHI learning Private limited, NewDelhi, India.pp.129-133. | | Trease, G.E, Evans , W.C.(1996). A textbook of Pharmacognosy.14th ed. ,BailliereTindall Ltd, London. | | Vyas GD (1999). Soil Fertility Deterioration in Crop Land Due to Pesticide. Journal of Indian Botanical Society., 78: 177-178. | | Zhang, S., Xu, Y., Zhang, J., Kong, D. and Hua, M (20ll). Composition analysis of volatile oil from different Ocimum basilicum (L.) by gc-ms and antimicrobial activity. Chinese journal of pharmaceuticals;6:009. |