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GC-MS IDENTIFICATION OF BIOACTIVE COMPOUNDS FROM SOLVENT EXTRACTS OF *DIOSPYROS FERREA* (WILLD.) BAKH, LEAF

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ABSTRACT
The present study was investigated forty phytocompounds in polarity based solvents that is Hexane, Ethylacetate and methanolic leaf extract of ***Diospyros ferrea*** (Ebenaceae). These compounds are proved to be antidiabetic and anti-inflammatory and hepatoprotective activity. The main objective of the work was to list out compounds with GC-MS chromatogram, remittance time and phytochemical analysis. The results showed that hexane solvent consists of seven compounds, of which Phenol 2,4 bis (1,1-dimethyl alkylated) phenolic group and remaining siloxone peaks are hydrocarbons). The ethylacetate and methanolic extract shows 33 (16+17) compounds, of which are phytol (Diterpene) fragrance alcohol; Squalene (Triterpene), α - tocopherol (vitamin -E); α-Amyrin(Triterpene), Friedeleon-3-one (Triterpene) where as methanolic extract shows Galactitol (sugar alcohol), Squalene (Triterpene), α- tocopherol (vitamin -E); α Amyrin (Triterpene) and all other siloxone peaks are hydrocarbons. So the phytochemical properties of these compounds are commercially important for inventing new drugs for curing human diseases. This paper gives details on the importance and isolation of compounds.

KEYWORDS: GC-MS chromatogram, Galactitol (sugar), Squalene (Triterpene), α - tocopherol (vitamin -E); α – Amyrin (Triterpene), siloxone peaks.

INTRODUCTION
Non-nutritive additives typically occur as bio active compounds shows an effect on the body as whole or on specific tissues. Plant based materials has become upswing due to their immense potential to heal life diseases. Bioactive compounds promote good health related to its ability to modulate one or more metabolic processes occur in small quantities but act quickly. Asolkar *et al* 1992 reported the active principal compounds responsible for medicinal properities present in 12 Indian *Diospyros* species. Majority of them are betulinic acid, beta sitosterol, lupeol, triterpenes, saponins and tannins. Betulinic acid is known to exhibit a variety of biological and medicinal properities Mansour *et al* 2012).

Diospyros is the largest genus of Ebenaceae family, are medicinally important as they contain active compounds with pharmacological significance. Out of the 500 *Diospyros* species widely distributed in the tropics, nearly 41 species occur in India. Phytochemical and pharmacological studies have been made on 130 *Diospyros* species only and the other species are yet to be investigated (Mallavadhani *et al.*, 1998). *Diospyros ferrea* is one such Indian species on which phytochemical and pharmacological studies now reported and hence, this *Diospyros* species has been selected for the present study.

Maridass *et al.*, (2008), Amrish & Tarasingh (2012) and Mohammed Abbas *et al.*, (2016) reported the presence of sterols, alkaloids, flavonoids, tannins, saponins, terpenoids, anthroquinones, glycosides and coumarins in *Diospyros buxifolia*, *D.malabarica* and *D.mespiliformis*. The active role of above identified compounds in the treatment of pain, inflammation, fungal, bacterial, parasitic and viral diseases has been highlighted by many investigators through their studies in many other plant species.

This species also reported to contain triterpenoids, naphthols and naphthaquinones such as diospyrin, isodiospyrin that are responsible for their medicinal properties. Hence, the phytochemical analytical studies proposed in the *Diospyros ferrea* species become very significant and pertinent research activity. The identification and study of medicinal bioactive novel phytocompounds have met with great success with the introduction of various analytical techniques and sophisticated instruments. High performance liquid Chromatography (HPLC), Gas Chromatography – Mass spectrometry (GC-MS) and Fourier Transform-Infrared

(FT-IR) spectrometry have been very useful in the identification and quantification of active compound from plant extracts. In the present study, phytochemical compounds were identified in the solvent *ferrea* by using GC-MS analytical technique.

MATERIALS AND METHODS

Collection and Solvent extraction of leaf

The plant material i.e., leaf of *Diospyros ferrea* was collected from the hill slopes of Seshachala forest, Tirupathi, Andhra Pradesh, India, as the plant is an evergreen bushy shrubs, branchlets glabrous, bark is grey to black, leaves are obovate – spathulate or elliptic, entire, obtuse or emarginated, base is acute to attenuate and glabrous variety.

Leaves *of D.ferrea* were subjected to selective sequential extraction using solvents of increased order of polarity. Three analytical grade solvents viz., Hexane, Ethylacetate and Methanol were used, each of the extract samples were further subjected to GC–MS (gas chromatogram–mass spectrometry) studies. Crude plant extract was prepared by Soxhlet extraction method, 100g leaf of *Diospyros ferrea* was uniformly packed separately into the thimble and extracted with 300 ml of different solvents separately. The process of extraction continued till the solvent in siphon tube of extractor became colourless. 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^{o}C for their future use in phytochemical analysis.

Qualitative Phytochemical screening was done by the method of (Evans 1989; Gokhlae *et al.,* 1993, Trease and Evans 1996; Harborne 1984; Shanmugam *et al.,* 2010) to identify Alcoloids, Flavonoids seponins glycosides and phenols etc.

GC-MS Analysis and Identification of compounds

Phytochemicals were analyzed by GC-MS (SHIMADZU QP 2010) employing the electron impact (EI) mode at an ionizing potential of 70 eV with a 30m × 0.32 mm, film thickness and 1.8µm capillary column (Resteck-624 MS) packed with 5% phenyl dimethyl silicone at an ion source temperature of 200°C. For further analysis, GC/MS settings were set to the initial column temperature at 45°C and held for 4 min; the temperature was raised to 50°C and then increased up to 175°C at a rate of 100^{o}C/min for 2 minutes, and then finally programmed to 240°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 at 280°C (Zhang *et al.,* 2011).

Mass spectrum of GC-MS interpretation was conducted using the database of National Institute of Standards and Technology (NIST) which consists of more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known component inherent in the NIST library. The name, molecular weight, retention time and structure of the components of the test materials were ascertained.

RESULTS

Qualitative phytochemical screening of compounds from *D. ferrea* leaf extracts

Phytochemical screening for biologically active compounds and their activity assessment are the two essential steps in the exploration of useful medicinal herbs. The use of organic solvents is proved advantageous for the preparation of plant extract. The polarity of organic solvents make the extraction of bioactive compounds more effective without alter their activity. Three different solvents viz., Hexane, Ethyl acetate and Methanol that exhibit polarity in the increasing order were used in the preparation of *D.ferrea*. The phytochemical screening tests that distinguish qualitatively the biologically active compounds such as alkaloids, phlobatannins, coumarins, anthraquinones, tannins, glycosides, phytosterols, flavonoids, phenols, saponins and terpenoids were employed.

The hexane extract of the *D.ferrea* leaf was tested positive for seven categories of compounds viz., alkaloids, phlobatannins, glycosides, flavonoids, phenols, saponins and terpenoids while ethyl acetate and methanolic leaf extract revealed the presence of only four groups of compounds in each extract. Except glycosides, no other single group of compounds was tested positive uniformly in the three solvents extracts.

Alkaloids were tested positive in both hexane and ethyl acetate solvent extract but negative in methanolic extract. Phlobatannins were detected only in hexane leaf extract. Tannins were tested positive with methanolic leaf extract only. Similarly flavonoids and terpenoids were detected in hexane and ethyl acetate extracts only. The phenols and saponins were identified in hexane and methanolic extracts. The ethyl acetate extract tested negative for these compoundsOf the different groups of biological active compounds tested for their presence, the coumarins, anthraquinones and phytosterols were not detected in the three solvents leaf extracts of *Diospyros ferrea.*

Table I: Qualitative Phytochemical screening for major compounds from *D. ferrea* leaf extract in hexane, ethylacetate and methanol solvents.

S.No	Category of compounds & test employed	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's test	+	-	-
	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**	+	+	-

+ Present – Absent

GC-MS Phytochemical analysis

Gas-Chromatography-Mass Spectroscopy (GC-MS) is an instrumental technique by which different chemical compounds can be separated, identified and quantified from complex mixture and extracts. In the present study leaf extracts of ***Diospyros ferrea*** in three solvents (hexane, ethyl acetate and methanol) were subjected to GC-MS analysis. The names of different compounds present in each solvent extract were presented in tables 3, 4 and 5 along with their molecular weight (MW), molecular formula (MF), retention time (RT) and Chromatograms. Seven major chemical compounds were identified in hexane extract (Table-2 & Figure-1) and in ethylacetate extract sixteen different compounds were detected (Table-3 & Figure-2). The highest numbers of seventeen compounds were identified in methanolic extract (Table-4 & Figure-3).

In hexane extract, out of the seven peaks detected six were siloxone peaks representing hydrocarbons and one (phenol 3, 5-bis (1,1-dimethyl) represents alkylated phenolic group. The GC-MS chromatogram of ethyLaceate extract revealed 16 different peaks representing significant compounds such as phytol, squalene, tocopherol, α-amyrin, betulin and friedelan besides other hydrocarbons In methanolic extract galactitol, cetene, citronellol, tocopherol, pregnenolone, olen and thunbergol are some of the important compounds identified (of the above identified compounds majority of them belong to hydrocarbon group, while some of them are triterpenes and a few are phenolic compounds.

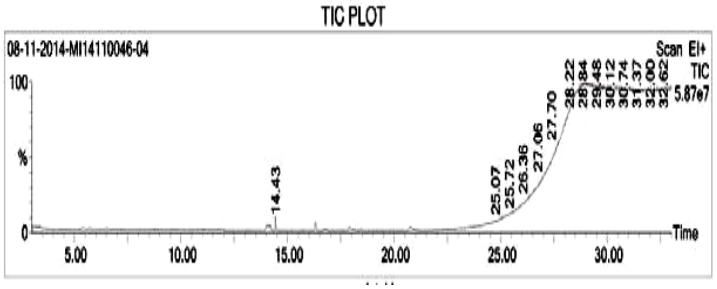

Fig. 1: GC-MS Chromatogram of Hexane leaf extract of *Diospyros ferrea.*

Table 2: Phytocompounds in Hexane leaf extract of *D. ferrea* identified by GC-MS analysis.

S.No.	Retention Time	Name of the Compound	Molecular Weight	Molecular Formula	Area%
1.	14.171	3-Butoxy-1,1,1,7,7,7-Hexamethyl -3, 5, 5-tris tetra siloxane	590	$C_{19}H_{54}O_7Si7$	8.302
2.	14.431	Phenol, 3,5-bis[1,1- dimethyle ethyl]	206	$C_{14}H_{22}O$	13.88
3.	16.301	Saline, [{4-{1,2,-bis{(trimethyl silyl) oxy ethyl {1,2-phenylene} bis (oxy) trimethyl	458	$C_{20}H_{42}O_4Si_4$	12.128
4.	16.751	Benzoic acid, 2,4 bis, (trimethyl silyl) oxy-trimethylsilyl 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 octene, 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

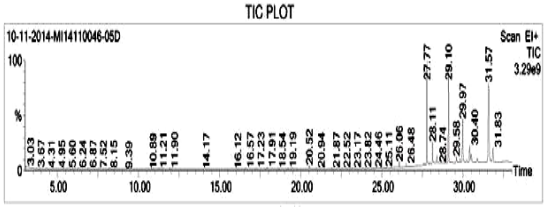

Fig. 2: GC-MS Chromatogram of Ethylacetate leaf extract of *Diospyros ferrea*.

Table 3: Phytocompounds in Ethylacetate leaf extract of *D. ferrea* identified by GC-MS analysis.

S.No.	Retention Time	Name of the Compound	Molecular weight	Molecular formula	Area%
1.	20.517	Phytol	296.0	$C_{20}H_{40}O$	0.794
2.	26.058	Trans-Geranylgeraniol	290.0	$C_{20}H_{34}O$	0.796
3.	27.766	Nonadecane, 2-methyl-	282.0	$C_{20}H_{42}$	14.573
4.	28.112	dl- α Tocopherol	430.0	$C_{29}H_{50}O_2$	3.424
5.	28.379	Heneicosane	296.0	$C_{21}H_{44}$	1.201
6.	29.099	Heptadecane, 2,3-dimethyl-	268.0	$C_{19}H_{40}$	17.536
7.	29.579	Cholest-5-en 3-ol[3a]-tetradecanoate	596.0	$C_{41}H_{72}O_2$	1.443
8.	29.799	c-HIMACHALENE	204.0	$C_{15}H_{24}$	0.628
9.	29.966	α -Amyrin	426.0	$C_{30}H_{50}O$	10.618
10.	30.213	Betulin	442.0	$C_{30}H_{50}O_2$	0.736
11.	30.400	α -Amyrin,trimethylsilyl ether	498.0	$C_{33}H_{58}OSi$	4.272
12.	30.493	Androstan-6-one, [5á]	274.0	$C_{19}H_{30}O$	2.157
13.	30.873	Geranyl isovalerate	238.0	$C_{15}H_{26}O_2$	1.201
14.	31.567	Friedelan-3-one	426.0	$C_{30}H_{50}O$	23.626
15.	31.833	Tetracosapentaene, 2,6,10,15,19,23-hexamethyl-	412.0	$C_{30}H_{52}$	3.682
16.	32.620	Ethyl iso-allocholate	436.0	$C_{26}H_{44}O_5$	0.922

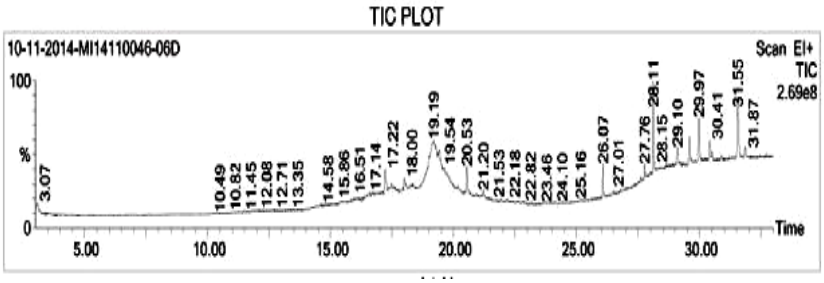

Fig. 3: GC-MS Chromatogram of methanolic leaf extract of *Diospyros ferrea*.

Table 4: GC- MS Compounds of Methanolic Leaf Extract.

S.No.	Retention Time	Name of the Compound	Molecular weight	Molecular formula	Area%
1.	17.216	E-9-Methyl-8-tridecen-2-ol, acetate	254.0	$C_{16}H_{30}O_2$	1.900
2.	17.996	Tetra hydr ionone	196.0	$C_{13}H_{24}O$	1.116
3.	18.063	1-Dimethyldodecylsiyloxypent-2-en-4-yne	308.0	$C_{19}H_{36}OSi$	1.067
4.	18.310	1,4-Di-O-acetyl-2,3,5-tri-O-methylribitol	278.0	$C_{12}H_{22}O_7$	2.356
5.	19.190	Galactitlol	182.0	$C_6H_{14}O_6$	29.940
6.	19.417	Cetene	224.0	$C_{16}H_{32}$	20.007
7.	20.170	Decane,2-cyclohexyl-	224.0	$C_{16}H_{32}$	2.265
8.	20.530	Citronellol	156.0	$C_{10}H_{20}O$	3.366
9.	21.204	Eicosyl acetate	340.0	$C_{22}H_{44}O_2$	0.841
10.	26.072	Farnesol isomera	222.0	$C_{15}H_{26}O$	1.090
11.	28.112	a-Tocopheryl acetate	472.0	$C_{31}H_{52}O_3$	3.122
12.	29.099	Stigmastan – 6,22-dien,3,5-dedihydro-	394.0	$C_{29}H_{46}$	1.390
13.	29.579	Pregnenolone	316.0	$C_{21}H_{32}O_2$	1.712
14.	29.966	Olen-12-ene	410.0	$C_{30}H_5O$	2.671
15.	30.406	Urs-12-en-24-oigc acid,3-oxo-, methylester,[+]-	468.0	$C_{31}H_{48}O_3$	1.287
16.	31.553	Thunbergol	290.0	$C_{20}H_{34}O$	4.381
17.	31.840	D.A-Friedooleanan-28-l,3-oxo-	428.0	$C_{30}H_{52}O$	0.817

CONCLUSION

Qualitative phytochemical screening for compounds in *Diospyros ferrea* leaf extract using hexane, ethylacetate and methanol solvents revealed the presence of alkaloids, phlobatannins, glycosides, flavonoids, phenols, saponins and terpenoids. Previously, the above catagories of compounds were reported in *D. mespiliformis, D.wallichii, D.malabarica, D.melonoxylon, D.chloroxylon and D.mespiliformis* by Alizera Nematollahi *et al.,* 2011, Amrish & Tarasingh (2012), Mohammed Abbas *et al.,* (2016) respectively.

In the light of above research findings the medicinal importance of *Diospyros* species lies in their possession of chemical compounds namely flavonoids, phenols, tannins, terpenoids, saponins etc In GC-MS analysis seven compounds were screened in hexane extract, Sixteen different compounds were identified in the ethylacetate extract where as seventeen compounds from methanolic leaf extract. Phytol, friedelan-3-one, squalene, tocopherol, α-amyrin, betulin, citronellol, pregnenolene, olen and thunbergol are the important compounds identified from *Diospyros ferrea* leaf extracts. These compounds are broadly grouped under hydrocarbons, terpens and phenolic compounds.

Betulin is also well known as anti-inflammatory, anti-bacterial, anti-cancer, anti-malarial, anti-protozoal, anti-HIV, anti-feedant, anti-obese and anti-plasmodial compound (Mansour *et al.,* 2012). The α-amyrin and friedelan-3-one are the triterpenes identified in the *D.ferrea* leaf extract. The terpenoids are generally used in the prevention and therapy of inflammatory and diabetic diseases (Wagner and Elmadfa 2003). Tocoferol is a well known antioxidant present in many plants and it is also present in the leaf extract of *D.ferrea*.

SUMMARY

Diospyros species constitute the chief source of very important bioactive compounds viz., diospyrin, betulinic acid, napahthoquinones and triterpenes responsible for varied medicinal properties. Hence, there is a need to carry out phytochemical and pharmacological studies in *Diospyros* species in which such enquires have not been made so far. The present work on *D.ferrea* is one such attempt in that direction carried out with the following objectives.

i). To identify major classes of compounds present in the leaf extracts of *D.ferrea.*
ii). To identify different phytochemicals in leaf extracts of *D.ferrea* by GC-MS analysis.

Preliminary phytochemical analysis of *D.ferrea* leaf extracts revealed the presence of major classes of compounds. The hexane leaf extracts showed the presence of alkaloids, phlobatannins, glycosides, flavonoids, phenolos, saponins and terpenoids. In the ethylacetate extract glycosides, flavonoids and terpenoids were found. Tannins, glycosides and saponins were found in the methanolic leaf extracts of *D.ferrea*.

GC-MS phytochemical anaylisis of *D.ferrea* leaf extracts resulted in the identification of different compounds. The three solvent leaf extracts differ in the number of compounds identified in each extract. A maximum of seventeen compounds were identified in methanolic leaf extract, where as sixteen compounds in ethyl acetate solvent and only seven compounds in hexane solvent were observed. Phytol, friedelan-3-one, squalene, tocopherol, α-amyrin, betulin, citronellol, pregnenolene, olen and thunbergol are the important phytoconstituents identified from leaf extracts. These compounds are grouped under hydrocarbons, terpenes and phenolic compounds.

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