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Journal of Applied Pharmaceutical Science

ISSN: 2231-3354 Received on: 18-01-2012 Revised on: 24-01-2012 Accepted on: 06-02-2012

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GC-MS Analysis of Bioactive Components of *Feronia elephantum* Correa (Rutaceae)

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ABSTRACT

Feronia elephantum belongs to the family Rutaceae is well known in Indian traditional system for its traditional uses. The present investigation was carried out to determine the possible bioactive components of leaves and bark of F. elephantum using GC-MS analysis. 18 components from leaves and 14 components from bark of the above said plant were identified. The prevailing compounds in the ethanol extract of leaves of F. elephantum were 7-Norbornadienyl t-butyl ether (17.26%) , 2-isopropyl-5-methyl-1- heptanol (11.40%), 1-(8.47%), Phenol, 4–[2–(dimethylamino)–ethyl]– 2.3 -Octanol.2-butvl (4.56%). Dimethylquinolin-4(1H)-one (3.58%), Ethyl iso-allocholate (1.63%). The ethanol extract of F. elephantum bark contained, 2-Propenenitrile, 3-(3.4-dimethoxyphenyl)-(60.72%) was found as major component followed by phenol, 4-(3-hydroxy-I-propenyl)-2-methoxy-(9.35%), 3-(2-N-Acetyl-N-methylaminoethyl)indol (1.15%), cholesta-8,24-dine-3-ol, 4-methyl-(3a'- 4a')-(0.86%) as the major components.

Keywords: F. elephantum, wood apple, GC-MS, bioactive components.

INTRODUCTION

From ancient days to recent civilization, human beings depend on nature for running their life smoothly from day to day. Plants remain a vital source of drugs and now-a- days much emphasis has been given to nutraceuticals. *F. elephantum* is one of the medicinally important plants belonging to Rutaceae, commonly known as wood apple. Various parts of wood – apple have been used against various ailments in ethnomedicine. Juice of young leaves is mixed with milk and sugar candy given as remedy for biliousness and intensive troubles of children. A powdered gum mixed with honey, is given to overcome dysentery and diarrhea in children. The leaves are used traditionally in Ayurveda as antiemetic, aromatic, expectorant, purgative, useful in anorexia, bronchitis, calculus, cardiac debility, cough, gastropathy, hiccup and in vitiated conditions of vayu (Gill *et al.*, 1998; Parajapat *et al.*, 2003).



Fruit pulp is sour, sweet, edible stomachic. The pulp is applied externally as a remedy for the bites of venous insects. Fruit pulp is also used by tribal of Rewa District of Madhya Pradesh against boils and amoebiasis. The bark is also aromatic and cooling, and useful in vitiated conditions of pitta. The bark is occasionally prescribed for biliousness and useful in liver diseases (Priya, 2000; Mohammed and Anwar, 2003). Taking into consideration of the medicinal importance of this plant, the ethanol extract of leaves and bark of *F. elephantum* were analyzed for the first time using GC – MS. This work will help to identify the compounds of therapeutic value. GC – MS is the best technique to identify the bioactive constituents of long chain hydrocarbons, alcohols, acids, ester, alkaloids, steroids, amino and nitro compound etc.

MATERIALS AND METHODS

Collection of plant material

Leaves and bark of *Feronia elephantum* Correa were collected in the month of February and March 2011 from the Agasthiarmalai Biosphere Reserve, Western Ghats, Tamil Nadu. The plant were identified with help of local flora, Voucher specimen preserved in the Ethnopharmacology Unit, Research Department of Botany, V.O.Chidambaram College, Tuticorin – 628 008, Tamil Nadu, India.

Preparation of powder and extract

Leaves and barks were cleaned, shade dried and pulverized to powder in a mechanical grinder. Required quantity of powder of leaves and bark were weighed respectively and transferred to Stoppard flask, and treated with ethanol until the powder is fully immersed. The flask was shaken every hour for the first 6 hrs and then it was kept aside and again shaken after 24 hrs. This process was repeated for 3 days and then the extract was filtered. The extract was collected and evaporated to dryness by using vacuum distillation unit. The final residue thus obtained was then subjected to GC – MS analysis.

GC - MS analysis

GC -MS analysis were carried out on a GC Clarus 500 Perkin Elmer system and Gas Chromatograph interfaced to a Mass Spectrometer (GC – MS) instrument employing the following conditions: column Elite – 1 fused silica capillary column (30m x 0.25 mm 1D X 1 µ df, composed of 100% Dimethyl Poly Siloxane). For GC – MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1 ml / min. and an injection volume of 2 μ l was employed (split ratio of 10 : 1) injector temperature-250° C; ion - source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min) with an increase of 10°C /min to 200°C/min, then 5°C/ min to 280°C /min, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5s and fragments from 40 to 550Da. Total GC running time was 36 minutes. The relative % amount of each component was calculated by comparing its average peak area to the total areas, software adopted to handle mass spectra and chromatogram was a Turbomass.

Identification of Components

Interpretation on mass spectrum of GC – MS was done using the database of National Institute of standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

RESULTS AND DISCUSSION

GC - MS analysis

The components present in the ethanol extracts of leaves and bark of *F. elephantum* was identified by GC - MS (Figures 1 and 2).

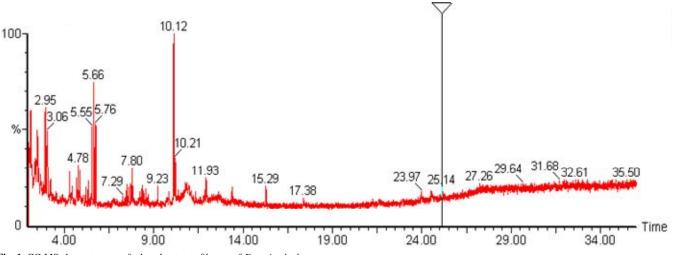


Fig. 1: GC-MS chromatogram of ethanol extract of leaves of Feronia elephantum.

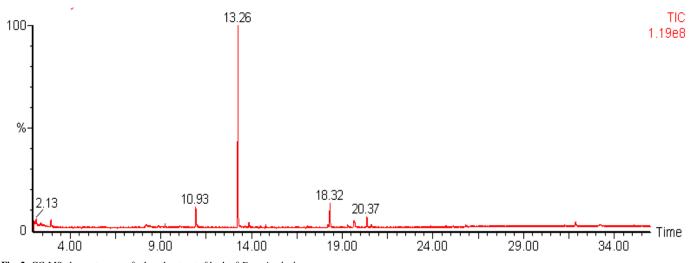


Fig. 2: GC-MS chromatogram of ethanol extract of bark of Feronia elephantum.

Table. 1: Components	detected in	Feronia	elephantum	leaf extract.
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S.No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1	2.50	Octane, 3,5-dimethyl-	C ₁₀ H ₂₂	142	10.42
2	2.95	Propane, 1,1,3-triethoxy-	C9H20O3	176	10.42
3	4.78	Dodecane	C ₁₂ H ₂₆	170	3.26
4	5.35	Octadecane,3-ethyl-5-(2-ethylbutyl)-	C26H54	366	2.93
5	5.55	9-Octadecene, (E)-	C18H36	252	8.14
6	5.66	2-Isopropyl-5-methyl-1-heptanol	C ₁₁ H ₂₄ O	172	11.40
7	5.76	1-Octanol, 2-butyl-	C ₁₂ H ₂₆ O	186	8.47
8	7.80	Phenol, 4-[2-(dimethylamino)ethyl]-	C ₁₀ H ₁₅ NO	165	4.56
9	8.36	2-Nonadecanone2, 4-dinitrophenylhydrazine	C25H42N4O4	462	1.95
10	9.23	9,12,15-Octadecatrienoic acid, 2-phenyl-1, 3-dioxan-5-yl ester	C ₂₈ H ₄₀ O ₄	440	2.93
11	10.12	7-Norbornadienyl t-butyl ether	C ₁₁ H ₁₆ O	164	17.26
12	10.21	9-Octadecen-12-ynoic acid, methyl ester	C ₁₉ H ₃₂ O ₂	292	3.58
13	11.93	2,3-Dimethylquinolin-4(1H)-one	C ₁₁ H ₁₁ NO	173	3.58
14	13.39	Octadecanoic acid, 4-hydroxybutyl ester	C22H44O3	356	2.61
15	15.29	2-Nonadecanone2,4-dinitrophenyl- hydrazine	C25H42N4O4	462	2.61
16	17.38	Ethyl iso-allocholate	C ₂₆ H ₄₄ O ₅	436	1.63
17	23.97	1,1 Cyclobutanedi carboxamide, 2-phenyl-N,N'-bis(1-phenylethyl)-	C ₂₈ H ₃₀ N ₂ O ₂	426	2.28
18	25.14	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-, (E,E)-	C ₁₅ H ₂₆ O	222	1.95

Table. 2: Components detected in Feronia elephantum bark extract.

S. No	RT	Name of the compound	Molecular Formua	MW	Peak Area %
1	2.94	Propane, 1,1-diethoxy-	C7H16O2	132	2.37
2	9.23	Propane, 1,1,3-triethoxy-	C9H20O3	176	1.15
3	10.93	Phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy-	C10H12O3	180	9.35
4	13.26	2-Propenenitrile, 3-(3,4-dimethoxyphenyl)-	C ₁₁ H ₁₁ NO ₂	189	60.72
5	18.32	Columbianetin	C ₁₄ H ₁₄ O ₄	246	9.78
6	19.31	3-(2-N-Acetyl-N-methylaminoethyl)indol	C ₁₃ H ₁₆ N ₂ O	216	1.15
7	19.63	Tricyclo[4.2.1.1(2,5)]dec-3-ene-9,10-dione	C ₁₀ H ₁₀ O ₂	162	2.30
8	20.37	Tetracyclo[6.3.0.0(2,11).0(3,7)undecane-5,10-dione, 7-methyl-3-[(2-methoxyethoxy) methoxy]-	C ₁₆ H ₂₂ O ₅	294	4.03
9	20.61	Tryptamine, N-[4-hydroxyhydrocinnamoyl]-	C ₁₉ H ₂₀ N ₂ O ₂	308	1.58
10	23.89	Sarreroside	C ₃₀ H ₄₂ O ₁₀	562	2.54
11	24.72	9,12,15-Octadecatrienoic acid, 2-phenyl-1,3-dioxan-5-yl ester	C ₂₈ H ₄₀ O ₄	440	0.58
12	25.14	Cholesta-8,24-dien-3-ol, 4-methyl-, (3á,4à)-	C ₂₈ H ₄₆ O	398	0.86
13	31.87	24,25-Dihydroxyvitamin D3	C ₂₇ H ₄₄ O ₃	416	1.87
14	33.22	Ethyl iso-allocholate	C ₂₆ H ₄₄ O ₅	436	1.72

The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) in the ethanol extracts of leaves and bark of F. elephantum are presented in Tables 1 and 2. Eighteen compounds were identified in the ethanol extracts of leaves of F. elephantum. The prevailing compounds were 7-Norbornadienyl t-butyl ether (17.26%), 2isopropyl-5-methyl-1- heptanol (11.40%), 1-Octanol,2-butyl (8.47%), Phenol, 4-[2-(dimethylamino)-ethyl]- (4.56%), 2,3-Dimethylquinolin-4(1H)-one (3.58%), Ethyl iso-allocholate (1.63%). Fig 3 and 4 shows the mass spectrum and structures of iso-allocholate, 2-Nonadecanone Ethyl 2,4dinitrophenylhydrazine. Fourteen compounds were identified in the ethanol extract of bark of F. elephantum.

The results revealed that, 2–Propenenitrile, 3–(3.4dimethoxyphenyl)–(60.72%) was found as major component followed by phenol, 4–(3-hydroxy-I-propenyl)-2-methoxy– (9.35%), 3-(2-N-Acetyl–N-methylaminoethyl)indol (1.15%), cholesta–8,24–dine–3–ol, 4–methyl–(3a'- 4a')– (0.86%) in the ethanol extract of bark of *F. elephantum*.

Fig 5, 6 and 7 shows the mass spectrum and structures of cholesta-8, 24-dien-3-ol, 4- methyl- (3a', 4a'), 2-propenenitrile, 3-(3,4-dimethoxyphenyl)-, phenol, 4- (3- hydroxyl- 1- propenyl) -2-methoxy-respectively.

Major phytocompounds and its biological activities obtained through the GC–MS study of leaves and bark of F. *elephantum* have been tabulated (Table 3 and 4).

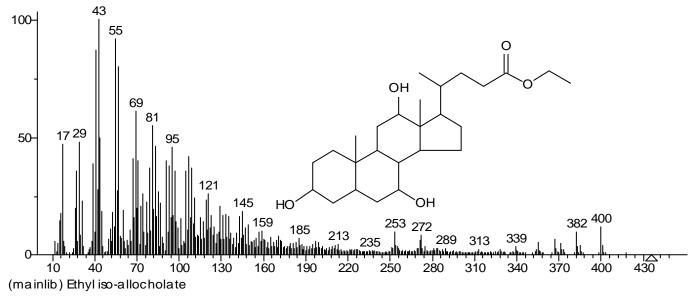


Fig. 3: Mass Spectrum of Ethyl iso-allocholate (RT:17.38).

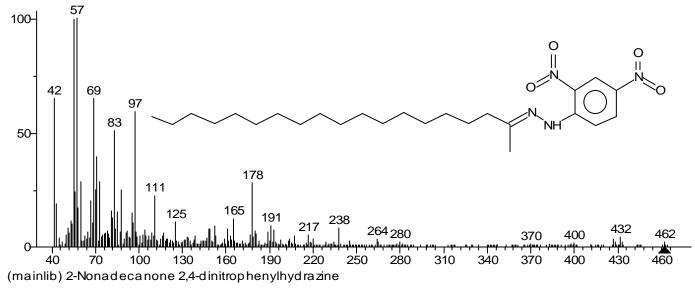


Fig. 4: Mass Spectrum of 2-Nonadecanone 2,4-dinitropheylhydrazine(RT: 8.36).

Table. 3: Activity of phytocomponents identified in the ethanol leaf extract of Feronia elephantum.

S.No	Name of the compound	Nature of compound	**Activity
1	2-Isopropyl-5-methyl-1-heptanol	Alcoholic compound	Antimicrobial
2	1-Octanol, 2-butyl-	Alcoholic compound	Antimicrobial
3	Phenol,4-[2-(dimethylamino)	Phenolic compound	Antimicrobial
	ethyl] -	*	Antioxidant
			Anti-inflammatory
4	2-Nonadecanone2,4-initrophenyl hydrazine	Nitrogen compound	Antimicrobial
5	2,3-Dimethylquinolin-4(1H)-one	Alkaloid	Antimicrobial
			Anti-inflammatory
6	Ethyl iso-allocholate	Steroid	Antimicrobial
			Diuretic
			Anti-inflammatory
			Antiasthma
7	1,1-Cyclobutanedicarboxamide, 2-phenyl-N,N'-bis(1-phenylethyl)-	Amino compound	Antimicrobial
8	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-, (E,E)-	Alcoholic compound	Antimicrobial

**Activity Source: Dr.Duke's Phytochemical and Ethnobotanical Databases

Table. 4: Activity of phytocomponents identified in the ethanol bark ex	stract of Feronia elephantum.
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S.No	S.No Name of the compound Nature of compound		*Activity
1	Phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy-	Phenolic compound	Antimicrobial
			Antioxidant
			Anti-inflammatory
2	2-Propenenitrile, 3-(3, 4-dimethoxy-phenyl)-	Nitrogen compound	Antimicrobial
3	Columbianetin	Ketone compound	Colour pigment
4	3-(2-N-Acetyl-N-methylaminoethyl)indol	Steroid	Antimicrobial
			Diuretic
			Anti-inflammatory
			Antiasthma
5	Tryptamine, N-[4-hydroxyhydro- cinnamoyl]-	Amino compound	Antimicrobial
6	Cholesta-8,24-dien-3-ol,4-methyl-, (3á,4à)-	Steroid	Antimicrobial
			Diuretic
			Anti-inflammatory
			Antiasthma
7	24,25-Dihydroxyvitamin D3	Vitamin compound	Nutrient
8	Ethyl iso-allocholate	Steroid	Antimicrobial
	-		Diuretic
			Anti-inflammatory
			Antiasthma

**Activity Source: Dr.Duke's Phytochemical and Ethnobotanical Databases.

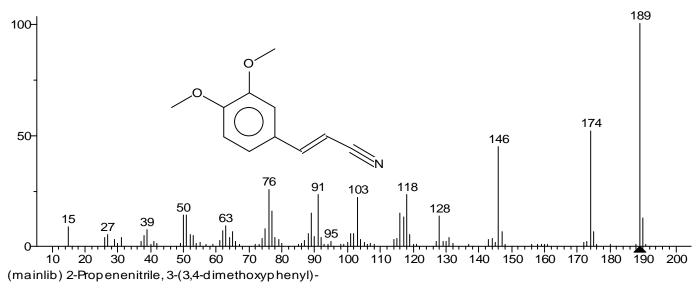


Fig. 5: Mass Spectrum of Cholesta-8,24-dien-3-ol,4-methyl-,(3a',4a')-

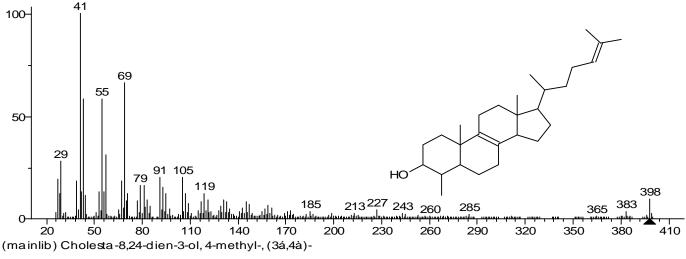
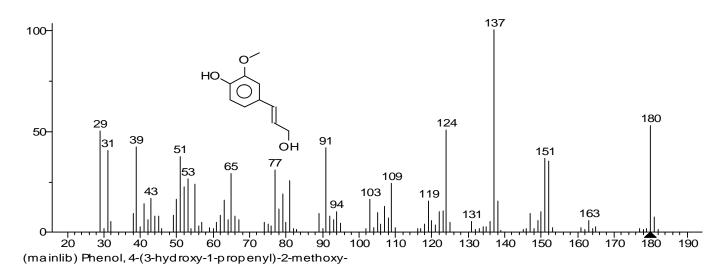


Fig. 6: Mass Spectrum of 2-propenenitnle, 3-(3-4-dimethoxyphenyl)-



CONCLUSION

In the present study, 18 components from leaves and 22 components from bark of the *F. elephantum*, were identified by Gas Chromatography– Mass Spectrometry (GC – MS) analysis. The presence of various bioactive compounds justifies the use of this plant for various ailments by traditional practitioners. However, isolation of individual photochemical constituents and subjecting it to biological activity will definitely give fruitful results. It could be concluded that, *F. elephantum* contains various bioactive compounds. So it is recommended as a plant of phytopharmaceutical importance. However, further studies are needed to undertake its bioactivity and toxicity profile.

ACKNOWLEDGEMENT

We would like to thank Mr. S. Kumaravel, Senior Scientist, Indian Institute of Crop Processing Technology (Ministry of Food Processing Industries, Government of India), Tanjavore, Tamil Nadu for providing all the facilities and support used to carry out the work.

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