Research Article

GC-MS Analysis of Bioactive Components from the Ethanolic Leaf Extract of *Flueggea leucopyrus* Wild

Rajeswari B, Muthuirulappan Srinivasan*
Department of Biochemistry, Centre for Research and Development, PRIST University, Thanjavur, Tamil Nadu, India.
***Corresponding author's E-mail:** sriniclic@gmail.com

Accepted on: 23-05-2015; **Finalized on:** 30-06-2015.

ABSTRACT

The bioactive components of *Flueggea leucopyrus* leaves have been evaluated using GC/MS. The chemical compositions of the ethanolic extract of *Flueggea leucopyrus* leaves were investigated using Perkin-Elmer Gas Chromatography–Mass Spectrometry, while the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library. GC/MS analysis of ethanolic extract of *Flueggea leucopyrus* leaves revealed the existence of 9-Octadecenoic ACID (Z) (20.547), I-(+)-Ascorbic acid 2,6-dihexadecanoate (22.030), Heptadecanoic (23.09), 9-Octadecenoic acid, methyl ester, (E) (23.550), 9,12-Octadecadienoic acid (Z,Z) (25.042), Oleic Acid $$ 9-Octadecenoic (26.800). The results of this study offer a platform of using *Flueggea leucopyrus* leaves as herbal alternative for various diseases.

Keywords: *Flueggea leucopyrus*, GC/MS, Bioactive components.

INTRODUCTION

Phytochemistry or plant chemistry has developed in recent years as a distinct discipline, somewhere in between natural product organic chemistry and plant biochemistry and is closely related to both. It is concerned with the enormous variety of organic substances that are elaborated with and accumulated by plants and deals with the chemical structures of these substances, their biosynthesis, turn over and metabolism, their natural distribution and their biological function[1]. India is called the botanical garden of the world for its rich natural resources. Over 6,000 plants in India are used in traditional, folklore and herbal medicine. The Indian system of medicine has identified 1500 medicinal plants of which 500 are commonly used[2].

Phytochemicals are the chemicals extracted from plants. These organic chemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Primary constituents include the common sugars, aminoacids, proteins, purines and pyrimidines of nucleic acids, chlrophyll's etc. Secondary constituents are the remaining plant chemicals such as alkaloids (derived from aminoacids), terpenes (a group of lipids) and phenolics (derived from carbohydrates)[3]. Plant produces these chemicals to protect itself but recent research demonstrates that emphasizes the plant source of most of these protective, disease-preventing compounds. A true nutritional role for phytochemicals is becoming more probable every day as research uncovers more of their remarkable benefits[4].

Within a decade, there was a number of dramatic advances in analytical techniques including TLC, UV, NMR and GC-MS that were powerful tools for separation identification and structure determination of phytochemicals[5]. The aim of this study is to determine the organic compounds present in the *Flueggea leucopyrus* leaves extract with the aid of GC-MS Technique, which may provide an insight in its use in tradition medicine.

MATERIALS AND METHODS

Plant Materials

The fully mature *Flueggea leucopyrus* leaves were collected in April 2013 from Tamil University, Thanjavur District, Tamil Nadu, India from a single herb. The leaves were identified and authenticated by Botanist, Dr. M. Jagadeesan, M.Sc., Ph.D., Department of environmental and herbal science, Tamil University, Thanjavur, Tamil nadu, India. A Voucher specimen has been deposited at the Herbarium, Tamil University, Tamil Nadu, India.

Plant Sample Extraction

The collected *Flueggea leucopyrus* leaves were washed several times with distilled water to remove the traces of impurities from the leaves. The leaves were dried at room temperature and coarsely powdered. The powder was extracted with 70% ethanol for 48 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The *Flueggea leucopyrus* leaves extract was stored in refrigerator until used. Preliminary phytochemical tests were carried out on the ehtanolic extract of *Flueggea leucopyrus* leaves using standard procedures to identify the constituents as described by Sofowara[6], Trease and Evans[7] and Harborne[8,9].

GC –MS Analysis

GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column

Elite-1 fused silica capillary column (30 x 0.25mm ID x 1µMdf, composed of 100% Dimethyl polydiloxane), operating in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1 ml /min and an injection volume of 0.5 µl 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, then 5°C/min to 280°C, ending with a 9min isothermal at 280°C. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time is 36min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a TurboMass Ver 5.2.0.

RESULTS AND DISCUSSION

Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen substituted derivatives. Most are secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total. These substances serve as plant defense mechanisms against, insects and herbivores. Flavonoids exhibit several biological effects such as anti-inflammatory, anti-fungal, anti-hepatotoxic and anti-ulcer actions[10].

Identification of Components

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The 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.

GC-MS Analysis

Nine compounds were identified in *Flueggea leucopyrus* leaves by GC-MS analysis. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in (Table 1 and Fig 1). The prevailing compounds were 9-Octadecenoic acid (Z) (20.547), l-(+)-Ascorbic acid 2,6-dihexadecanoate (22.030), Heptadecanoic (23.09), 9-Octadecenoic acid, methyl ester, (E) (23.550), 9,12-Octadecadienoic acid (Z,Z) (25.042), Oleic Acid $$ 9-Octadecenoic (26.800). The biological activities listed (Table 2) are based on Dr. Duke's phytochemical and ethnobotanical Databases by Dr. Jim Duke of the Agricultural Research Service/USDA.

In the present study twenty chemical constituents have been identified from ethanolic extract of the plant of *Flueggea leucopyrus* by Gas Chromatogram- Mass spectrometry (GC-MS) analysis. The presence of various bioactive compounds justifies the use of the whole plant for various ailments by traditional practitioners. However isolation of individual phytochemical constituents and subjecting it to biological activity will definitely give fruitful results.

It could be concluded that *Flueggea leucopyrus* contains various bioactive compounds. So it is recommended as a plant of phytopharmaceutical importance. However, further studies will need to be undertaken to ascertain fully its bioactivity, toxicity profile, effects on the biological system and agricultural products.

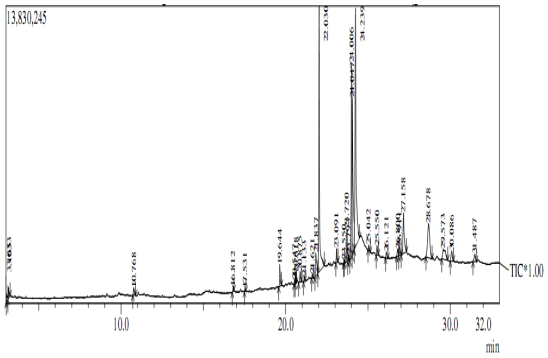

Figure 1: Analysis of bioactive compounds in *Flueggea leucopyrus* leaf by GC MS

Table 1: Analysis of bioactive compounds in *Flueggea leucopyrus* leaf by GC MS

Peak	R.Time	Area%	Name of Compound	Molecular Formula	Molecular Weight
1	3.033	0.52	Imidazole, 2-amino-5-[(2-carboxy)vinyl]	$C_6H_7N_3O_2$	153
2	3.165	0.73	1H-Pyrrole, 1-methyl	C_5H_7N	81
3	10.768	0.53	Benzofuran, 2,3-dihydro	C_8H_8O	120
4	16.812	0.29	Dodecanoic acid	$C_{12}H_{24}O_2$	200
5	17.531	0.15	Diethyl Phthalate	$C_{12}H_{14}O_4$	222
6	19.644	1.33	Tetradecanoic acid	$C_{24}H_{48}O_2$	368
7	20.547	0.19	9-Octadecenoic acid (Z)	$C_{18}H_{36}O_2$	284
8	20.618	0.54	2,6,10-Trimethyl,14-ethylene-14-pen	$C_{20}H_{38}$	278
9	20.875	0.76	Pentadecanoic acid	$C_{15}H_{30}O_2$	242
10	21.133	0.27	Cyclopropanenonanoic acid, 2-[(2-butylcyclopr	C_7H_{12}	96
11	21.621	0.21	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270
12	21.837	2.38	Cyclopentadecanone, 2-hydroxy	$C_8H_{12}O_2$	140

13	22.030	19.31	l-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	652
14	23.091	0.82	Heptadecanoic acid	$C_{17}H_{34}O_2$	270
15	23.550	0.18	9-Octadecenoic acid, methyl ester, (E)	$C_{18}H_{34}O_2$	282
16	23.720	2.24	2-Hexadecen-1-OL, 3,7,11,15-tetrame	$C_{20}H_{40}O$	296
17	23.793	0.33	Methyl stearate	$C_{19}H_{38}O_2$	298
18	24.006	15.29	9-Octadecenoic acid	$C_{18}H_{34}O_2$	282
19	24.047	12.40	9,12,15-Octadecatrienoic acid	$C_{18}H_{32}O$	264
20	24.239	23.02	Octadecanoic acid	$C_{18}H_{36}O_2$	284
21	25.042	0.31	9,12-Octadecadienoic acid (Z,Z)	$C_{18}H_{32}O_2$	280
22	26.121	0.70	Nonadecanoic acid	$C_{19}H_{36}O_2$	296
23	26.121	0.50	Hexadecanoic acid, 2-hydroxy-1,3-	$C_{37}H_{74}NO_8P$	691
24	26.800	0.75	Oleic Acid $$ 9-Octadecenoic	$C_{18}H_{34}O_2$	282
25	26.911	0.83	Cyclopentadecanone, 2-hydroxy-	C12H20	164
26	27.158	4.32	Icosanoic acid	$C_{20}H_{40}O_2$	321
27	28.678	6.62	Solanesol	$C_{45}H_{74}O$	360
28	29.573	2.47	1,5,9-Decatriene, 2,3,5,8-tetramethyl	$C_{14}H_{24}$	192
29	30.086	1.10	Glycidol stearate	$C_{21}H_{40}O_3$	340
30	31.487	0.90	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390

Table 2: Biological activity of compounds in *Flueggea leucopyrus* leaf extract

S.No.	R. Time	Area%	Name of the Compound	Molecular Formula	Molecular Weight	Biological Activity**
1.	19.644	1.33	Tetradecanoic acid	$C_{24}H_{48}O_2$	368	Antioxidant, Lubricant, Hypercholesterolemic, Cancer_preventive, Cosmetic.
2.	22.030	19.31	l-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	652	Anticancer, Antioxidant, Cardioprotective, Antiarthritis, Antiageing, Hypolipidemic
3.	23.091	0.82	Heptadecanoic acid	$C_{17}H_{34}O_2$	270	Antioxidant
4.	23.550	0.18	9-Octadecenoic acid, methyl ester, (E)	$C_{18}H_{34}O_2$	282	Antioxidant, hypocholesterolemic nematicide, pesticide, anti-androgenic flavor, hemolytic, 5-Alpha reductase inhibitor
5.	24.047	12.40	9,12,15-Octadecatrienoic acid	$C_{18}H_{32}O$	264	Antibacterial Antifungal
6.	24.239	23.02	Octadecanoic acid	$C_{18}H_{36}O_2$	284	Cosmetic, Flavor, Hypocholesterolemic, Lubricant, Perfumery, Prophetic, Suppository
7.	25.042	0.31	9,12-Octadecadienoic acid (Z,Z)	$C_{18}H_{32}O_2$	280	Antiarteriosclerotic, Antiarthritic, antianaphylactic, Antieczemic, Cancer preventive, antiprostatic, hepatoprotective, Hypocholesterolemic, Metastatic, Nematicide
8.	26.800	0.75	Oleic Acid $$ 9-Octadecenoic	$C_{18}H_{34}O_2$	282	5-Alpha-Reductase-Inhibitor, Allergenic, Alpha-Reductase-Inhibitor, Anemiagenic, Antialopecic, Antiandrogenic, Antiinflammatory, Antileukotriene-D4 (Anti-platelet activating factor), Cancer-Preventive, Choleretic, Dermatitigenic Flavor, Hypocholesterolemic, Insectifuge Irritant, Percutaneostimulant, Perfumery, Propecic

**Source: Dr. Duke's phytochemical and ethnobotanical database (online database)

Acknowledgement: The authors are grateful to Dr. S. Velavan, Director, Harman Institute of Science Education and Research (www.harmanresearchcentre.com), Thanjavur, Tamil Nadu for providing necessary facility to complete this work.

REFERENCES

1. Harborne JB. Plant flavonoids in biology and medicine: Biochemical pharmacological, and structure–activity relationships. NY, USA: Alan R. Liss. 1986, 15–24.
2. Agrawal OP, Raju PS. Global market of herbal products: Opportunities for Indian Traditional System of Medicine. New Delhi, India, Narcosa Publishing House, 2006, 5-10.
3. Liu RH. Potential synergy of phytochemicals in cancer prevention: Mechanism of action. Journal of Nutrition, 134(12 Suppl.), 2004, p3479S–3485S.
4. Hamburger M, Hostettmann, K. Bioactivity in plants: the link between phytochemistry and medicine. Phytochemistry. 30, 1991, 3864È74.
5. Roberts JKM, Xia JH. High-resolution NMR methods for study of higher plants, Methods Cell Biol. 49, 1995, 245–258.
6. Sofowara A. Medicinal plants and Traditional medicine in Africa. Spectrum Books Ltd, Ibadan, Nigeria. 1993, 191-289.
7. Trease GE, Evans WC. Phenols and Phenolic glycosides. In: Textbook of Pharmacognosy. (12th ed.). Balliese, Tindall and Co Publishers, London. 1989, 343-383.
8. Harborne JB. Phytochemical methods, London. Chapman and Hall, Ltd. 1973, 49-188.
9. Harborne JB. Phytochemical Methods. A Guide to Modern Technique of Plant Analysis. London: Chapman and Hall. 1984, 78-210.
10. de-Fatima A, Modolo LV, Conegero LS, Pilli RA, Ferreira CV, Kohn LK, de-Carvalho JE. Lactones and their derivatives: biological activities, mechanisms of action and potential leads for drug design. Curr. Med. Chem. 13, 2006, 3371-3384.

Source of Support: Nil, **Conflict of Interest:** None.