Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(5): 157-160
Received: 25-07-2019
Accepted: 27-08-2019

R Shyamala
Research Scholar, P.G and Research Department of Chemistry, AVVM Sri Pushpam College, Poondi, Thanjavur, Tamil Nadu India

R Manikandan
Associate Professor, P.G and Research Department of Chemistry, AVVM Sri Pushpam College, Poondi, Thanjavur, Tamil Nadu India

Correspondence
R Manikandan
Associate Professor, P.G and Research Department of Chemistry, AVVM Sri Pushpam College, Poondi, Thanjavur, Tamil Nadu India

Determination of bioactive compounds in *Ziziphus oenoplia* leaves extract using gas chromatography and mass spectroscopic technique

R Shyamala and R Manikandan

Abstract
Phytochemicals are the chemicals extracted from plants. These organic chemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. GC-MS method used for the analysis of the obtained extract can be an interesting tool for testing the amount of some active principles in herbs used in various industries. The aim of this study was to carry out for identification of bioactive compounds from ethanolic extract of *Ziziphus oenoplia* leaves by Gas chromatography and Mass spectroscopy (GC-MS). GCMS analysis of ethanolic extract was done by standard protocol using the equipment 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. The GC-MS analysis of *Ziziphus oenoplia* leaves extract revealed the presence of various compounds like Heptadecane, Hexadecanoic acid, ethyl ester, Pentadecanoic acid, 2-Hexadecen-1-ol, 3,7,11,15-Tetramethyl, 1,2-Benzenedicarboxylic acid, dihexyl ester and 9-Octadecenoic acid. These findings support the traditional use of *Ziziphus oenoplia* leaves in various disorders.

Keywords: Gas chromatography and mass spectroscopy, *Ziziphus oenoplia* leaves, phytochemistry

1. 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 (Harborne, 1986) [8]. Plants have been an important source of medicine with qualities for thousands of years. Plants are used medicinally in different countries, and they are the source of many potent and powerful drugs. Mainly on traditional remedies such as herbs for their history, they have been used as popular folk medicines (Sathyaprabha *et al.*, 2010) [16]. It has been shown that *in vitro* screening methods could provide the needed preliminary observations necessary to elect crude plant extracts with potentially useful properties for further chemical and pharmacological investigations (Mathekaga and Meyer, 1998) [12].

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) (Liu, 2004) [11]. 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 (Hamburger and Hostettmann, 1991) [7]. Within a decade, there were a number of dramatic advances in analytical techniques including TLC, UV, NMR and GC-MS that were powerful tools for separation, identification and structural determination of phytochemicals (Roberts and Xia, 1995) [13].

Gas Chromatography Mass Spectroscopy (GC-MS) a hyphenated system which is a very compatible technique and the most commonly used technique for the identification and quantification of biochemical components of medicinal plants (Ronald Hites, 1997) [14]. The chosen medicinal plant namely as *Ziziphus oenoplia* leaves belongs to Rhamnaceae Family. *Ziziphus oenoplia* leaves it is widely distributed from the Indian subcontinent through southern China and Southeast Asia to northern Australia. It grows along roadside forests and thickets.

The aim of this study is to determine the organic compounds present in *Ziziphus oenoplia* leaves with the aid of GC-MS Technique.

2. Material and Methods

2.1 Plant materials: The *Ziziphus oenoplia* leaves were collected from Thanjavur district, Tamil Nadu, India from a herb. The plant were identified and authenticated by Dr. S. John Britto, The Director, the Rapinat Herbarium and center for molecular systematics, St. Joseph's college Trichy-Tamil Nadu. India. A Voucher specimen has been deposited at the Rabinat Herbarium, St. Josephs College, Thiruchirappalli, Tamil nadu, India.

2.2 Preparation of extracts:

The collected *Ziziphus oenoplia* leaves were washed several times with distilled water to remove the traces of impurities from the leaves. Then examined carefully old, infected and fungus damaged portion of the leaves were removed. Healthy leaves were spread out in a plain paper and shade dried at room temperature for about 10 days and ground in to fine powder using mechanical grinder. The powder was extracted with ethanol for 24 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The *Ziziphus oenoplia* leaves extract was stored in refrigerator until used.

2.3 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 µI was employed (split ratio of 10:1) injector temperature 250 ºC; ion-source temperature 280 ºC. The oven temperature was programmed from 110 ºC (isothermal for 2 min), with an increase of 10 ºC/min, to 200 ºC, then 5 ºC/min to 280 ºC, ending with a 9min isothermal at 280 ºC. 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. min. 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

3. Results and discussion

Gas chromatography – mass spectrometry (GC-MS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample (Kell *et al*., 2005) [10]. In the last few years, GC-MS has become firmly established as a key technological platform for secondary metabolite profiling in both plant and non-plant species (Fernie *et al*., 2004) [6]. 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 (De-Fatima *et al*., 2006)[4]. 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.

Twenty compounds were identified in *Ziziphus oenoplia* 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 Heptadecane, Hexadecanoic acid, ethyl ester, Pentadecanoic acid, 2-Hexadecen-1-Ol, 3, 7, 11, 15-Tetramethyl, 1, 2-Benzenedicarboxylic acid, dihexyl ester and 9-Octadecenoic acid. The biological activities of selected compounds were listed (Table 2) are based on Dr.Duke's Phytochemical and Ethnobotanical Databases by Dr. Jim Duke of the Agricultural Research Service/USDA.

Among the identified phytochemicals hexadecanoic acid is suggested to be a Hexadecanoic acid, ethyl ester and it may employed as antioxidant, antimicrobial, flavor, hypocholesterolemic agent and larvicidal activities (Bodoprost and Rosemeyer, 2007; Falodun *et al*., 2009) [3, 5]. 1, 2- benzenedicarboxylic acid, dihexyl ester is a plasticizer compound and acts as antimicrobial and antifouling agent (Heinonen *et al*., 1998) [9].

Compounds like n-hexadecanoic acid, 12-octadecanoic acid, dodecanoic acid, tetradecanoic acid, 1,2-Benzenedicarboxylic acid, dibutyl ester, hexadecanoic acid, ethyl ester and 9,12-octadecadienoic acid (Z,Z) were identified in the ethanolic leaf extract of *Vitex altissima*, a Verbenaceae member (Sathish *et al*., 2012) [15]. Likewise, hexadecane, dodecanoic acid, nonadecane, eicosane, tetradecanoic acid, oleic acid, heptacosane, 9,12- octadecenoic acid, ethyl ester; n-hexadecanoic acid; 1,2-benzenedicarboxylic acid and 9-octadecenoic acid (Z)-ethyl ester were reported in *Clerodendrum inerme* and *C. phlomidis* leaves (Anandhi and Ushadevi, 2013; Balaji and Kilimozhi, 2014) [1, 2].

The investigation concluded that the stronger extraction capacity of ethanol could have been produced number of active constituents responsible for many biological activities. So that those might be utilized for the development of traditional medicines and further investigation needs to elute novel active compounds from the medicinal plants which may be created a new way to treat many incurable diseases.

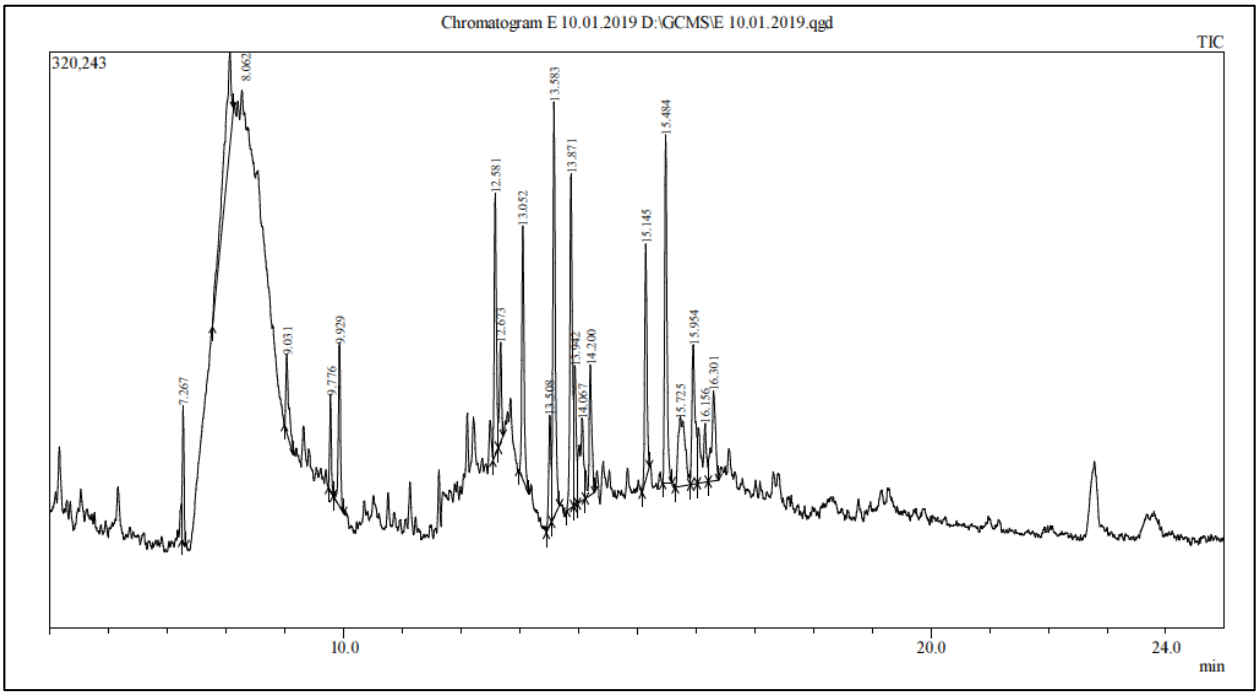

Fig 1: GC-MS Chromatogram of *Ziziphus oenoplia* leaves extract

Table 1: Identification of bioactive compounds in ethanolic extract of *Ziziphus oenoplia* leaves using GC MS

Peak#	R.Time	Area %	Molecular formula	Molecular weight	Name of the compound
1	7.267	2.38	$C_{17}H_{36}$	240	Heptadecane
2	8.062	8.77	$C_{12}H_{26}O$	186	1-Octanol, 2-butyl-
3	9.031	2.44	$C_{15}H_{24}$	204	Beta.-Farnesene
4	9.776	1.68	$C_{17}H_{36}$	240	Heptadecane
5	9.929	3.76	$C_{20}H_{26}O_4$	330	1,2-Benzoldicarbonsaeure,
6	12.581	6.25	$C_{20}H_{38}$	278	3-Eicosyne
7	12.673	2.24	$C_{12}H_{18}O$	178	6-Dodecanone
8	13.052	6.29	$C_{18}H_{36}O$	268	9-Octadecen-1-ol
9	13.508	2.82	$C_{12}H_{26}O$	186	1-Octanol, 2-butyl
10	13.583	10.71	$C_{14}H_{22}N_2O$	234	Xycaine
11	13.871	9.25	$C_{15}H_{30}O_2$	242	Pentadecanoic acid
12	13.942	3.44	$C_{14}H_{30}$	198	Dodecane, 4,6-dimethyl
13	14.067	3.85	$C_{20}H_{30}O_4$	334	1,2-Benzenedicarboxylic acid, dihexyl ester
14	14.200	3.80	$C_{18}H_{36}O_2$	284	Hexadecanoic acid, ethyl ester
15	15.145	5.70	$C_{18}H_{36}$	252	9-Octadecene
16	15.484	8.80	$C_{20}H_{40}O$	296	2-Hexadecen-1-ol,3,7,11,15-Tetramethyl
17	15.725	5.34	$C_{18}H_{34}O_2$	282	9-Octadecenoic acid
18	15.954	5.08	$C18H_{34}O_2$	282	9-Octadecenoic acid
19	16.156	3.50	$C_{13}H_{28}$	184	Nonane, 5-methyl-5-propyl
20	16.301	3.90	$C_{18}H_{36}O_2$	284	Hexadecanoic acid, ethyl ester

Table 2: Biological activity of Phytocomponents identified in ethanolic extract of *Ziziphus oenoplia* leaves

S. No	Compound Name	Biological activity**
1.	Heptadecane	Antioxidant
2.	Hexadecanoic acid, ethyl ester	Nematicide, Pesticide, Lubricant, Antiandrogenic, antimicrobial, Antioxidant, hypocholesterolemic nematicide, pesticide, anti-androgenic flavor, hemolytic, 5-Alpha reductase inhibitor.
3.	Pentadecanoic acid	Antimicrobial, antifungal, Antioxidant
4.	2-Hexadecen-1-Ol, 3,7,11,15-Tetramethyl	Antimicrobial, Sedatives and Anesthetics
5.	1,2-Benzenedicarboxylic acid, dihexyl ester	Reduces β-induced neurotoxicity
6.	9-Octadecenoic acid	5-α reductase inhibitor, allergenic, α-reductase inhibitor, anti-inflammatory, anti-androgenic, cancer preventive, anemiagenic, anti-alopecic, antileukotriene-D4, choleretic, dermatitigenic, hypocholestrolemic, insectifuge, perfumery, propecic, flavour Antioxidant,

**Duke's. Phytochemical and Ethnobotanical Databases, www.ars-gov/cgi-bin/duke/, 2013.

4. Conclusion

The investigation concluded that the stronger extraction capacity of ethanol could have been produced number of active constituents responsible for many biological activities. From this study it can be concluded that *Ziziphus oenoplia* may serve as a new potential source of therapeutic drugs due to the presence of numerous important phytochemical bioactive compounds.

5. Reference

1. Anandhi K, Ushadevi T. Analysis of phytochemical constituents and antibacterial activities of *Clerodendrum inerme* L. against some selected pathogens. *International* Journal of Biotechnology and Allied Fields, 2013; 1(7):387-393.
2. Balaji K, Kilimozhi D. GC-MS analysis of various extracts of *Clerodendrum phlomidis* leaf. Responsible responsible for many biological activities and its beneficial effects could be utilized to create an International Journal of Pharmacy and Pharmaceutical Sciences. 2014; 6(1):226-232.
3. Bodoprost J, Rosemeyer H. Analysis of phenacylester derivatives of fatty acids from human skin surface by reversed-phase HPTLC: Chromatography mobility as a function of physicochemical properties. International Journal of Molecular Sciences. 2007; 8:1111-1124.
4. De-Fatima A, Modolo LV, Conegero LS, Pilli RA, Ferreira CV, Kohn LK *et al*. Lactones and their derivatives: biological activities, mechanisms of action and potential leads for drug design. Curr. Med. Chem. 2006; 13:3371-3384.
5. Falodun A, Siraj R, Choudary MI. GC-MS analysis of insecticidal leaf essential oil of Pyrenacanthastaudtii Hutch and Dalz (Icacinaceae). Tropical Journal of Pharmaceutical Research. 2009; 8:139-143.
6. Fernie AR, Trethewey RN, Krotzky AJ, Willmitzer L. Metabolite profiling: From diagnostics to systems biology. Nat Rev Mol Cell Biol. 2004; 5:763-9.
7. Hamburger M, Hostettmann K. Bioactivity in plants: the link between phytochemistry and medicine. Phytochemistry. 1991; 30:3864È74.
8. Harborne JB. Plant flavonoids in biology and medicine: Biochemical pharmacological, and structure–activity relationships. NY, USA: Alan R. Liss, 1986, 15-24.
9. Heinonen OP, Alnanes D, Virtamo T. Prostate cancer and supplementation with alpha- tocopherol and beta-carotene: Incidence and mortality in a controlled trial. Journal of the National Cancer Institute. 1998; 90(6):440-446.
10. Kell DB, Brown M, Davey HM, Dunn WB, Spasic I, Oliver SG. Metabolic footprinting and systems biology: The medium is the message. Nat Rev Microbiol. 2005; 3:557-65.
11. Liu RH. Potential synergy of phytochemicals in cancer prevention: Mechanism of action. Journal of Nutrition. 2004; 134(12):3479S-3485S.
12. Mathekaga AD, Meyer JJM. Antibacterial activity of South African *Helichrysum* species. South Afr J Bot. 1998; 64:293-5.
13. Roberts JKM, Xia JH. High-resolution NMR methods for study of higher plants, Methods Cell Biol. 1995; 49:245-258.
14. Ronald Hites A. Gas Chromatography Mass Spectroscopy: Handbook of Instrumental Techniques for Analytical Chemistry, 1997, 609-611.
15. Sathish SS, Janakiraman N, Johnson M. Phytochemical analysis of *Vitex altissima* L. Using UV-VIS, FTIR and GC-MS. International Journal of Pharmaceutical Sciences and Drug Research. 2012; 4(1):56-62.
16. Sathyaprabha G, Kumaravel S, Ruffina D, Praveenkumar PA. comparative study on antioxidant, proximate analysis, antimicrobial activity and phytochemical analysis of *Aloe vera* and *Cissus quadrangularis* by GC-MS. J Pharma Res. 2010; 3:2970-3.