WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 6, 691-698. Research Article ISSN 2277– 7105

DETERMINATION OF PHYTO-CONSTITUENTS IN *TEPHROSIA PURPURA* FLOWER EXTRTACT USING GC-MS

R. Kavitha*[1] and A. M. Uduman Mohideen[2]

[1]Department of Chemistry, Kunthavai Naacchiyaar Government Arts College for Women (Autonomous) Thanjavur, 613007, Tamil Nadu, India.

[2]P.G. and Research Department of Chemistry, Khadir Mohideen College, Adirampattinam, Thiruvarur District, 614 701, Tamil Nadu, India.

Article Received on 27 Jan. 2018,
Revised on 17 Feb. 2018,
Accepted on 09 March 2018,
DOI: 10.20959/wjpr20186-11415

***Corresponding Author**
R. Kavitha
Department of Chemistry, Kunthavai Naacchiyaar Government Arts College for Women (Autonomous) Thanjavur, 613007, Tamil Nadu, India.

ABSTRACT

GC-MS has become a highly recommended tool for monitoring and tracking organic compounds in the environment. GC-MS is exclusively used for the analysis of esters, fatty acids, alcohols, aldehydes, terpenes etc. The aim of this study was to carry out for identification of phyto-compounds from the methanolic extract of *Tephrosia purpura* flower by Gas chromatography and Mass spectroscopy (GC-MS). GCMS analysis of methanolic 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 revealed the presence of various compounds like tetradecane, Tetradecane, Pentadecane, Tetradecanoic Acid, Hexanoic Acid and tridecanoic acid in the methanolic extract of *Tephrosia purpura*. Hence, the *Tephrosia purpura* may use anticancer, anti-microbial activity, antioxidant and antiinflammatory activity due to the presence of secondary metabolites in the methanolic extract. These findings support the traditional use of *Tephrosia purpura* in various disorders.

KEYWORD: Gas chromatography and Mass spectroscopy, *Tephrosia purpura*, Phytochemistry.

INTRODUCTION

Medicinal plants have been used in virtually all cultures as a source of medicine. Assurance of the safety, quality, and efficacy of medicinal plants and herbal products has now become a key issue in industrialized and in developing countries. The widespread use of herbal remedies and healthcare preparations is described in the Vedas and the Bible. Medicinal Plants have been used for thousands of years to flavor and conserve food, to treat health disorders and to prevent diseases including epidemics. The knowledge of their healing properties has been transmitted over the centuries within and among human communities. Active compounds produced during secondary metabolism are usually responsible for the biological properties of plant species used throughout the globe for various purposes. Phytochemicals are the chemicals extracted from plants. These organic chemicals are classified as primary or secondary constituents, depending on their role in plant metabolism (Krishnaiah *et al*., 2011).

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) 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) Within a decade, there were a number of dramatic advances in analytical techniques were powerful tools for separation, identification and structural determination of phytochemicals (Roberts and Xia, 1995).

Tephrosia purpura Medic. (English name: Kolingi; Tamil: Kolingi) is a species of flowering plant in the family of fabaceae. This plant has pinnate leaves, white and purplish flowers and flat hairy pods. It is a common waste land weed, even grown in sides of the roads in many parts of India and Srilanka. It is under cultivation as green manure crops in poor soils The aim of this paper is to determine the organic compounds present in the *Tephrosia purpura* flower extract with the aid of GC-MS Technique, which may provide an insight in its use in tradition medicine. This plant was used as a traditional medicine for curing many diseases like leprosy, ulcers, asthma, tumors, liver, spleen, heart and blood related diseases (Rao and Raju, 1984; Saleem et al., 1999; Saxena and Choubey, 1997).

MATERIAL AND METHODS

Plant materials

The flowers of *Tephrosia purpura* were collected from Thanjavur, Thanjavur District, Tamil Nadu, India from a single tree.

Preparation of extracts

The *Tephrosia purpura* flowers were first washed well and dust was removed from the flower. Then the flowers were dried at room temperature and coarsely powdered. The powder was extracted with methanol for 24 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in desiccator until used.

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 250ºC; ion-source temperature 280ºC. The oven temperature was programmed from 110ºC (isothermal for 2 min), with an increase of 10ºC/min, to 200ºC, then 5ºC/min to 280ºC, ending with a 9min isothermal at 280ºC. 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 Turbo Mass 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 (Dsse-Fatima *et al*., 2006).

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. 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.

GC-MS ANALYSIS

Gas chromatography has a very wide field of applications. But, its first and main area of use is in the separation and analysis of multi component mixtures such as essential oils, hydrocarbons and solvents (Kadhim *et al*., 2016). Intrinsically, with the use of the flame ionization detector and the electron capture detector (which have very high sensitivities) gas chromatography can quantitatively determine materials present at very low concentrations. It follows, that the second most important application area is in pollution studies, forensic work and general trace analysis. Because of its simplicity, sensitivity, and effectiveness in separating components of mixtures, gas chromatography is one of the most important tools in chemistry (Altameme *et al*., 2015). It is widely used for quantitative and qualitative analysis of mixtures, for the purification of compounds, and for the determination of such thermo chemical constants as heats of solution and vaporization, vapor pressure, and activity coefficients (Andrew Marston, 2007). A knowledge of the chemical constituents of plants is desirable not only for the discovery of therapeutic agents, but also because such information may be of great value in disclosing new sources of economic phytocompounds for the synthesis of complex chemical substances and for discovering the actual significance of folkloric remedies. Higher 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 chemotherapeutants, these are non-phytotoxic, more systemic and easily biodegradable (Bliesner, 2006).

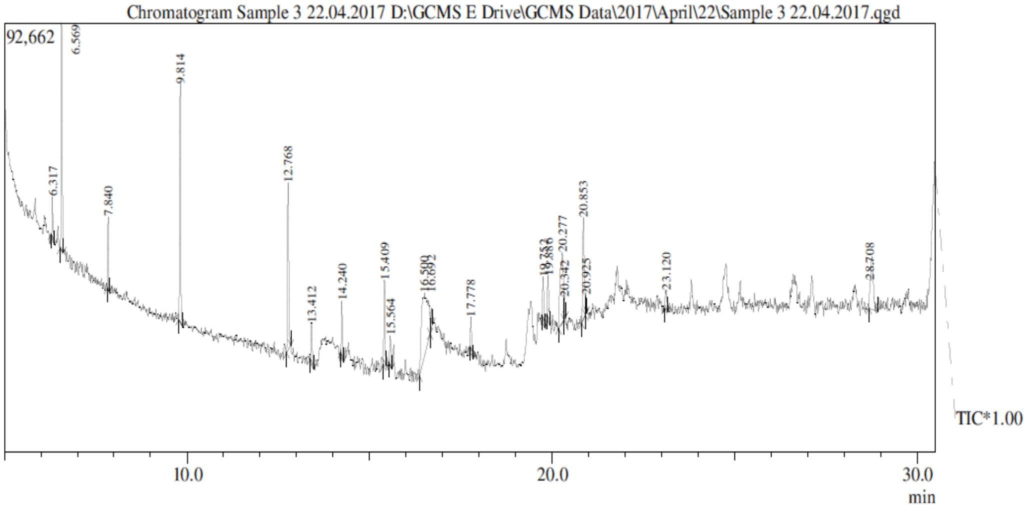

Figure 1: Chromatogram obtained from the GC/MS with the extract of *Tephrosia purpura.*

Table 1 Shows the components identified in methanolic extract of *Tephrosia purpura* (GC MS study).

Peak#	R.Time	Area%	Height%	Molecular formal	Name of the compounds
1	6.317	1.45	15.94	$C_3H_5ClO_3$	Propanoic acid, chloro-2-hydroxy-
2	6.569	0.69	15.94	$C_{11}H_{20}O_4$	Oxalic acid, isobutyl pentyl ester
3	7.840	2.61	5.11	$C_9H_{20}O_3$	Propane, 1,1,3-triethoxy-
4	9.814	11.39	16.73	$C_{14}H_{30}$	Tetradecane
5	12.768	8.91	2.82	$C_{15}H_{32}$	Pentadecane
6	13.412	2.28	2.82	$C_{12}H_{18}O$	Mixture Of 2-Hydroxy-1,4,6,7-Tetramethyleneoctane And 2-Hydroxy-1,4,5,7-Tetramethyleneoctane
7	14.240	2.38	3.92	$C_{12}H_{14}Br_2O_4$	(1a,3b,5b,7a)-4,4-Dibromo-8,8-Dimethyltricyclo[5.1.0.03,5]Octane-1,7-Dicarboxylic Acid
8	15.409	4.49	5.99	$C_{14}H_{30}$	Tetradecane
9	15.564	1.44	2.07	$C_8H_7NO_4$	Terephthalic monohydroxamic acid
10	16.500	18.82	4.49	$C_7H_{14}O_2$	Hexanoic Acid
11	16.692	1.24	1.47	$C_{10}H_{18}O_3$	2-Methylbutanoic anhydride
12	17.778	1.53	2.33	$C_9H_{19}I$	Nonane, 1-iodo-
13	19.752	2.01	3.03	$C_{24}H_{38}O_4$	1,2-Benzenedicarboxylic Acid, Diisooctyl Ester
14	19.886	2.68	3.03	$C_{16}H_{32}O_2$	Tetradecanoic Acid,
15	20.277	10.26	4.93	$C_{11}H_{24}$	2,2,6,6-Tetramethylheptane
16	20.342	0.71	1.53	$C_{20}H_{36}D_4O_2$	2-Octadec-1''-Enyloxy-1,1,2,2-Tetradeutero Ethanol
17	20.853	7.79	7.17	$C_{13}H_{24}O$	1-Tridecyn-4-Ol
18	20.925	0.60	1.29	$C_3H_3N_3O$	1H-1,2,3-Triazole-4-carboxaldehyde
19	23.120	1.48	1.39	$C_7H_{16}O_3$	1,1,3-Trimethoxybutane
20	28.708	7.23	2.05	$C_{31}H_{48}O_2$	2,2'-Methylene-Bis(4-Methyl-6-Tert-Octylphenol) -
		100.00	100.00		

Table 2: Activity of phyto-components identified in the methanolic extracts of the *Tephrosia purpura* by GC-MS.

Peak No.	R.Time	Height%	Name of the Compounds	Biological Activity**
1	9.814	11.39	Tetradecane	Antimicrobial activity
2	19.667	2.00	Tridecanoic Acid	Antimicrobial, Antifouling, Anti fouling

**Source: Dr. Duke's phytochemical and ethnobotanical databases [Online database].

The principle of gas chromatography is adsorption and partition. Within the family of chromatography-based methods gas chromatography (GC) is one of the most widely used techniques. GC-MS has become a highly recommended tool for monitoring and tracking organic pollutants in the environment. GC-MS is exclusively used for the analysis of esters, fatty acids, alcohols, aldehydes, terpenes etc. (Abeer Fauzi Al-Rubaye *et al.*, 2017).

In the present study, twenty compounds were identified in *Tephrosia purpura* flower by GC-MS analysis. The active compounds with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in (Table 1 and Fig 1). The prevailing compounds were tetradecane, Tetradecane, Pentadecane, Tetradecanoic Acid, Hexanoic Acid and tridecanoic acid.

Anandhi and Ushadevi, (2013) and Balaji and Kilimozhi, (2014) identified the 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.

CONCLUSION

GC-MS is widely used in pharmaceutical industries for analytical research and development, quality control, quality assurance, production, pilot plants departments for active pharmaceutical ingredients (API), bulk drugs and formulations. The prevailing compounds were tetradecane, Tetradecane, Pentadecane, Tetradecanoic Acid, Hexanoic Acid and tridecanoic acid were identified in *Tephrosia purpura* flower by GC-MS analysis. 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.

4. REFERENCE

1. Abeer Fauzi Al-Rubaye, Imad Hadi Hameed, Mohanad Jawad Kadhim. A Review: Uses of Gas Chromatography-Mass Spectrometry (GC-MS) Technique for Analysis of Bioactive Natural Compounds of Some Plants. International Journal of Toxicological and Pharmacological Research, 2017; 9(1): 81-85.
2. Altameme HJ, Hadi MY, Hameed IH. Phytochemical analysis of Urtica dioica leaves by fourier-transform infrared spectroscopy and gas chromatography-mass spectrometry. Journal of Pharmacognosy and Phytotherapy, 2015; 7(10): 238-252.
3. Anandhi K and 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.
4. Andrew Marston: Role of advances in chromatographic techniques in phytochemistry. Phytochemistry, 2007; 68: 2785-2797.
5. Azhagumurugan C and Rajan MK. Effect of leaf extract of Nilakumil, (Gmelina asiatica) against the root knot Nematode (Meloidogyne Incognita). *Research Journal of Recent Sciences*, 2014; 3: 264-266.
6. Balaji K and Kilimozhi D. GC-MS analysis of various extracts of Clerodendrum phlomidis leaf responsible for many biological activities and its beneficial effects could be utilized to create a *International Journal of Pharmacy and Pharmaceutical Sciences*, 2014; 6(1): 226-232.
7. Bliesner DM (2006) Validating Chromatographic Methods: A Practical Guide. John Wiley and Sons.
8. Bodoprost J and 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.
9. Bown D. Encyclopaedia of Herbs and their Uses. Dorling Kindersley: London, 1995.
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.*, 2006; 13: 3371-3384.
11. Du Z, Clery RA, Hammond CJ. Volatile organic nitrogen-containing constituents in ambrette seed Abelmoschus purpura Medik (Malvaceae). *J Agric Food Chem*., 2008; 56: 7388–7392.
12. Falodun A, Siraj R and Choudary MI. GC- MS analysis of insecticidal leaf essential oil of yrenacanthastaudtii Hutch and Dalz (Icacinaceae).*Tropical Journal of Pharmaceutical Research*, 2009; 8: 139-143.

13. Hamburger M, Hostettmann, K. Bioactivity in plants: the link between phytochemistry and medicine. *Phytochemistry*, 1991; 30: 3864È74.
14. Harborne, J.B. Plant flavonoids in biology and medicine: Biochemical pharmacological, and structure–activity relationships. NY, USA: *Alan R. Liss*, 1986; 15–24.
15. Kadhim MJ, Sosa AA, Hameed IH. Evaluation of anti-bacterial activity and bioactive chemical analysis of *Ocimum basilicum* using Fourier transform infrared (FT-IR) and gas chromatography-mass spectrometry (GC-MS) techniques. International Journal of Pharmacognosy and Phytochemical Research, 2016; 8(6): 127-146.
16. Krishnaiah, D Rosalam S, Nithyanandam, R. A review of the antioxidant potential of medicinal plant species, Food, 2011; 89(3): 217–233.
17. Liu IM., Thing-Fong Tzeng, Shorong-Shii Liou. *Abelmoschus purpura* (Malvaceae), an aromatic plant, suitable for medical or food uses to improve insulin sensitivity, *Phytotherapy Research*, 2010; 24(2): 233.
18. Liu RH. Potential synergy of phytochemicals in cancer prevention: Mechanism of action. *Journal of Nutrition*, 2004; 134(12): 3479S–3485S.
19. Mathekaga, AD, and Meyer JJM. Antibacterial activity of South African *Helichrysum* species. *South Afr J Bot*., 1998; 64: 293-5.
20. Rao EV and Raju NR. 2 flavonoids from *Tephrosia purpurea*. Phytochemistry, 1984; 23(10): 2339-2342.
21. Roberts JKM, Xia JH. High-resolution NMR methods for study of higher plants, *Methods Cell Biol.*, 1995; 49: 245–258.
22. Saleem M, Alam A, et al. *Tephrosia purpurea* ameliorates benzoyl peroxide-induced cutaneous toxicity in mice: Diminution of oxidative stress. Pharmacy and Pharmacology Communications, 1999; 5(7): 455-461.
23. Sathish SS, Janakiraman N and Johnson M. Phytochemical analysis of Vitex altissimaL. using UV-VIS, FTIR and GC-MS. *International Journal of Pharmaceutical Sciences and Drug Research*, 2012; 4(1): 56-62.
24. Sathyaprabha G, Kumaravel S, Ruffina D, Praveenkumar P. A 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.
25. Saxena VK and Choubey A. A novel neoflavonoid glycoside from *Tephrosia purpurea* stem. Fitoterapia, 1997; 68(4): 359-360.