



Bio-active compounds analysis and characterization in Ethanolic plant extracts of *Justicia tranquebariensis* L. (Acanthaceae) – using GC-MS

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Abstract : Phytocompounds in Ethanolic extract of *Justicia tranquebariensis* was elucidated using Gas chromatography – Mass spectrometry method. Fifteen compounds were identified. The major constituents are Hexadecane, Dibutyl phthalate, Dotriacontane and Hexadecanoic acid, and ethyl ester. From this study it is obvious that *Justicia tranquebariensis* plant extracts contains many biologically active compounds, such as Antimicrobial activity, antioxidant, antiviral and Cytotoxic activity.

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Introduction

India is the largest producer of medicinal herbs and is appropriately called the botanical garden of the world¹. Plants are capable of synthesizing an overwhelming variety of low-molecular weight organic compound² called secondary metabolites, usually with unique and complex structures. Many metabolites have been found to possess interesting biological activities and find applications, such as pharmaceuticals, insecticides, dyes, flavors and fragrances. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases³.

In recent years, Gas Chromatography and Mass Spectrum (GC-MS)⁴ has been applied unambiguously to identify the structures of different phytoconstituents from plant extracts and biological samples with great success^{5,6}.

Justicia tranquebariensis is a small shrub, which is widely distributed in southern parts of India. It is a perennial plant, leaves opposite, oval, entire⁷. Therapeutic value of this plant ranges from anti tumoral, anti viral⁸, analgesic to anti-inflammatory activities. The juice is used as cooling and aperients, and is prescribed for the children to cure smallpox, and bruised leaves are also applied to external injuries.

Some species of the genus *Justicia* have been used in the traditional system of medicines for the treatment of fever, pain, inflammation, diabetes, diarrhoea and liver diseases. They also possess anti-

inflammatory, anti-allergic, anti-tumoral, anti-viral and analgesic activities. The leaf juice of *J. tranquebariensis* has been used to treat jaundice and leaf paste is applied over affected area to treat skin diseases⁹.

Materials and Methods

Collection of samples

Justicia tranquebariensis were selected based on their ethno medical importance. Healthy disease free leaves, stems of *Justicia tranquebariensis* were collected from Semmalai, Karur district, Tamilnadu, India. The plant materials were shade dried, pulverized and stored at 4°C. Until further use.

Preparation of extracts

30g of air dried powder of plant material were infused in ethanol (100ml) until complete exhaustion. The infusion was filtered with four layered muslin cloth and stored at 4°C.

Gas chromatography and Mass spectrum analysis

Gas chromatography analysis was carried out at South Indian Textile Institute (SITRA), Coimbatore. It is one of the key techniques generally used for screening / identification of many groups of plant phytochemicals. Often irreplaceable tool in the phytochemical analysis even at trace level of plant chemical compounds. 5ml of ethanol extract was evaporated to dryness and reconstituted in 2µl ethanol. The extracts were then subjected into GC-MS analysis. Chromatographic separation was carried out with CE GC 8000 top MSMD 8000 Fyson instrument with Db 35mr column (10m x 0.5mm, 0.25 µm film thicknesses). Heating programs were executed from 100 - 250°C at 3 minutes by using the helium as a carrier with the injector heater at 250°C¹⁰. Injection temperature at 250°C, interface temperature at 200°C, quadruples temperature at 150°C and ion source temperature at 230°C were maintained. Injection was performed in split less mode. The mass spectra of compounds in samples were obtained by electron ionization (EI) at 70eV and the detector operated in scan mode from 20 to 600 atomic mass units (amu). Identifications were based on the molecular structure, molecular mass and calculated fragmentations. Resolved spectra were identified for phytochemicals by using the standard mass spectral database of WILEY and NIST¹¹.

Results and discussion

Ethnomedicinal Uses

Local people use this plant against inflammations. Leaf is used as expectorant, in cold, cough and nasal disorders. Leaf juice, about 15-20 ml, is administered orally for every one hour up to half of the day and keeping of leaf paste externally on the sight of snake bite work as an antidote for Cobra bite. Leaf juice is given orally to treat jaundice and leaf paste is applied over affected area to treat skin disease¹². The active principles with their retention time (RT), Molecular formula, molecular weight (MW), peak area in percentage are presented below (Table 1).

This typical gas chromatogram shows the relative concentrations of various compounds getting eluted as a function of retention time¹³. The heights of the peak indicate the relative concentrations of the compounds present in the plant. The numbers at various peaks are the retention time in minutes. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds¹⁴.

The large compound fragments into small compounds giving rise to appearance of peaks at different m/z ratios. The GC-MS analysis of 4 compounds and its biological activity were presented in (Table 2). Major compounds are Tetradecanal, Hexadecanoic acid, ethyl ester (CAS), Dotriacontane, and Pentatriacontane (Figure -1).

Tetradecanal compounds have the property of Antibacterial activity¹⁵. The identified compounds possess many biological properties for instance Hexadecanoic acid^{16, 17}, ethyl ester (CAS) has antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant, ant androgenic, flavor, hemolytic 5-Alpha reductase

inhibitor¹⁸. Dotriacontane and Pentatriacontane have antimicrobial, antioxidant, antispasmodic, antibacterial, and antiviral¹⁹.

Table 1: Phytoconstituents present in *Justicia tranquebariensis* as identified by GC-MS

S. no	Retention time (RT)	% of Peak Area	Compound Name	Molecular formula (MF)	Molecular weight (MW)
1.	7.80	3.73	Dodecane	C12H26	170
2.	10.79	4.57	Tetradecane	C14H30	198
3.	14.90	2.98	Hexadecane	C16H34	226
4.	15.18	3.37	Tetradecanal (CAS)	C14H28O	212
5.	19.14	1.67	Octadecane	C18H38	254
6.	22.22	5.61	Dibutyl phthalate	C16H22O4	278
7.	23.01	1.37	Hexadecanoic acid, ethyl ester (CAS)	C18H36O2	284
8.	24.15	1.48	Triacontane	C30H62	422
9.	24.61	9.55	Tetratriacontane	C34H70	478
10.	29.82	1.21	Hexatriacontane (CAS)	C36H74	506
11.	30.99	1.8	Pentacosane (CAS)	C25H52	352
12.	31.36	3.57	Di-(2-ethylhexyl)phthalate	C24H38O4	390
13.	32.05	22.30	Dotriacontane	C32H66	450
14.	33.86	2.09	Pentatriacontane	C35H72	492
15.	35.78	2.82	Octacosane (CAS)	C28H58	394

Table 2: Biological properties of GC-MS compounds

S. No	Name of the compound	Biological properties
1.	Tetradecanal	Antibacterial activity.
2.	Hexadecanoic acid, ethyl ester (CAS)	Antioxidant, Hypocholesterolemic Nematicide, Pesticide, Lubricant, Ant androgenic, Flavor, Hemolytic 5-Alpha reductase inhibitor
3.	Dotriacontane	Antimicrobial, antispasmodic, antioxidant,
4.	Pentatriacontane	Antibacterial, Antiviral

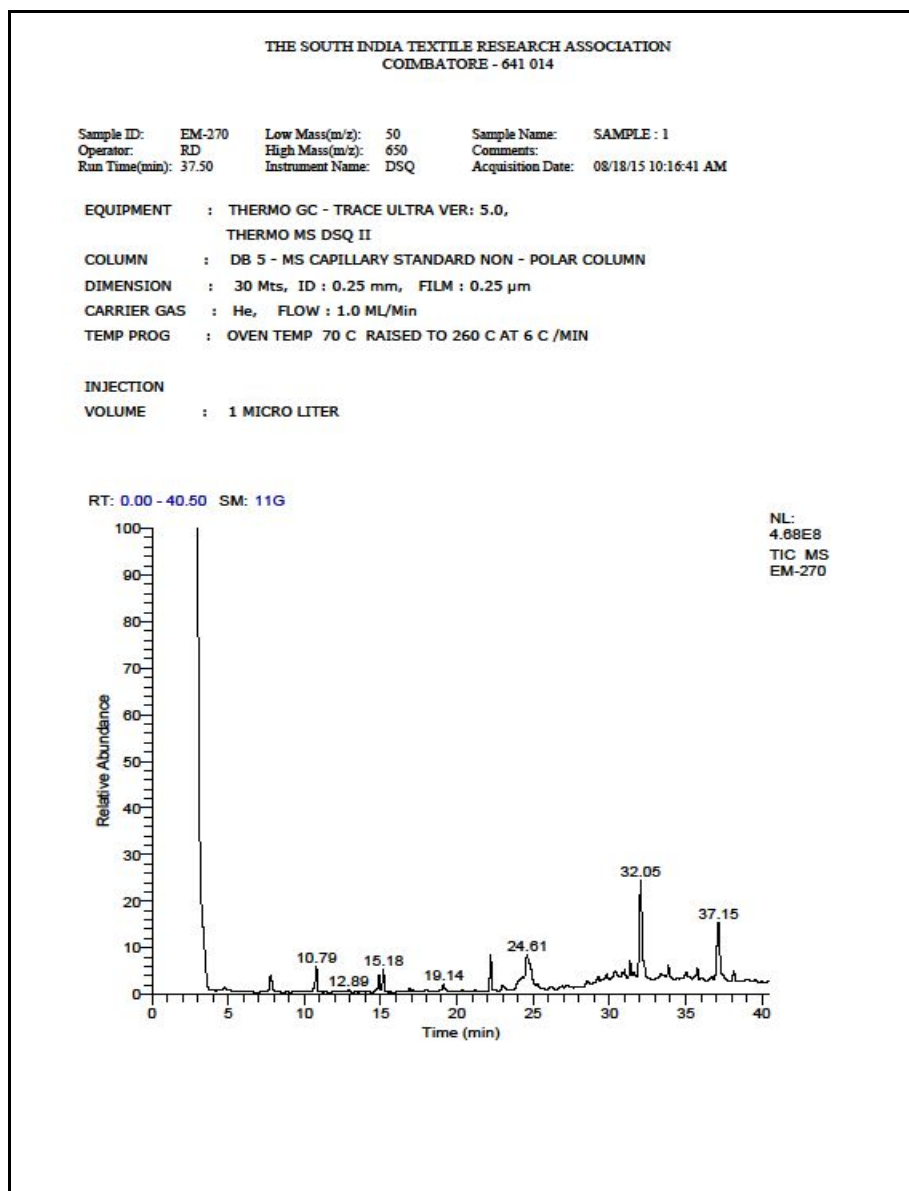


Figure -1 Gas chromatography and mass spectroscopy (GC-MS) analysis of Ethanolic sample of *Justicia tranquebariensis*

Conclusion

The GC-MS analysis of the Ethanolic extract of *J. tranquebareinsis* reveals the presence of phytoconstituents belonging to the, esters, alcohols, ethers²⁰ and the compounds are Tetradecane, Hexadecane, Tetradecanal (CAS), Octadecane, Dibutyl phthalate, Hexadecanoic acid, ethyl ester (CAS), Triacontane, Tetratriacontane, Hexatriacontane (CAS), Pentacosane (CAS), Di-(2-ethylhexyl) phthalate, Dotriacontane, Pentatriacontane, and Octacosane (CAS). The plant holds promise for the production of novel²¹ pharmaceuticals as well as a nutraceutical. It would be worthwhile to further isolate the compounds and determine their specific activity and also to understand the synergistic effect of compounds for their therapeutic roles²².

Here we report the presence of some important compounds in this plant isolated by GC-MS analysis. Thus, this type of study may give information on nature of active principles present in the medicinal plants. These identified phytoconstituents presumed to be responsible for eliciting the traditional activity of *Justicia tranquebariensis*.

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References

1. Ahmedull, M., Nayar, MP. Red data book for Indian plants. Botanical Survey of India. 1999, 4.
2. Dhanalakshmi R, Manavalan R, Bioactive Compounds in Leaves of *Corchorus trilocularis* L. BY GC-MS Analysis, International Journal of PharmTech Research, 2014, Vol.6, No.7, pp 1991-1998.
3. Duraipandiyan, V., Ayyanar, M., Ignacimuthu, S. Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. BMC Complement. Altern. Med 2006, 6, 35-41.
4. M.G.Kulkarni M.G.and Sathe P.S. Phytochemical and GC-MS analysis of *Hamiltonia suaveolens* (ROXB), International Journal of ChemTech Research, 2013, Vol.5, No.1, pp 212-219,
5. Prasain, J.K., Wang, C.-C., and S. Barnes, S. Mass spectroscopic methods for the Determination of flavonoids in biological samples. Free Radical Biology & Medicine, 2004, 37, 1324-50.
6. De Rijke, E., Out, P., Neissen, W.M.A., Ariese, F., Gooijer, C., and Brinkman, U.A. The analytical separation and detection methods for flavonoids. Journal of Chromatography, 2006, 1112, 31-63.
7. Robbins, Cotron, Vinay, k., Abdul, KA, and Nelson, F. Pathologic Basis of Disease. Elsevier publication. 2008, 7th edition, 1306-1308.
8. Asano, J., Chiba, K., Tad, M., Yoshi, T. Phytochemical analysis. Phytochemistry. 1996, 42, 713.
9. Poongodi, A., Thilagavathi, S., Aravindhan, V., Rajendran, A. Observations on some ethnomedicinal plants in Sathyamangalam forests of Erode district, Tamil Nadu, India. Journal of Medicinal Plants Research. 2011, 5(19), 4709-4714.
10. Chandra MohanS, Dinakar S, Anand T, ElayarajaR, Sathiyapriya B, Phytochemical, GC-MS analysis and Antibacterial activity of a Medicinal Plant *Acalypha indica* International Journal of PharmTech Research, 2012, Vol.4, No.3, pp 1050-1054.
11. Thiripura Salini, S, Shankar, S. Phytochemical and GCMS Analysis of *Canthium coromandelicum* Leaves Extract. International Journal of PharmTech Research, 2014, Vol.6, No.5, pp 1731-1735.
12. Yoganasimhan, S.N. Medicinal Plants of India, Regional Research Institute, Bangalore, 2000, 2.
13. Sarada, K, Jothibai Margret, Rand Mohan, V.R. GC – MS Determination of Bioactive Components of *Naringi crenulata* (Roxb) Nicolson. International Journal of ChemTech Research, 2011, Vol. 3, No.3, pp 1548-1555.
14. Rajeswari J, Rani S, Gc-Ms Analysis of Whole Plant of *Leptadenia Reticulata*, International Journal of PharmTech Research, 2014, Vol.6, No.7, pp 2043-2050.
15. Subbaiyan, B, Samydurai, P, Karthik prabu, M, and Thangapandian, V. Gas chromatography and Mass spectrum analysis of *Catharanthus pusillus* Murray g. don (apocyanaceae). Int. Res J Pharm. App Sci., 2014, 4(2):48-52.
16. Janani S.R, and Singaravadivel K, Screening of Phytochemical and GC-MS Analysis of some Bioactive constituents of *Asparagus racemosus*, International Journal of PharmTech Research, 2014, Vol.6, No.2, pp 428-432.
17. Bharathy V and F. Uthayakumari, Bioactive Components in leaves of *Jatropha tanjorensis* J.L.Ellis & Saroja by GC-MS Analysis, International Journal of PharmTech Research, 2013, Vol.5, No.4, pp 1839-1843.
18. Praveen kumar, P, Kumaravel, S and Lalitha, C. Screening of antioxidant activity, total phenolic and GC-MS study of *Vitex negundo*. Afr. J. Biochemistry. 2010, 4 (7): 191-195.
19. Nithya T.G, Jayanthi J and Raghunathan MG, Phytochemical, Antibacterial and GC MS analysis of a floating fern *Salvinia molesta* D.S.Mitchell (1972), International Journal of PharmTech Research, 2015, Vol.8, No.9, pp 85-90.
20. Maruthupandian A and Mohan V.R, GC-MS analysis of some bioactive constituents of *Pterocarpus marsupium* Roxb. International Journal of ChemTech Research, 2011, Vol. 3, No.3, pp 1652-1657.
21. Shree Devi M.S, Sathiyarajeswaran P, Kannan M, Mohanasrinivasan. V, Subathra Devi.C, Jemimah Naine.S, Vaishnavi.B, GC-MS, FT-IR Analysis and Anti Bacterial Study of Bioactive Compounds of Chundaivatral Chooranam - A Siddha Poly Herbal Formulation, International Journal of PharmTech Research, 2015, Vol.8, No.10, pp 204-209.

22. Ravinder Singh C, Nelson R, Muthu Krishnan P and Pargavi B, Identification of Volatile Constituents from *Premna serratifolia* L. through GC-MS, International Journal of PharmTech Research, 2011, Vol. 3, No.2, pp 1050-1058.
