



**GC-MS ANALYSIS OF PHYTOCOMPONENTS IN THE METHANOLIC EXTRACT
OF *JUSTICIA WYNAADENSIS* (NEES) T. ANDERS****S. U. PONNAMMA* AND K. MANJUNATH***Department of Microbiology and Biotechnology, Bangalore University, Bangalore, India.***ABSTRACT**

Justicia wynaadensis, locally called “Maddu Thoppu” belongs to the family *Acanthaceae*. The present study was carried out to identify the phytocomponents present in the methanolic extract of *Justicia wynaadensis* by GC-MS analysis to ascertain its usage by the local community as ‘a plant possessing medicinal properties’. Twenty four compounds were identified. The major constituents are Dihydrocoumarin, Phytol and Palmitic acid. Significant quantities of Linoleic acid, Stearic acid, Squalene and phytosterols such as Campesterol and Stigmasterol were also present.

KEY WORDS : GC-MS, *Justicia*, *Acanthaceae*, medicinal, Phytocomponents, methanolic**S. U. PONNAMMA**

Department of Microbiology and Biotechnology, Bangalore University, Bangalore, India.

INTRODUCTION

Justicia wynaadensis belongs to *Acanthaceae* and is endemic to the rainforest region of the Western Ghats¹ and common in forests of Irpu². Traditionally, the extract of this plant is consumed by the local community during the monsoons by incorporating it into a dessert, as it is said to possess maximum medicinal properties during the season and is said to keep them healthy throughout the year. The aqueous extract prepared by crushing and soaking the stem and leaves in water, possesses a unique flavor and blue color. The use of this plant by the Kuruchiar tribes of Tirunelli forest, Wayanad district, Kerala as an external application over rheumatic swellings has been reported³. The plant is said to possess catalase and peroxidase activity; also polyphenols and flavanoids have been identified and estimated⁴. Regarding the specific phytochemicals and chemical activity, there has not been any report on this plant except that the plant extract lowers cellular cholesterol and cholesteryl ester concentration; further studies had also shown a novel inhibitory effect on the uptake of ox-LDL by human macrophage cell line⁵. The most studied species among the *Justicia* are *Justicia pectoralis* Jacq., *Justicia procumbens* L., *Justicia gendarussa* Burm. f., and *Justicia anselliana* (Nees) T. Anderson⁶. The present work was carried out to identify some of the phytochemicals present in the methanolic extract of the aerial parts of *J. wynaadensis* by GC-MS technique, to ascertain the medicinal properties of the plant.

MATERIALS AND METHODS

Collection of the plant material

The aerial parts of *Justicia wynaadensis* plant was collected from the region surrounding the Irpu Hills, Western Ghats, Karnataka, on the 18th day of the Hindu calendar month of Aadi which falls during monsoon. The aerial parts of the plant was identified and authenticated at

the National Ayurveda Dietetics Research Institute, Bangalore.

Preparation of the extract

The leaves and stem of *Justicia wynaadensis* was shade dried, crushed by hand and ground into coarse powder using an electric grinder. Twenty grams of the powdered plant material was subjected to three methanol washes followed by filtration of the combined filtrate through the filter paper and evaporated to give a final yield of 7% extract. The extract was subjected to GC-MS analysis.

GC-MS analysis

The GC-MS analysis of the sample was performed using a Shimadzu GCMS-QP2010 gas chromatograph-mass spectrometer interfaced with a Turbo Mass quadrupole mass spectrometer, fitted with an Rtx-5 fused silica capillary column (30 X 0.25 mm, with 1 µm film thickness). The oven temperature was programmed from 100°C to 320° C at 100°C/min and a hold for 10 min. Helium was used as carrier gas at flow 1.0 mL/min. The injector temperature was 250 °C, injection size 1 µL neat, with split ratio 1:10. The interface and MS ion source were maintained at 320°C and 200°C, respectively, the mass spectra were taken at 70eV with a mass scan range of 40-700 amu. Data handling was done using GCMS solution software. The identification of compounds was based on comparison of their mass spectra with those of WILEY and NIST Libraries.

RESULTS AND DISCUSSION

GC-MS analysis

GC-MS chromatogram of the methanolic extract of *Justicia wynaadensis* showed 30 peaks (Figure 1) and have been identified after comparison of the mass spectra with WILEY and NIST libraries (Table 1), indicating

the presence of 24 phytochemicals. From the results, it was observed that Hexadecanoic acid (synonym: Palmitic acid), 2H-1-Benzopyran-2-one,3,4-dihydro- (synonym: Dihydrocoumarin/ Melitol) and 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Synonym: Phytol), were the major components in the extract. The phytochemicals that contribute to the medicinal property of the plant is listed in Table 2. Palmitic acid is reported to be an antioxidant⁷, a nematicide⁸ and a pesticide while Melitol and Phytol are said to be cancer-preventive. Other antioxidants present were Tetradecanoic acid; Hexadecanoic acid; Heptadecanoic acid; 2,6,10,14,18,22-tetracosahexaene,2,6,10,15,19,23-hexamethyl- (synonym: Squalene); gamma-

Tocopherol; Vitamin E; Ergost-5-en-3.Beta.-ol (synonym: Campesterol) and Stigmasta-5,22-dien-3.beta.-ol,(3.beta.,22E)- (synonym: Stigmasterol). The presence of so many antioxidants in *Justicia wynaadensis* justifies the high antioxidant property observed⁴. The unique flavor could be attributed to the presence of flavor or aroma compounds such as 2-Propenoic acid,3-(2-hydroxyphenyl)-; Benzeneacetic acid; 2H-1-Benzopyran-2-one,3,4-dihydro-; Hexadecanoic acid; Octadecanoic acid and Squalene. Hypocholesterolemic compounds such as 9,12-Octadecadienoic acid(Z,Z)-; Octadecanoic acid; gamma-Tocopherol; Vitamin E; Ergost-5-en-3.Beta.-ol and Stigmasta-5, 22-dien-3.beta.-ol were also present.

Figure 1
GC-MS chromatogram of the methanolic extract of the aerial parts of *Justicia wynaadensis*.

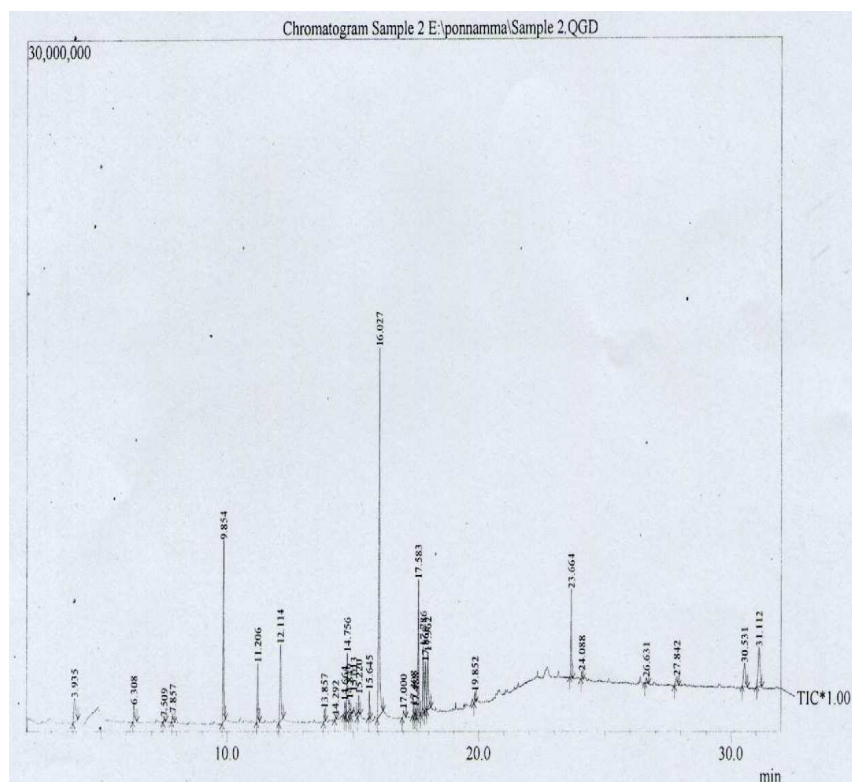


Table 1
Phytocomponents identified in the methanolic extracts of *Justicia wynaadensis*
by GC-MS.

No.	RT	Name of the Compound	Mol. formula	MW	Peak Area %
1	3.935	1,2,3-Propanetriol	C ₃ H ₈ O ₃	92	3.27
2	6.308	2-Propenoic acid,3-(2-hydroxyphenyl)-,(E)-	C ₉ H ₈ O ₃	164	1.51
3	7.509	Benzeneacetic acid	C ₈ H ₈ O ₂	136	0.27
4	7.857	1,2,3,4-Tetrahydroxybutane	C ₄ H ₁₀ O ₄	122	0.64
5	9.854	2H-1-Benzopyran-2-one,3,4-dihydro-	C ₉ H ₈ O ₂	148	10.58*
6	11.206	2H-1-Benzopyran-2-one,3,4-dihydro-	C ₉ H ₈ O ₂	148	3.01
7	12.114	2H-1-Benzopyran-2-one,3,4-dihydro-	C ₉ H ₈ O ₂	148	6.18
8	13.857	Tetradecanoic acid	C ₁₄ H ₂₈ O ₂	228	0.63
9	14.292	(-)-Loliolide	C ₁₁ H ₁₆ O ₃	196	0.18
10	14.664	2-Cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butenyl)-	C ₁₃ H ₁₈ O ₃	222	0.71
11	14.756	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296	4.80
12	14.849	2-Pentadecanone,6,10,14-trimethyl-	C ₁₈ H ₃₆ O	268	1.05
13	15.013	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296	1.21
14	15.220	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296	1.26
15	15.645	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270	1.19
16	16.027	Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	24.96*
17	17.000	Heptadecanoic acid	C ₁₇ H ₃₄ O ₂	270	0.57
18	17.408	Octadeca-9, 12, dienoic acid methyl ester	C ₁₉ H ₃₄ O ₂	294	0.50
19	17.488	9,12,15-Octadecatrienoic acid, methyl ester,(Z,Z,Z) -	C ₁₉ H ₃₂ O ₂	292	0.35
20	17.583	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296	7.51*
21	17.786	9,12-Octadecadienoic acid (Z,Z)-	C ₁₈ H ₃₂ O ₂	280	4.79
22	17.868	9,12,15-Octadecatrienoic acid, methyl ester,(Z,Z,Z) -	C ₁₉ H ₃₂ O ₂	292	3.64
23	17.962	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	284	4.30
24	19.852	3-Methyl-(2,6-dimethylheptyl)-2-penten-5-olide	C ₁₅ H ₂₆ O ₂	238	0.71
25	23.664	2,6,10,14,18,22-tetracosahexaene, 2,6,10,15,19,23 - hexamethyl-	C ₃₀ H ₅₀	410	5.37
26	24.088	Heptacosane	C ₂₇ H ₅₆	380	0.48
27	26.631	gamma-Tocopherol	C ₂₈ H ₄₈ O ₂	416	0.43
28	27.842	Vitamin E	C ₂₉ H ₅₀ O ₂	430	1.19
29	30.531	Ergost-5-en-3.Beta.-ol	C ₂₈ H ₄₈ O	400	3.40
30	31.112	Stigmasta-5, 22-dien-3.beta.-ol, (3.beta.,22E)-	C ₂₉ H ₄₈ O	412	5.33

*Major Phytocomponents

Table 2
Activity of some of the phytocomponents identified in the methanolic extracts of
***Justicia wynaadensis* by GC-MS.**

No	RT	Name of the Compound / Synonym	Activity**
1	6.308	2-Propenoic acid,3-(2-hydroxyphenyl)-/ Cinnamic acid dihydro	Antibacterial, flavor, Aldose-Reductase-Inhibitor, Allergenic, Anesthetic, Antiinflammatory, Antimutagenic, Antispasmodic, Cancer- Preventive; Choloretic; Dermatitigenic, Fungicide, Herbicide, Laxative, Pesticide, Lipoxygenase-Inhibitor, Pesticide, Tyrosinase- Inhibitor, Vermifuge
2	7.509	Benzeneacetic acid / Phenyl acetic acid	Flavor, Perfumery
3	9.854	2H-1-Benzopyran-2-one,3,4-dihydro-/ Dihydrocoumarin	Cancer-preventive, Flavor
4	13.857	Tetradecanoic acid / Myristic acid	Antioxidant, Lubricant, Hypercholesterolemic, Cancer-preventive, Cosmetic
5	14.292	(-)-Loliolide	Pesticide, Ant-repellent, Nematicide
6	14.756	3,7,11,15-Tetramethyl-2-hexadecen-1-ol 2-Pentadecanone,6,10,14-trimethyl/ Phytol	Cancer-preventive
7	16.027	Hexadecanoic acid / Palmitic acid	Antioxidant, Pesticide, Flavor, 5-Alpha- Reductase-inhibitor, Antifibrinolytic, Hemolytic, Lubricant, Nematicide, Antiallopecic
8	17.000	Heptadecanoic acid / Margaric acid	Antioxidant
9	17.786	9,12-Octadecadienoic acid(Z,Z)- / Linoleic	Anticoronary, Antiallopecic,

		acid	
			Antiarteriosclerotic, Antiarthritic, antianaphylactic, Antieczemic, Cancer-preventive, antiprostatic, hepatoprotective, Hypocholesterolemic, Metastatic, Nematicide
10	17.962	Octadecanoic acid / Stearic acid	Cosmetic, Flavor, Hypocholesterolemic, Lubricant, Perfumery, Propepic, Suppository
11	23.664	2,6,10,14,18,22-tetracosahexaene, 2,6,10,15,19,23 -hexamethyl- / Squalene	Antibacterial, Antioxidant, Cancer-preventive, Antitumor, Immunostimulant, perfumery, Pesticide, Sunscreen
12	26.631	gamma-Tocopherol	Anticancer, Antioxidant, Antitumor, Antiinflammatory, Hypocholesterolemic, Cardioprotective
13	27.842	Vitamin E	Antiaging, Antialzheimeran, Antidermatitic, Antidiabetic, Antioxidant, Antitumor, Cancer-preventive, Hypocholesterolemic, Immunostimulant
14	30.531	Ergost-5-en-3.Beta.-ol / Campesterol	Antioxidant, Hypocholesterolemic
15	31.112	Stigmasta-5,22-dien-3.beta.-ol,(3.beta.,22E)-/ Stigmasterol	Antihepatotoxic, Antiviral, Antioxidant, Cancer-preventive, Hypocholesterolemic

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

Campesterol and stigmasterol, the anticancerous⁹ phytosterols have also been detected in *Justicia anselliana* and an accurate and sensitive method for their quantification in the plant has been validated¹⁰. Phytosterols have been clinically

proved to reduce blood cholesterol and scientific reports suggest that they possess antioxidant activity¹¹. A separate study carried out on the ethanolic extract of *Mussaenda frondosa* Linn by GC-MS analysis has led to

the identification of twenty chemical constituents¹².

CONCLUSION

The presence of so many phytochemicals in *Justicia wynaadensis* lends credence to its use by the local community as a plant with

'medicinal properties' and also holds promise for the production of novel 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 therapeutic roles.

REFERENCES

1. Gamble J S. Flora of the Presidency of Madras. Adlard and Sons Ltd: 755-756, (1967).
2. Keshava murthy, K. R. and Yoganarasimhan, S. N, Ed. Flora of Coorg (Kodagu), Karnataka, India, with data on medicinal plants and chemical constituents. Vimsat Publishers: 333-335, (1990).
3. Udayan P. S, Harinarayanan M.K, Tushar K.V and Indira Balachandran. Some common plants used by the Kuruchiar tribes of Thirunelli forest, Wayanad district, Kerala in medicine and other traditional uses. Indian J of Traditional Knowledge, 7(2): 250-255, (2008).
4. Medapa S., Singh G.R.J and Ravikumar,V..The phytochemical and antioxidant screening of *Justicia wynaadensis* . Afr J Plant Sci, 5(9): 489-492, (2011).
5. M.T.R. Subbiah and E.J. Norman. Rain forest plant extract with cellular cholesterol lowering properties, U.S. Patent, US 6,365,411 B1, 2002.
6. Correa G.M and Alcantara A. F.C. Chemical constituents and biological activities of species of *Justicia* – A review. Rev bras farmacogn, 22(1):220-238, (2012).
7. Cho K.H., Hong J.H and Lee K.T. Monoacylglycerol (MAG)-oleic acid has stronger antioxidant , anti-atherosclerotic, and protein glycation inhibitory activities than MAG-palmitic acid. J Med Food, 13(1):99-107, (2010).
8. K. Munakata. Nematocidal natural products. In: D.L Whitehead and W.S Bowers (eds.), *Natural Products for Innovative Pest Management*, Oxford: Pergamon, 1983, pp. 299-310.
9. Bradford P.G. and Awad A.B. Phytosterols as anticancer compounds. Mol Nutr Food Res, 51 (2): 161-70, (2007).
10. Kpoviessi D.S., Gbaguidi F., Gbenou J., Accrombessi G., Moudachirou M., Rozet E., Hubert P and Quetin-Leclercq J . Validation of a method for the determination of sterols and triterpenes in the aerial part of *Justicia anselliana* (Nees) T. Anders by capillary gas chromatography. J Pharm Biomed Anal, 48(4):1127-1135, (2008).
11. J. Zawistowski. Tangible health benefits of phytosterol functional foods. In: J. Smith and E.Charter (eds.), *Functional Food Product Development*, Wiley Blackwell, 2010, pp. 362-372.
12. Gopalakrishnan, S and Vadivel, E. GC-MS analysis of some bioactive constituents of *Mussaenda frondosa* Linn. Int J Pharm Bio Sci, 2(1):313-320, (2011).