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Evaluation Of Bioactive Compounds In *Justicia Gendarussa* Leaves Extract Using Gas Chromatography And Mass Spectroscopy

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ABSTRACT

The current study was aimed to various the phytochemical profile and GC-MS analysis of *Justicia gendarussa* leaf extracts. Powder of *Justicia gendarussa* leaves showed the presence of tannin, flavonoids, polyphenol and terpenoids and saponin. A significant tannin, flavonoids, polyphenol and terpenoids and saponin were present in *J. gendarussa* leaves powder. The identification of the chemical compounds and their histolocalizatin, provides support to their quality control. The GC-MS analysis of *J. gendarussa* leaves revealed the presence of 25 compounds. The identified compounds possess many biological properties.

Keywords: *Justicia gendarussa*, Histochemical and GCMS

INTRODUCTION

Plants are used as medicines in various cultures and serve as a source of many potent drugs due to the presence of certain bioactive compounds for pharmaceutical industries (Gopalakrishnan and Udayakumar, 2014). Phytochemicals are useful in the treatment of certain disorders by their individual, additive, or synergic actions to improve health (Patel, 2015; Mahomoodally, 2013). These phytochemicals or secondary metabolites are usually large groups of chemicals that are not essential for the plant growth but important in providing defence response against predators through their unpalatably due to particular flavours and aromas (Barbieri *et al.*, 2017). The reason of medicinal use of many plants is assigned to the active parts of each plant, where some biologically active compounds are stored by the plants [biological activities like- antimicrobial, antioxidant, anti-inflammatory, antiseborrheic, antipsoriatic and anti-acne, etc.] (Motamarri *et al*., 2012; Gerometta *et al.,* 2020).

Gas chromatography-mass spectroscopy (GC-MS) is a combined analytical technique used to determine and identify compounds present in a plant sample (Uma and Balasubramaniam, 2012). GCMS analysis of many plants has helped in the identification and characterization of phytochemicals present in plant extracts (Eswaraiah *et al*., 2020). Our present research is focused on the histochemical screening of different extracts and identification of the biological compounds in the leaves of *J. gendarussa* by GC-MS.

MATERIALS AND METHODS

Collection of plant materials

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The leaves of *Justicia gendarussa* were collected in January 2020 from Kadukaval, Thanjavur district, Tamil Nadu, India.

Phytochemical analysis

The powder was extracted with aqueous, benzene, ethanol and hexane for 24 hours using the maceration method with "intermittent shaking" to obtain an extract. The extract was filtered using Whatman filter No 1 paper and filtrate was used for phytochemical analysis.

Histochemical tests (John Peter Paul, 2014; Gersbach *et al.*, 2001).

A small quantity of dried and finely *J. gendarussa* leaves powder was placed on a grease free microscopic slide and treated with specific chemicals and reagents and waited for 1-2 minutes. A positive test for histochemical was indicated by the appearance of the appropriate colour change after application of the reagent. Using a light microscope to observe and record any colour changes.

The *J. gendarussa* leaves powder was treated with diluted ammonia and H_2SO_4 gave yellow colour indicates flavonoids. *J. gendarussa* leaves powder treated with ferric chloride to gave Dark blue to black indicates the presence of tannin. *J. gendarussa* leaves powder treated with Toludine blue to give Blue green/Red colour indicates the presence of polyphenol. *J. gendarussa* leaves powder treated with Dinitrophenol hydrazine (few drops) to give Orange colour indicates the presence of Terpenoids. *J. gendarussa* leaves powder treated with H_2SO_4 (few drops) to give Yellow colour indicates the presence of Saponin.

GC-MS Analysis

GC-MS analysis was carried out on Shimadzu 2010 plus comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column RTX 5Ms (Column diameter is 0.32 mm, column length is 30m, column thickness 0.50 µm), operating in electron impact mode at 70eV; Helium gas (99.99 %) was used as carrier gas at a constant flow of 1.73 ml /min and an injection volume of 5µl was employed (split ratio of 10:1), injector temperature 270ºC; ion source temperature 200ºC. The oven temperature was programmed from 40ºC (isothermal for 2 min), with an increase of 8ºC/min, to 150 ºC, then 8 ºC/min to 250ºC, ending with a 20 min 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 51.25 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 TurboMassVer 5.2.0 (Srinivasan and Ramarao, 2013). Interpretation on GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. (Dukes, 2013).

RESULTS AND DISCUSSION

Phytochemical analysis

Present study to investigate the histochemical analysis of *Justicia gendarussa* leaves powder. A significant amount of tannin, flavonoids, polyphenol and terpenoids and saponin were present in *J. gendarussa* leaves powder. The identification of the chemical compounds and their histolocalization, provides support to their quality control (Table 1 and Plate 1).

Table 1: Histochemical analysis of *Justicia gendarussa* leaves powder

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11 , Iss 6, 2022

S. No	Phytochemicals	Results	
		Colour observation	Present
1	Tannin	Dark blue to black	++
2	Flavonoids	Yellow	+
3	Polyphenol	Blue and green	++
4	Terpenoids	Orange	++
5	Saponin	Light yellow	+

Note: (+) Presence; (++) present with high intensity of the colour

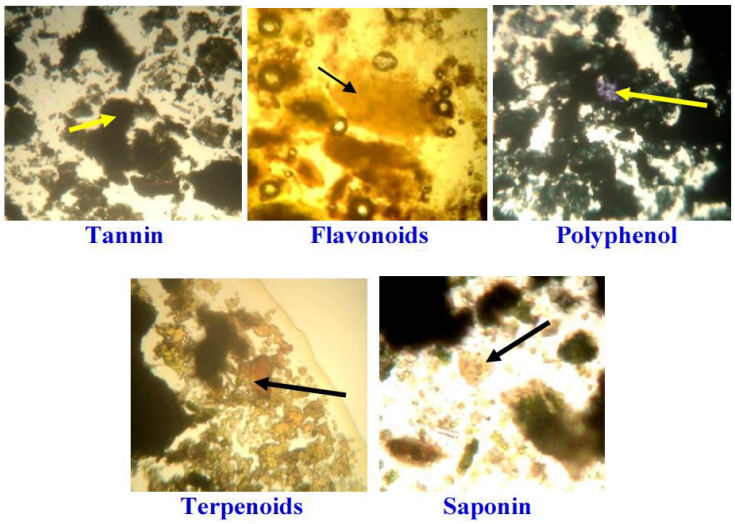

Plate 1: Histochemical analysis of *Justicia gendarussa* leaves powder

Compounds belonging to the respective groups have been reported to impart various medicinal characteristics to the plants. Due to the presence of flavonoids plants possess antioxidant properties as well as anticancer activities (Yadav and Agarwala, 2011). While terpenoids were well known for antibacterial, anti-inflammatory and anticancer properties (Chung *et al.,* 1998). Tannins have anti-cancerous and antibacterial activities (Radhika *et al.,* 2013). Phenolic compounds and phytosterol present in plants are responsible for antimicrobial, antiallergic, antidiabetic, antioxidant, anti-inflammatory, antimutagenic and anticarcinogenic properties (Khan *et al.,* 2015). The presence of saponins in plant is very important because of their anticancer, antifungal, antioxidant, antibacterial and weight loss (Lira *et al.,* 2017).

GC-MS Analysis

In the present study, twenty-five compounds were identified in *Justicia gendarussa* leaves extract. The principle governs the molecular formula, molecular weight and concentration (%) with their corresponding retention time (RT). The prevailing compounds were 1,2-benzene dicarboxylic acid, diethyl ester (cas) ethyl phthalate, tetradecanoic acid, neophytadiene, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, n-hexadecanoic acid, hexadecanoic acid (cas) palmitic acid, phytol, 9,12,15-octadecatrienoic acid, (z,z,z)-, 9-octadecenoic acid (z)- (cas) oleic acid, 1,2-benzenedicarboxylic acid, bis(2-ethylhexyl) ester (cas) bis(2-ethylhexyl) phthalate (Table 4, 5 and Figure 1).

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11 , Iss 6, 2022

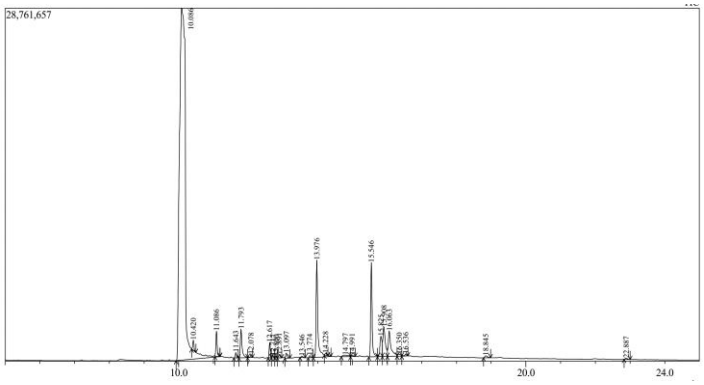

Figure 1: GC-MS Chromatogram of *Justicia gendarussa* leaves extract

Table 4: Identification of bioactive compounds in *Justicia gendarussa* leaves extract by GC-MS analysis

Peak #	R. Time	Area %	Height %	M. formula	M. weight (g/mol)	Molecular Name
1	10.086	75.51	47.95	$C_{12}H_{14}O_4$	222	Amirnone
2	10.420	0.46	1.41	$C_{11}H_9NO$	171	Pyridinium, 3-hydroxy-1-phenyl, hydroxide, inner salt
3	11.086	1.15	3.44	$C_{12}H_{14}O_4$	222	1,2-Benzenedicarboxylic acid, diethyl ester (CAS) Ethyl phthalate
4	11.643	0.25	0.74	$C_{17}H_{24}O_4$	292	Phthalic acid, 5-methylhex-2-yl ethyl ester
5	11.793	1.84	3.94	$C_{14}H_{28}O_2$	228	Tetradecanoic acid
6	12.078	0.16	0.44	$C_{16}H_{34}O$	242	1-Hexadecanol
7	12.617	0.65	2.09	$C_{20}H_{38}$	278	NEOPHYTADIENE
8	12.717	0.11	0.26	$C_{18}H_{36}O$	268	2-Pentadecanone, 6,10,14-trimethyl-
9	12.800	0.06	0.20	$C_{13}H_{16}O_4$	236	2-((3-Methylbutan-2-yloxy)carbonyl)benzoic acid
10	12.891	0.19	0.44	$C_{20}H_{40}O$	296	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
11	13.097	0.27	0.68	$C_{18}H_{34}$	250	1-Octadecyne
12	13.546	0.08	0.16	$C_{19}H_{38}O_2$	298	Butyl Ester Of 2,4,6,8-Tetramethylundecanoic Acid

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11 , Iss 6, 2022

13	13.774	0.13	0.28	$C_{17}H_{24}O_3$	276	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione
14	13.976	6.28	13.26	$C_{16}H_{32}O_2$	256	n-Hexadecanoic acid
15	14.228	0.11	0.27	$C_{22}H_{44}$	308	1-Docosene
16	14.797	0.32	0.21	$C_8H_5N_5O$	187	1,2,3,5,9b-Pentaazacyclopenta[a]naphthalen-4-ol
17	14.991	0.10	0.19	$C_{16}H_{32}O_2$	256	Hexadecanoic acid (CAS) Palmitic acid
18	15.546	5.00	12.83	$C_{20}H_{40}O$	296	Phytol
19	15.825	1.61	2.76	$C_{16}H_{28}O_2$	252	Oxacycloheptadec-8-en-2-one (CAS) Ambrettolide
20	15.908	2.56	4.03	$C_{18}H_{30}O_2$	278	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-
21	16.063	2.52	3.46	$C_{18}H_{34}O_2$	282	9-Octadecenoic acid (Z)- (CAS) Oleic acid
22	16.350	0.22	0.26	$C_{24}H_{48}O_2$	368	Docosanoic acid, ethyl ester
23	16.536	0.14	0.27	$C_{15}H_{26}O$	222	FARNESOL 2
24	18.845	0.20	0.26	$C_{19}H_{38}O_2$	298	Nonadecanoic acid (CAS)
25	22.887	0.10	0.14	$C_{24}H_{38}O_4$	390	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (CAS) Bis(2-ethylhexyl) phthalate

Table 5: Biological activity of phyto-components identified in the extract of the *Justicia gendarussa* leaves by GC-MS

S. No	Compound Name	Biological activity*
1	1,2-Benzenedicarboxylic acid, diethyl ester (CAS) Ethyl phthalate	Plasticizers
2	Tetradecanoic acid	**Antioxidant, Cancer preventive**, Nematicide, Hypocholesterolemic, Lubricant
3	NEOPHYTADIENE	Antihelmeththic Properties
4	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	**Cancer-preventive**
5	n-Hexadecanoic acid	**Antioxidant,** Nematicide, 5-Alpha-Reductase-Inhibitor Flavor, Hemolytic, Hypercholesterolemic Pesticide, Antialopecic, Antiandrogenic,

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11 , Iss 6, 2022

		Antifibrinolytic.
6	Hexadecanoic acid (CAS) Palmitic acid	**Antioxidant**, Pesticide, Flavor, 5-AlphaReductase-inhibitor, Antifibrinolytic, Hemolytic, Lubricant, Nematicide, Antialopecic.
7	Phytol	Antimicrobial, **Anticancer, Cancer preventive**, Diuretic, **Antiinflammatory**
8	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	**Antiinflammatory**, Insectifuge Hypocholesterolemic, **Cancer preventive**, Nematicide, Hepatoprotective, Insectifuge, Antihistaminic, Antieczemic, Antiacne, 5-Alpha reductase inhibitor, Antiandrogenic, **Antiarthritic,** Anticoronary.
9	9-Octadecenoic acid (Z)-(CAS) Oleic acid	**Antiinflammatory,** Antiandrogenic, **Cancer preventive,** Dermatitigenic Hypocholesterolemic, 5-Alpha reductase inhibitor, Anemiagenic, Insectifuge, Flavor
10	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (CAS) Bis(2-ethylhexyl) phthalate	Plasticizer

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

Uraku (2015) investigated the Chemical Compositions of Cymbopogon citrates Leaves by Gas Chromatography-Mass Spectrometry (GC-MS) Method. Six compounds were identified in the methanol leaf extract and they include; hexadecanoic acid (8.11%), hepta-9,10,11-trienoic acid (17.43%), octadecenoic acid (8.41%), 2-ethenyltetradecan-1-ol (13.28%), eicosane aldehyde (37.56%) and 1-ethoxyoctadecane (15.20%) as the major chemical constituents.

Das and Sudhakar Swamy (2016) determined the bioactive compounds by GC-MS in fruit methanol extracts -a comparative analysis of three Atalantia species from South India. Twenty seven compounds were identified from the mass spectra obtained. 1,3,4,5 Tetrahydroxycyclohexanecarboxylic acid was the major compound.

CONCLUSION

In the present study, *J. gendarussa* leaves have shown to have various secondary metabolites which possess many pharmacological properties. The GC-MS analysis showed the presence of 25 bioactive compounds that have been identified from the *J. gendarussa*. However, further research is needed in order to analyze its bioactivity and pharmaceutical applications in a quantitative and qualitative approach.

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