



**PRELIMINARY PHYTOCHEMICAL INVESTIGATION AND ESTIMATION OF
POLYPHENOLICS IN DIFFERENT PARTS OF SELECTED SPECIES OF JUSTICIA**

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Article Received on 22/01/2018

Article Revised on 11/02/2018

Article Accepted on 03/03/2018

ABSTRACT

The present research work aimed at gaining comparative phytochemical data set of different plant parts of selected *Justicia* species from Kerala, i.e. *J. adhatoda*, *J. beddomei*, *J. betonica*, *J. carnea*, *J. gendarussa*, *J. montana*, and *J. wayanadensis*. The polyphenolic content of seven species of *Justicia* are determined in the present work. The phenolic content was determined using Folin-Ciocalteu assay. Phytochemical investigation was carried out using standardized phytochemical tests. The results show that different parts of selected *Justicia* species contains significant amount of phenolic content. Highest phenolic content was estimated in aqueous extracts of various parts in comparison to that in absolute methanol extracts. Highest phenolic content in aqueous extract was shown by leaf of *J. adhatoda* (18.67 mg/GAE/g) and lowest for root of *J. gendarussa* (1.62 mg/GAE/g). In the case of absolute methanolic extracts, highest value of phenolic content was shown by root of *J. beddomei* (10.11 mg/GAE/g) and least phenolic content was as shown by root of *J. carnea* (1.69 mg/GAE/g). Different parts of selected *Justicia* species revealed the presence of alkaloids, phytosterols, phenolic and flavonoid compounds, tannins, proteins, oils, saponins etc. after phytochemical analysis.

KEYWORD: *Justicia*, Phytochemical, polyphenolic content, aqueous extract, absolute methanolic extracts.

1. INTRODUCTION

The demand for traditional healthcare preparations is snowballing in the Indian and foreign markets. The medicinal plant industry is posturing great threat due to the Unavailability of genuine raw drugs and the use of several substitutes or adulterants as the source plant. Overexploitation of these resources for prolonged periods can exhaust natural stocks to the point where they are unable to recuperate within a short time. Improved access and techniques for collection are causing the exploitation of many species beyond sustainable levels.

Acanthaceae family is an important source of therapeutic drugs and ethanopharmacological knowledge. *Justicia* is largest genus of Acanthaceae with estimated of 600 species.^[1] *Justicia adhatoda* is a highly valued Indian medicinal plant which is used in treatment of diseases like asthma, cough, bronchitis and tuberculosis due to the presence of various biologically active compounds in the plant. Polyphenolics are group of phytochemicals that account for most of biological activity in the plant products.

Present research is envisioned with obtaining an estimation of total polyphenolics present in different part of selected species of *Justicia* such as *J. adhatoda*, *J. beddomei*, *J. betonica*, *J. carnea*, *J. gendarussa*, *J. montana* and *J. wayanadensis*. Comparative analyses of total phenolic content present in different parts of various species of *Justicia*, using aqueous and absolute methanolic extracts were carried.

2 MATERIALS AND METHODS

2.1 Collection of plant materials

Different species of *Justicia* used in the present investigation, were collected from different forest/non-forest regions of Kerala. Various parts (stem, leaf and root) of different species were collected from the plants maintained in the Botanical Garden of St Joseph's college, Devagiri. The plants were authenticated by the plant systematics and genetic resources division of CMPR AVS, Kottakkal, Kerala, India and the voucher specimens are deposited at 'CMPR' Herbarium.

2.2 Extraction of Sample

The shade dried ground plant materials such as root, stem and leaf (5 g for each sample) were extracted with 100 ml each of the solvents - absolute methanol and water for

6 hours by reflux method. Filtrate obtained was made up to 100ml in standard flask and stored at 4°C for future use.

2.3 Estimation of Total Phenolics

Total Phenolic content in the plant material was determined by Folin Ciocalteu assay.^[2] An aliquot (1 ml) of extracts or standard solution of Gallic acid (20, 40, 60, 80 and 100 µg/ml) was added to 25 ml of volumetric flask, containing 9 ml of distilled water. Reagent blank using distilled water was prepared. 1 ml of Folin-Ciocalteu phenol reagent was added to the mixture and shaken. After 5 minutes 10 ml of 7% Na₂CO₃ solution was added to the mixture. The volume was then made up to the mark. After incubation for 90 minutes at room temperature, the absorbance against the reagent blank was determined at 550 nm with an UV-Visible spectrophotometer. Total phenolics content is expressed in mg Gallic Acid Equivalents (GAE).

2.4 Phytochemical screening

Qualitative analysis of phytochemicals

Major constituents of methanol extract were screened qualitatively as per standard procedure described by Jamil *et al.* 2012.^[3] Major constituents analyzed were alkaloids, tannins, flavonoids, protein, carbohydrate, saponins and glycosides.

3. RESULT AND DISCUSSION

Substantial variation in Total phenolic content between species and parts in different solvents were observed (Table I). Detection of alkaloids, tannins, flavonoids etc. in different parts of various species of *Justicia* indicates the presence bioactive metabolites (Table II). These secondary metabolites contribute to potent use of *Justicia* species in healthcare industries.

Table-I: Total Phenolics in different parts of various species of *Justicia*.

SL. No	Species	Plant Part	TOTAL PHENOLICS mg GAE/gm	
			Water	Absolute methanol
1	<i>J. adhatoda</i>	STEM	8	4.59
		ROOT	8.67	6.67
		LEAF	18.67	6.2
2	<i>J. beddomei</i>	STEM	13.03	3.25
		ROOT	13.32	10.11
		LEAF	18.53	3.45
3	<i>J. betonica</i>	STEM	2.57	2.22
		ROOT	2.11	1.77
		LEAF	9.31	4.95
4	<i>J. carnea</i>	STEM	3.28	1.81
		ROOT	3.13	1.69
		LEAF	6.05	2.54
5	<i>J. gendarussa</i>	STEM	2.75	2.32
		ROOT	1.62	2.68
		LEAF	4.53	5.04
6	<i>J. montana</i>	STEM	5.38	2.23
		ROOT	4.77	2.95
		LEAF	9.45	3.85
7	<i>J. wayanadensis</i>	STEM	3.69	1.75
		ROOT	4.58	2.27
		LEAF	3.48	2.48

Table-II: Qualitative analysis of different classes of phytochemicals.

SL. No.	Species	Plant Part	ALKALOIDS		FLAVIONIDS		GLYCOSIDES	FIXED OILS AND FAT	CARBOHYDRATE	SAPONINS	GUM & MUCILAGE	STEROLS	PROTEIN	PHENOLICS AND TANNINS					
			MAYERS TEST	DRAGEN TEST	Aq. NaOH Test	Conc. H ₂ SO ₄	BORNTRANGE TEST	SPOT TEST	SAPONIFICATION TEST	MOLISCH TEST	FEHLING TEST	FORM TEST	PRECIPTATION TEST	LIBERMANN BURCHARD TEST	LIBERMANN STEROL TEST	BIURET TEST	NINHYDRIN TEST	FERIC CHLORIDE TEST	LEAD ACETATE TEST
1	<i>J. adhatoda</i>	STEM	+	+++	++	+++	+++	++	+++	++	+	-	---	-	-	++	+		
		ROOT	+++	+	-	+++	+	-	+++	+++	+++	-	+	-	-	+	++	+	
		LEAF	++	++	+	+++	++	++	+++	+	+	-	++	-	-	-	+++	+++	
2	<i>J. beddomei</i>	STEM	+	+++	+	+++	+	+	++	+	+	-	---	-	-	+	+		
		ROOT	+++	-	+	++	-	-	-	+	+++	-	+	+++	-	-	+++	++	++
		LEAF	++	++	-	-	+++	+++	+++	+	+	-	+++	+	-	-	+++	++	
3	<i>J. betonica</i>	STEM	+	+	+	+++	++	++	++	+	++	-	---	-	-	++	+	+	
		ROOT	-	-	-	+++	+	-	-	+++	+++	-	+++	-	-	++	++	+	++
		LEAF	+	++	++	+	+++	++	++	+	+	-	++	-	-	++	+	++	++
4	<i>J. carnea</i>	STEM	+++	+	+	+++	+++	++	+++	++	+	+	---	-	-	++	+	+	+
		ROOT	-	-	-	++	-	-	-	+++	+	-	++	++	-	++	++	+	+++
		LEAF	++	+	+	+	+	+++	++++	++	+	-	+++	++	++	++	+	+++	++

5	<i>J. gendarussa</i>	STEM	+	+	++	+++	+++	+	+++	+++	+++	-	---	-		++	+	+	+
		ROOT	++	-	-	+	-	-	-	+++	++	-	++	+	-	++	+++	+	+
		LEAF	+	+++	+++	+++	+++	++	++	++	++	-	++	+	++	+	+	+++	+++
6	<i>J. montana</i>	STEM	+	++	+	+++	+++	++	+++	++	+++	-	--	--		++	+	+	+
		ROOT	++	+	-	+++	-	-	-	+++	++	++	+++	+++	+	++	++	++	++
		LEAF	+	++	+	+	-	+	++	+	+	-	+++	-			-	+++	+++
7	<i>J. wayanadensis</i>	STEM	+	+++	+	+++	+++	+	++	+++	++	+	++	-	++	+	+	+	++
		ROOT	+	-	-	+	-	-	-	+++	++	+	+++	+	-	++	++	+	+++
		LEAF	+	++	++	++	+++	++	+++	+	+	-	++	-	-	-	++	++	-

Critical analysis of the results shows that both supporting and contradicting literatures are available. John *et al.*, 2013^[4] has reported that highest phenolic content was shown by leaf of *J. adhatoda* (38.75 mg GAE) and the least phenolics were observed for root of *J. beddomei* (22.65 mg GAE). In the current study, highest TPC was obtained for leaf of *Justica adhatoda* in water extract (18.67 mg/ GAE/gm). Lowest TPC was observed in root samples of *J. gendarussa* in water (1.62 mg/ GAE/gm). Among aqueous and methanolic extract of leaf samples of different species of *Justicia*, highest TPC was observed in *J. adhatoda* and least in the *J. wayanadensis*. In the case of methanolic and aqueous extract of root samples highest TPC was observed in *J. beddomei*. Least TPC was found in the *J. gendarrussa* water extract. While in the methanolic extract least TPC was found in *J. carnea*. Within the water extract stem samples of different species of *Justicia* showed highest TPC in *J. beddomei* and least in *J. betonica*.

Akhtar *et al.*, 2015 observed that Methanol/chloroform (1:1) proved to be a better solvent system for extraction of wide range of metabolites from medicinal plants.^[5] Jayapriya *et al.*, 2015 found out that methanol extract showed higher extractive value when compared to other solvent.^[6]

Phenolic contents may be affected by seasons and climatic changes. Variation of phenolic content may be due to various reasons such as variation in maturity of plant materials, weather, geographic location of the plants and procedure used for their phytochemical extraction. Katalinic *et al.*, 2006 while studying phenolic content of 70 medicinal plants observed a significant variation^[7]. Seasonal variation in the antioxidant activity and content of phenolic compounds *Sorbus aucuparia* leaf extract was reported earlier by Olszewska.^[8] Rahiman *et al.*, 2013 reported that air drying time can also cause variation in total phenolic content in some medicinal plants.^[9]

CONCLUSION

Result showed that the content of total phenols expressed as gallic acid equivalents (GAE/GM) dry weight of plant ranged from 1.69 to 10.11 mg/ GAE/gm in methanolic extract and 1.62 to 18.69 mg/GAE/gm in aqueous extract. In the present study, aqueous solvent is proved to be a better solvent system for extraction of polyphenols in comparison to methanol. It has been shown that all the three parts of selected seven species of *Justicia* are sources of polyphenolic compounds and other phytochemicals. The phytochemical information in the present work may inspire new biomedical application for species of *Justicia*.

ACKNOWLEDGEMENTS

The authors are thankful to the authorities of Centre for Medicinal Plants Research, AVS, Kottakkal, Kerala especially Dr. Sulaiman C. T., Senior Scientist,

Phytochemistry Division for providing necessary facilities for carrying out this work.

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