

## Phytochemical Screening of *Justicia Gendarussa*

**Muameead Ramees K, Hamath k, Nileena F, Rosemary K, Sinju P  
Raju, Nobin C Joy and Suresh J\***

Department of Pharmacognosy, JSS College of Pharmacy, JSS Academy of Higher Education & Research, India

**\*Corresponding author:** Suresh Joghee, Department of Pharmacognosy, JSS College of Pharmacy, JSS Academy of Higher Education & Research, Mysuru, Karnataka, India, E-mail: jsuresh@jssuni.edu.in

### Research Article

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### Abstract

*Justicia gendarussa* belongs to family Acanthaceae is commonly known as Nili-Nirgundi. It is traditionally used to treat various diseases such as the wound healing, antioxidant, anti-proliferative etc. The aim of the present study was to investigate the qualitative and quantitative estimation of phytoconstituents of aerial parts of alcoholic extract of *Justicia gendarussa*. Physiochemical constant, preliminary phytochemical analysis for carbohydrates, glycosides, alkaloids, flavonoid, phenols, tannins and saponin and quantitative analysis for total flavonoid and total alkaloid content were carried out by standard procedures. The physiochemical constituents like total ash and acid insoluble ash, water soluble ash were found to be 14.25%w/w, 05%w/w, 7.30%w/w respectively. The results confirmed the presence of carbohydrates, glycosides, alkaloids, flavonoid, phenols, tannins and saponin and the plant extracts shows higher yield of total phenolic content and also flavonoid content.

**Keywords:** *Justicia Gendarussa*; Ash Value; Flavonoid Content and Alkaloid Content

### Introduction

*Justicia gendarussa* belongs to family Acanthaceae is commonly known as Nili-Nirgundi. The plant is up to one meter height and found in tropical and subtropical parts of Asia and in India at seashore area like Valsad, Surat and Hills like Khasi hills, Pavagarh. *J. gendarussa* herb is cultivated in Indianguardens for its attractive foliage and flowers. The herb is cultivated in Indian gardens for its attractive foliage and flowers [1-3]. Most are tropical herbs, shrubs or twining vines, some are epiphytes. Only a few species are distributed in temperate region. The four main centres of distribution are; Indonesia, Malaysia, Africa, Brazil and Central America [1-3]. Warrer willow is an erect, branched, smooth undershrub, 0.8-1.5 m high. Leaves are opposite, lanceolate, green or variegated with short

petioles, 7-14 cms long, 1-2.5 cm wide, pointed at the ends. Flowers are small, in 4-12 long spikes, terminal or in axils of leaves. Calyx-teeth are smooth, linear, and about 3mm long. Corolla is nearly smooth, 1.5cms long white or pink with purple spots. Capsule is club shaped, about 12mm long and smooth [1].

Kirtikar CR and Basu BD, Indian Medicinal Plants, F. L. M.Basu had reported the sequential screening for phytochemicals of *j.gendarussa* leaves are rich in alkaloids, flavanoids, saponin, carbohydrates, steroids, triterpenoids, carotenoids, aminoacids, tannins, phenolics, coumarines and Anthraquinones. Leaves contain a bitter alkaloid (justiciine) and they are rich in potassium salts. Root is bitter in taste considered anodyne, antiperiodic,

antiplasmodic, carminative, diaphoretic, diuretic, emetic, febrifuge and laxative [2].

An extract of the leaves or young shoots is used as an emetic in coughs and asthma in the Philippines, whereas fresh leaves are applied as topical to cure oedema of beriberi and rheumatism. A decoction of the leaves is used for bathing during childbirth. In Malaysia, the leaves are much applied for politicking to treat headache and pains, as a lotion to treat swellings and rheumatism, and in a bath after confinement, the roots for treating thrush and cough [4]. The leaves are also used in preparations to treat gonorrhoea, amenorrhoea and malaria. In Indonesia, the leaves are used to treat headache, rheumatism and pain. In Vietnam, the leaves are applied externally, as a poultice, decoction or tincture, to treat rheumatic arthritis and swellings.

In Thailand, the roots are used against diuretic, diarrhoea and as antivenin; the bark is used as antipyretic, anti cough, diuretic and anti-amoebic, in the treatment of wounds and allergy; the leaves are taken internally against cough, fever and as a cardiogenic, and used externally to treat inflammation, wounds and allergy. Numerous medicinal uses are recorded from India and China; the roots are used to treat rheumatism, dysuria, fever, carbuncles, jaundice and diarrhoea, the leaves as a diaphoretic and febrifuge and to treat lumbago, amenorrhoea, swellings, coughs, asthma, eczema, cephalalgia, hemiplegic, facial paralysis, earache and hemicranias, and the bark as emetic. Magical uses are reported for Indonesia, Malaysia and the Philippines. *Justicia gendarussa* is cultivated as an ornamental, and is often used for living fences.

The phytochemical analysis of the plants is very important commercially and has great interest in pharmaceutical companies for the production of the new drugs for curing of various diseases. The phytochemical research approach is considered effective in discovering bioactive profile of plants of therapeutic importance [5].

## Material and Method

### Collection and Authentication of Plant Material

The fresh aerial parts of *Justicia gendarussa* was collected in the month of October from kozhikode in Kerala and sample was authenticated by Dr.M.N.Naganandini, Asst. Professor, Dept.of Pharmacognosy, J.S.S.C.P, Mysore. After authentication the plant materials were dried under shade and after optimum drying, coarsely powdered and stored in well closed container till further use.

### Determination of Physicochemical Constants [6,7]

Determination of Ash values, extractive values, foreign organic matter and moisture content were carried out by using standard procedure.

### Preliminary Phytochemical Analysis [8]

A systematic and complete study of crude drugs should include a complete investigation of both primary and secondary metabolites derived from plant metabolism. The different qualitative chemical tests are to be performed for establishing profiles of the given extracts for their nature of chemical composition. Following chemical tests were carried out for different extracts of *Justicia gendarussa* to identify the presence of various phytochemical constituents.

**Tests for Carbohydrates [9]:** Small quantities of alcoholic extract was dissolved in little quantity of distilled water and filtered separately. The filtrates were used to test the presence of carbohydrates.

**Molisch's Test:** The extracts were treated with Molisch reagent and concentrated sulphuric acid was added along the sides of the test tube to form a layer. A reddish violet ring shows the presence of carbohydrates.

**Fehling's Test:** Filtrates were hydrolysed with dilute hydrochloric acid, neutralized with alkali and heated with equal amounts of Fehling's A and B solutions. Formation of green to yellow to red precipitate indicates the presence of reducing sugars.

**Benedict's Test:** Mix equal volume of Benedict's reagent and extract in the test tube and heat in a boiling water bath. Solution appears green, yellow or red, indicates the presence of reducing sugars.

### Test for Proteins

**Biuret Test:** To 3ml of Extract add 4% NaOH and few drops of 1% CuSO<sub>4</sub> solution. Violet or Pink colour indicates the presence of proteins.

**Millon's Test:** Mix the extract with Millon's Reagent, white precipitate is formed which turns into brick red on warming which indicates the presence of proteins.

### Test for Amino Acids

**Ninhydrin Test:** Heat 3ml extract and 3 drops of Ninhydrin solution in boiling water bath for 10 min. Purple or bluish colour indicates the presence of aminoacids.

### Tests for Sterols

Alcoholic extract were dissolved in chloroform, filtered and the filtrate was tested for sterols and tri-terpenes.

**Salkowski Test:** Few drops of concentrated sulphuric acid was added to the chloroform solution, shaken and allowed

to stand, appearance of red colour in lower layer indicates the presence of sterols.

**Liebermann-Burchard Test:** To the chloroform solution, few drops of acetic anhydride was added and mixed well. 1 ml of concentrated sulphuric acid was added from the sides of the test tube, appearance of reddish brown ring indicates the presence of sterols.

#### Test for Saponins

**Foam Test:** Small amount of alcoholic extract was shaken with little quantity of water, if the foam produced persists for 10 minutes; it indicates the presence of the saponins.

**Haemolysis Test:** To 2 ml of 1.8% sodium chloride solution in two test tubes, 2 ml distilled water was added to one of the test tube and to the other 2 ml of 1% sample of alcoholic extract was added. 5 drops of blood was added to each tube and gently mixed the contents. If the haemolysis observed under the microscope in the tube containing the extract it indicates the presence of saponins.

#### Tests for Alkaloids [10]

Alcoholic extract was basified with ammonia and extracted with chloroform. The chloroform solution was acidified with dilute hydrochloric acid. The acid layer was used for testing the alkaloids.

**Wagner's Test** (Iodine in Potassium Iodide): The acid layer was treated with few drops of Wagner's reagent. Formation of reddish brown precipitate indicates the presence of alkaloids.

**Mayer's Test** (Potassium Mercuric Iodide solution): The acid layer was treated with few drops of Mayer's reagent. Formation of creamy white precipitate indicates the presence of alkaloids.

**Dragendorff's Test** (Potassium Bismuth Iodide): The acid layer was treated with few drops of Dragendorff's reagent. Formation of reddish brown precipitate indicates the presence of alkaloids.

**Hager's Test:** The acid layer was treated with few drops of Hager's reagent. Formation of yellow precipitate indicates the presence of alkaloids.

#### Test for Tannins

**Ferric Chloride Test :** To the alcoholic extract a few drops of 1% neutral ferric chloride solution was added, formation of blue, green or brownish green colour indicates the presence of Tannins.

**Vanillin-Hcltest:** To the solution of alcoholic extract , 1-2 ml of Vanillin Hydrochloride reagent was added. Pink to red color indicates presence of Tannins.

#### Tests for Flavonoids [11]

**Shinoda Test:** To the alcoholic solution of alcoholic extract, a few fragments of magnesium ribbon and

concentrated hydrochloric acid were added. Appearance of magenta colour after few minutes indicates the presence of flavonoids.

**Ferric Chloride Test:** Few drops of neutral ferric chloride solution were added to little quantity of alcoholic extract. Formation of blackish green colour indicates the presence of phenolic nucleus.

**Lead Acetate Test:** Few drops of lead acetate solution (10%) were added to the alcoholic extract. Formation of yellow precipitate indicates the presence of flavonoids.

**Zinc-Hydrochloric Acid Reduction Test:** The alcoholic solution of alcoholic extract was treated with a pinch of zinc dust and few drops of concentrated hydrochloric acid. Formation of Magenta colour after few minutes indicates the presence of flavonoids.

**Naoh Test:** To alcoholic solution few drops of NaOH was added. Intense yellow color which disappeared after adding dilHCl indicates presence of flavonoids.

#### Test for Glycosides [8]

**Borntragers Test:** 2ml of chloroform solutions of the extracts of *Justicia gendarussa*. were treated with 1ml of dilute (10%) ammonia and shaken. Pink-red color in the ammoniacal (lower) layer indicates the presence of anthracene derivatives.

**Cardiac Glycosides:** 2ml of chloroform solutions of the extracts of *Justicia gendarussa*. were treated with conc. H<sub>2</sub>SO<sub>4</sub>. Appearance of ring at the interphase indicates the presence of deoxysugars in cardenolides.

### Estimation of Total Flavonoid Content [12,13]

Flavonoids are water-soluble polyphenolic compounds, which are extremely common and wide spread in the plant kingdom as their glycosides. The word 'flavonoid' is derived from the Latin word "flavus" meaning yellow and many flavonoids are indeed yellow in colour. It consists of a single benzene ring joined to a benzo-gamma-pyrone structure. The different classes within the group are distinguished by additional oxygen containing heterocyclic rings and hydroxyl groups. These include catechins, leucoanthocyanidins, flavonones, flavones, anthocyanidins, flavonols, chalcones, auronones and isoflavones. Total flavonol was determined by aluminium chloride colorimetric method. The principle of this method is that aluminium chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols. In addition, aluminium chloride forms acid labile complexes with the orthodihydroxyl groups in the A or B ring of flavonoid.

### Chemicals and Reagents

Aluminium chloride (10%): 10 g of aluminium chloride was dissolved in 100 ml of distilled water, filtered and used.

Potassium acetate (1 M): 98.1 g of potassium acetate was dissolved in 1 litre of distilled water and used.

### Preparation of Test Solutions

100 mg each of the extracts were dissolved in 10 ml of methanol to get 10 mg/ml solutions.

#### Preparation of Standard Solution

Rutin: 100 mg of rutin was dissolved in 100 ml of methanol to get 1000 µg/ml of solution. It was then serially diluted with methanol to obtain solutions ranging from 10 µg/ml to 250 µg/ml.

### Procedure

The extract (.5g) was mixed with 1.5 ml methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm with a Shimadzu UV-160A Spectrophotometer (Shimadzu Corporation, Japan). Using rutin, a standard curve was prepared and linearity was obtained in the range of 110 µg/ml. Using the standard curve the total flavonol content of extract was obtained. The total flavonol content was expressed as rutin equivalent in mg/g or % w/w of the extracts.

### Estimation of Total Alkaloid Content [14]

Plant extract dissolved in 2N HCl and then filter. 1ml of this solution was transferred the separating funnel and washed with 10 ml chloroform. The pH of the phosphate buffer solution was adjusted to neutral with 0.1N NaOH, 1ml of this solution was transferred to a separating funnel and then 5ml of bromocresol solution along with 5 ml phosphate buffer were added. The mixture was shaken and the mixture formed was fractioned with chloroform by vigorous shaking. The fraction were collected in 10 ml volumetric flask and diluted to volume with chloroform. The absorbance was measured at 470nm. All experiment

performed thrice, the results were averaged and reported in form of mean ± SEM.

## Results and Discussion

### Collection and Authentication of Plant Material

The fresh aerial parts of *Justiciagendarussa*. was collected in the month of October from kozhikode in Kerala and sample was identified and authenticated by Dr.M.N.Naganandini, Asst. Professor, Dept.of Pharmacognosy, J.S.S.C.P, Mysore. After authentication, the plant materials were dried under shade and after optimum drying, coarsely powdered and stored in well closed container till further use.

### Extraction of Plant Material

The percentage yield of ethanolic extract of aerial parts of *Justicia gendarussa* was found to be 10%w/w.

### Morphological Characters

Warrer willow is an erect, branched, and smooth under shrub, 0.8-1.5 m high. Leaves are opposite, lanceolate, green or variegated with short petioles, 7-14 cm long, 1-2.5 cm wide, pointed at the ends. Flowers are small, in 4-12 long spikes, terminal or in axils of leaves. Calyx-teeth are smooth, linear, and about 3mm long. Corolla is nearly smooth, 1.5cms long white or pink with purple spots. Capsule is club shaped, about 12mm long and smooth.

### Physicochemical Constants

Determination of physicochemical constants is important for the purpose of evaluation of crude drugs. The quality parameters of the drugs as raw material were established with the help of several official determination based on physiochemical studies. The studies were aimed at ensuring standardization of herbal drugs under investigation.

The physicochemical constants like ash value, extractive value, moisture content and foreign matter was determined. The dried aerial parts of *Justicia gendarussa* show higher percentage of total ash (14.1±0.705% w/w), acid insoluble ash (0.74±0.037) and water soluble ash (4.84±0.242% w/w). The results are shown in the Table 1.

Name of the species	Total Ash (%w/w)	Acid Insoluble Ash (% w/w)	Water Soluble Ash (%w/w)
<i>Justicia gendarussa</i>	14.1±0.705	0.74±0.037	4.84±0.242

Table 1: Physicochemical constants of the aerial parts of *Justicia gendarussa*.

### Extractive Value

The dried leaves of *Justicia gendarussa* showed alcohol soluble extractive (12.42±0.821) and water soluble extractive (16.52±0.626) respectively. The reports are shown in Table 2. Extractive values are useful for evaluation of crude drugs and gives idea about the nature of the chemical constituents present in them. The amount

of extractive, a drug yield to a given solvent, is often an approximate measure of a certain constituents of group of related constituents the drug contains. In some cases the amount of drug soluble in a given solvent is an index of its purity. Extractive values are primarily useful, for the determination of exhausted of adulterated drugs.

S.No	Parameters	Values(%w/w)
1.	Alcohol soluble extractive value (%w/w)	12.42±0.821
2.	Water soluble extractive value (%w/w)	16.52±0.626

Table 2: Alcohol soluble extractive (12.42±0.821) and water soluble extractive (16.52±0.626).

### Preliminary Phytochemical Analysis

Phytochemicals are chemical compounds that occur naturally in plants (phyto means "plant" in Greek). Some are responsible for colour and other organoleptic properties, such as the deep purple of blueberries and the smell of garlic. They are also biologically significant by playing an essential role in the plants to defend themselves against various pathogenic microbes by showing the antimicrobial activity by inhibition or killing mechanisms<sup>15</sup>. The secretion of these compounds is varying from plant to plant some produce more and some produce in minimal quantity. Sometimes they can be harmful and sometimes they can be very helpful. Preliminary phytochemical studies of *Justiciagendarussa* reveal the presence of carbohydrates, tannins, flavonoids, glycosides, phenols, saponins and amino acids. The results obtained were shown in Table 3.

Phytochemical Constituents	Alcoholic extract of <i>Justiciagendarussa</i>
Carbohydrate	+
Proteins	-
Amino acids	+
Steroids	-
Saponins	+
Alkaloids	+
Tannins	+
Flavonoids	+
Glycosides	+

(+ = Present in test, -- = Absence in test)

Table 3: Phytochemical investigation of *Justicia gendarussa*.

### Estimation of Flavonoid Content and Total Alkaloid:

The alcoholic extract was evaluated for total flavonoid content and the total content was found to be 3.25µg/ml as

shown in table 4. The total flavonoid contents can be determined in the sample extract by reaction with sodium nitrite followed by the development of coloured flavonoid aluminium complex formation using aluminium chloride in alkaline condition which can be monitored spectrophotometrically at wavelength of 415nm. Several studies show that flavonoids present in herbal drugs significantly contribute to their antioxidant activity.

The alcoholic extract was evaluated for total flavonoid content and the total content was found to be 0.420µg/ml as shown in table 4.

Sl.no	Plant extract	Flavonoid content µg/ml	Alkaloid content µg/ml
1	<i>Justicia gendarussa</i>	3.25	0.420

Table 4: Flavonoid content and Total alkaloids.

### Summary and Conclusion

In this present study the writer has investigated alcoholic extracts of the plant *Justicia gendarussa* for their preliminary phytochemical studies in a systematic way in order to give possible scientific support for the tribal claims.

The following are some of the important finding made in the present study.

- The quality control parameters of the plants, namely, ash values and extractive values, based on the physical and physico-chemical studies would ensure standardization of herbal drugs. These parameters observed in the present study may be useful for the proper identification of the plant species,
- The phytochemical studies of the plant extracts show the presence of carbohydrates, tannins, flavonoids, glycosides, phenols, saponins and amino acids
- The total flavonoid and alkaloids content was estimated quantitatively.

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