Botanical and Chemical Fingerprinting of Medicinal Roots of *Justicia gendarussa* Burm f.

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**ABSTRACT**

**Background:** Justicia gendarussa Burm f. of family Acanthaceae is medicinally important herb used in the treatment of inflammatory disorders, asthma, hepatic injuries, pathogenic infection and also shows antiproiferative activity against various cancer cell lines. **Materials and Methods:** Pharmacognostical evaluation (macro-microscopy, physicochemical analysis and preliminary phytochemical analysis), high-performance thin layer chromatography (HPTLC) fingerprinting and chemical profiling by gas chromatography-mass spectrometry (GCMS) of dried roots of *J. gendarussa* were done according to quality standard procedures. **Results:** Microscopic analysis revealed the compact arrangement of cells in cork region and thin-walled cortex beneath epidermis. Parenchymatous cells with xylem vessel were observed in the roots of *J. gendarussa*. Physicochemical studies revealed loss on drying (10.474%), total ash (2.990%), acid-insoluble ash (0.099%), water-soluble ash (15.28%), alcohol-soluble extractive value (0.564%) and water-soluble extractive value (4.11%) of the raw drug. Preliminary phytochemical analysis of ethanolic extract of *J. gendarussa* showed the presence of alkaloid, steroid, flavonoid, phenol, carbohydrate, saponin and quinone. *R* value of the spots and densitometric scan were recorded by HPTLC fingerprinting using toluene: ethyl acetate: formic acid (5.0:4.0:1.0). On photodocumentation, six spots were visualized under 254 nm, nine spots under 360 nm and six spots at 620 nm. Identification of components in ethanolic extract of *J. gendarussa* was done by GC-MS. GC-MS results in the presence of oleic acid, 9,12-octadecadienoic acid, 6,9,12-octadecatrienoic acid and estra-1,3,5(10)-trein-17-β-ol as bioactive compound.

**Key words:** Gas chromatography-mass spectrometry analysis, high-performance thin layer chromatography fingerprinting, Justicia gendarussa, pharmacognostic, quality control

**SUMMARY**

- Transverse section and powder of dried roots of *Justicia gendarussa* were examined microscopically. Microscopic observations showed the presence of well-developed cork and cortex. Presence of xylem vessels and parenchymatous rays were observed in transverse section. Parenchymatous cell and sclereids with vessel elements were found in powder microscopy
- Physicochemical studies revealed loss on drying (10.474%), total ash (2.990%), acid-insoluble ash (0.099%), water-soluble ash (15.28%), alcohol-soluble extractive (0.564%) and water-soluble extractive (4.11%)
- Preliminary phytochemical analysis of ethanolic extract of *J. gendarussa* showed the presence of alkaloid, steroid, flavonoid, phenol, carbohydrate, saponin and quinone

**INTRODUCTION**

*J. gendarussa* Burm f. Syn. *Gendarussa vulgaris* Nees is shade friendly, rapidly escalating and fragrant herb grown in India. *J. gendarussa* is commonly known as Nili nirgunthi and Krishna nirgundi in Hindi, Bakas and Kala aduls in Marathi, Kasanah and Vaidhyasinha in Sanskrit, and Karunochi in Tamil.[1-3] It is an erect, branched and smooth herb about one meter in height, leaves of *J. gendarussa* are linear-lanceolate and glabrous in appearance and flowers are small, white with pink or purple spots inside [Figure 1a and b].

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J. gendarussa is traditionally used in the treatment of chronic rheumatism, inflammations, bronchitis, eye diseases, fever, headache, earache, muscle pain, respiratory disorder and digestion problems.\(^4\)^

Roots of J. gendarussa are known to possess antipyretic, antiangiogenic, antimicrobial, antinociceptive and antiproliferative activity. Previous studies on J. gendarussa revealed the presence of active phytoconstituents such as flavonoids, alkaloids, triterpenoid saponins, amino acids, aromatic amines, stigmasterol and lupeol which help in reducing the oxidative stress.\(^2,9\)^

Ethanolic extract of this plant showed a significant antiarthritic activity against Freund's adjuvant-induced and collagen-induced arthritic rat models.\(^14\)^

Leaves and stem of J. gendarussa have been reported for anthelmintic activity and antibacterial activity against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Vibrio cholera.\(^15\)^

Methanolic extract of leaves of J. gendarussa is reported for its cytotoxic effect against some human cancer cell lines and also helps in ameliorating the CCl\(_4\)-induced hepatic injury.\(^19\)^

Earlier findings of expertise validate that methanolic extract of its roots possess anti-inflammatory potential against carrageenan-induced inflammation and an ethyl acetate fraction isolated from methanolic extract of roots of J. gendarussa showed the anti-inflammatory effect by inhibiting the expression of iNOS and COX-2 through NF-\(\kappa\)B pathway.\(^22\)^

The present study was designed to prepare a complete monograph for standardization and authentication of roots of J. gendarussa in dried form as this plant is still untouched drug in Ayurveda Pharmacopoeia of India and Quality Standards of Indian Medicinal Plants.

**MATERIALS AND METHODS**

**Macro-microscopic analysis**

Macroscopic characters of dried roots and powder were keenly observed under naked eyes to record the specific botanical characters. The external features of the test samples were documented using Canon IXUS digital camera.

Dried roots were preserved in formalin-acetic acid-alcohol preservative solution (5% formalin - 5 ml, 5% acetic acid - 5 ml and 50% ethyl alcohol - 90 ml).\(^{22}\) After 48 h, very thin transverse sections of root were obtained using sharp blade followed by safranin staining for microscopic visualization. Features were photographed using Zeiss AXIO trinocular microscope attached with Zeiss AxioCam camera under bright field light. Magnifications of figures are indicated in scale bars.

A pinch of coarse powder sifted through 80 pore size mesh was mixed with drops of choral hydrate on microscopic slides and mounted with a drop of glycerin-water. Slides were observed and characterized under Zeiss AXIO trinocular microscope. Magnifications are indicated by scale bars.\(^{22}\)

**Physicochemical analysis**

Physicochemical characterization such as loss on drying (LOD) at 105\(^\circ\)C, total ash (TA), acid-insoluble ash (AIA), water-soluble ash (WSA), alcohol-soluble extractive (ASE) value and water-soluble extractive (WSE) value were determined as per Quality Standard of Indian Medicinal Plants.\(^{26}\)

**Preliminary phytoconstituents screening**

Preliminary phytoconstituents screening was done to detect the presence of active constituents in the ethanolic extract of J. gendarussa.\(^{27}\)

**High-performance thin layer chromatography fingerprinting**

One gram of powdered roots was extracted with 10 ml ethanol and kept for cold percolation for 24 h and filtered. 4, 8 and 12 \(\mu\)l of the plant extract were applied on a precoated silica gel F254 on aluminum plates to a bandwidth of 7 mm using Linomat 5 (CAMAG, Muttenz, Switzerland) TLC applicator. J. gendarussa plate was developed using toluene:ethyl acetate:formic acid (5.0:4.0:1.0) as mobile phase in CAMAG twin trough chamber. The developed plate was visualized under short UV, long UV in CAMAG TLC photodocumentation unit, then derivatized with...
anisaldehyde-sulfuric acid reagent, and scanned under UV 254, 366 and 620 nm postderivatisation. \( R_f \) color of the spots and densitometric scan were recorded using CAMAG Scanner 4.

**Gas chromatography-mass spectrometry analysis**

Gas chromatography-mass spectrometry (GC-MS) analysis was carried out using Thermo Scientific Mass Spectrophotometer equipped with Triple Quad XLS. The column used was HP-5ms Ultra Inert (length: 30.0 m; diameter: 0.25 mm), with a film thickness of 0.25 \( \mu \)m. The carrier gas used was helium at a flow rate of 1.3 ml/min at a constant rate. Two microliters sample injection volume was utilized. The inlet temperature was maintained as 280°C. The oven temperature was programmed initially at 60°C for 3.5 min and then programmed to increase to 300°C at a rate of 10°C. Total run time was 22 min. The MS transfer line was maintained at a temperature of 240°C. MS was recorded using electron impact at fixed electron energy of 70eV and data were evaluated using total ion count for compound identification and quantification.

### RESULTS

**Macro-microscopic observations**

Macroscopically, the dried roots of *J. gendarussa* were about 10 cm long with a diameter of 0.5 cm. Dried roots were yellowish brown in color with rough and wrinkled surface and root scars. Powder of root was yellowish in color [Figure 1c-e] with pleasant odor. Transverse section of dried root showed elongated, compactly arranged cork cell and cortex of root was well developed under epidermis. Outer and inner region of root showed the presence of xylem vessels with some intracellular spaces. Xylem vessels were spherical and oval in shape. Large xylem vessels were found toward the outer region and their size was gradually decreased toward the inner region near pith. Parenchymatic rays were arranged in a uniseriate manner [Figure 1f-k]. Powder microscopy showed the presence of pitted lignified parenchymatous cells with lobed projection, sclereids of various dimensions were scattered and fiber sclereids with vessel elements were found. Group of stone cells were also observed in powder microscopy [Figure 2a-l].

**Physicochemical analysis**

Physicochemical characters such as LOD, TA value, AIA, water-insoluble ash, ASE and WSE are expressed in %w/w [Table 1].

**Preliminary phytochemical analysis**

According to Harborne’s methodology, phytoconstituents analysis revealed the presence of carbohydrates and some secondary metabolites such as alkaloids, steroids, flavonoids, phenols, saponins and quinone [Table 2].

**High-performance thin layer chromatography fingerprinting**

\( R_f \) values and color of the spots in chromatogram developed in toluene: ethyl acetate:formic acid (5:0.4:0.1:0.1) for ethanolic extract of dried roots were recorded [Table 3]. TLC photodocumentation revealed the presence of many phytoconstituents at different \( R_f \) values. High-performance thin layer chromatography (HPTLC) densitometric scan of the plates showed numerous bands under short UV, long UV and 620 nm (after derivatization). On photodocumentation, under short UV, six spots were observed; under long UV, there were nine spots and under 620 nm on postderivatization with anisaldehyde-sulfuric acid spray reagent, six spots were recorded [Figure 3a-c]. Densitometric scan at 254 nm revealed ten peaks corresponding to ten different compounds in the ethanolic extract, compounds with \( R_f \) 0.04 (40.01%), 0.17 (11.99%), 0.23 (26.47%), 0.40 (1.95%), 0.42 (1.71%), 0.53 (0.93%), 0.62 (9.59%), 0.67 (1.77%), 0.80 (1.96%) and 0.90 (3.62%) are shown in Figure 4a.

Densitometric scan at 366 nm [Figure 4b] showed six peaks such as \( R_f \) - 0.05 (3.71%), 0.15 (4.05%), 0.25 (2.20%), 0.58 (5.97%), 0.64 (2.61%) and 0.96 (81.46%). Figure 4c depicts seven peaks with \( R_f \) - 0.03 (23.91%), 0.23 (22.20%), 0.26 (14.84%), 0.48 (2.98%), 0.72 (6.91%), 0.81 (26.22%), and 0.86 (2.93%) after postderivatization at 620 nm.

### Table 1: Physicochemical analysis of dried roots of *Justicia gendarussa*

| Parameter (%w/w) | Mean±SE (n=3) |
|------------------|--------------|
| Loss on drying   | 10.47±0.002  |
| Total ash        | 2.990±0.004  |
| Acid-insoluble ash| 0.099±0.099  |
| Water-soluble ash | 1.528±0.034  |
| Alcohol-soluble extractive value | 0.56±0.122  |
| Water-soluble extractive value | 4.17±0.005  |

SE: Standard error

### Table 2: Preliminary phytochemical screening

| Test | Colour if positive | Inference |
|------|--------------------|-----------|
| Alkaloid | Orange red precipitate | +ve |
| Wagner's test | Reddish brown precipitate | +ve |
| Mayer's test | Dull white precipitate | +ve |
| Hagers test | Yellow precipitate | +ve |
| Steroid | | |
| Liebermann-Burchard test | Bluish green color | +ve |
| Salkowski test | Bluish red to cherry red color in chloroform layer and green fluorescence in acid layer | +ve |
| Carbohydrate | | |
| Molisch's test | Violet ring | +ve |
| Fehling's test | Brick red precipitate | +ve |
| Benedict's test | Red precipitate | +ve |
| Tannin | | |
| With FeCl₃ | Dark blue or green or brown | –ve |
| Flavonoids | | |
| Shinoda's test | Red or pink | +ve |
| Saponins | | |
| With NaHCO₃ | Stable froth | +ve |
| Terpenoid | | |
| Tin and thionyl chloride test | Pink | +ve |
| Coumarins | | |
| With 2N NaOH | Yellow | –ve |
| Phenol | | |
| With alcoholic ferric chloride | Blue to blue-black, brown | +ve |
| Carboxylic acid | | |
| With water and NaHCO₃ | Brisk effervescence | –ve |
| Amino acids | | |
| With Ninhydrin reagent | Purple colour | –ve |
| Resins | | |
| With aqueous acetone | Turbidity | –ve |
| Quinone | | |
| Concentrated sulfuric acid | Pink/purple/red | +ve |
were found in trace amounts [Table 4]. Compounds identified from GC-MS analysis were fatty acids (oleic acid, 9,12-octadecadienoic acid and 6,9,12-octadecatrienoic acid) and steroids (estra-1,3,5(10)-trein-17-β-ol), which correlate well with the results of phytochemical screening. Mass spectrum of ethanolic extract of *J. gendarussa* indicated the similarity of identified compounds and structures with different retention times as expressed in Figures 5a-d and 6.

**DISCUSSION**

Pharmacognostic analysis with physicochemical studies and HPTLC fingerprinting was done for authentication and quality control of drug. Macro-microscopic characters showed the presence of compactly arranged cork cell and cortex. Transverse section of root showed the presence of xylem vessels in a different shape. Gradually decreased xylem vessels were found. Parenchymatic cells with lobed projection, sclereids, fibers and group of stone cells were also observed in powder microscopy. The findings of the present study were supported by other expertise. The physicochemical constants of *J. gendarussa* were standardized to check for purity of drug. Deterioration time of drug depends on its water contents as LOD at 105°C was 10.474%. TA (2.990%) represents the inorganic residue after incineration. AIA percentage reveals the presence of siliceous substances in drug. By treating the TA with dilute hydrochloric acid, the percentage of AIA was determined; (0.099%) minimum AIA value percentage means less contamination with siliceous matter while the WSA (1.528%) indicates the inorganic contents after treatment of the TA with water. Secondary metabolites of plants are intended for their therapeutic values are extracted in suitable solvents (water and alcohol). The ASE values (0.564%) support the presence of polar components of the plant such as alkaloids, steroids, flavonoids and glycosides whereas the WSE value (4.11%) represents the presence of sugar and acids. HPTLC as quality assessment tool for the identification of variation in chemical constituents showed different *R*<sub>f</sub> at different wavelengths.[34] Values as *R*<sub>f</sub> at different wavelength under short UV, long UV and after postderivatization can serve as quality fingerprint for roots of *J. gendarussa*. GC-MS is the most commonly used technique for the identification and quantification purpose. Active constituents of plants material can be determined by GC-MS analysis and data interpretation can be done by matching the spectra with mass spectrum library such as NIST. GC-MS of ethanolic extract of dried rhizomes revealed the presence of four compounds out of which oleic acid is reported to induce apoptosis in carcinoma cells by increasing the intracellular ROS production or caspase-3 activity[35] and 9,12-octadecadienoic acid (linoleic acid) is reported to possess anti-inflammatory, nematicide, insectifuge, hypocholesterolemic, anticancer, hepatoprotective, antihistaminic, antiacne, antiarthritic and antieczemic activity.[36] GC-MS results of ethanolic extract of dried roots showed the presence of pharmacologically active components.
### Table 4: Details of compounds identified from ethanolic extract of *Justicia gendarussa* Burm. F. root

| Peak | %R | Percentage area | Name | Formula | MF | RMF |
|------|----|----------------|------|---------|----|-----|
| 1    | 6.099 | 51.19 | Oleic acid | C18H34O2 | 882 | 897 |
| 2    | 8.936 | 35.74 | 9,12-octadecadienoic acid | C18H32O2 | 811 | 847 |
| 3    | 13.27 | 5.22 | 6,9,12-octadecatrienoic acid | C19H34O2 | 770 | 770 |
| 4    | 14.18 | -   | - | - | - | - |
| 5    | 15.12 | 5.19 | Estra-1,3,5(10)-trein-17-β-ol | C18H28O | 744 | 767 |

*: Unidentified; RMF: Reverse match factor; MF: Match factor

### CONCLUSION

Macro-microscopic observations, physicochemical analysis, preliminary phytochemical screening and HPTLC fingerprinting could be utilized as reference limits for the quality control standards to study the roots of *J. gendarussa*. Chemical profiling using GC-MS revealed the presence of omega fatty acids and sterol in ethanolic extract of the dried roots of *J. gendarussa*. The data obtained from the study can be used to study the therapeutic efficacy of compounds on the pharmacological activity.
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Conflicts of interest

There are no conflicts of interest.

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