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ORIGINAL ARTICLE

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

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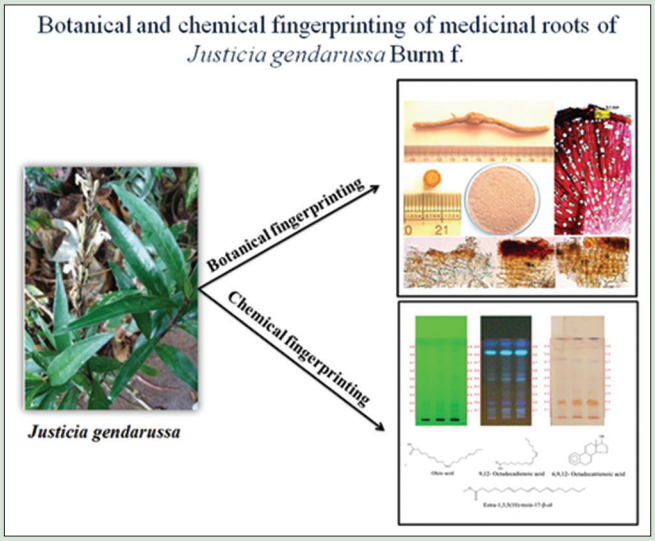
ABSTRACT

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 antiproliferative 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 (1.528%), 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_f, color 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 in ethanolic extract of *J. gendarussa.* **Conclusion:** These specific identities will be useful in identification and authentication of the raw drug in dried form.

Key words: Gas chromatography-mass specrtometry 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 parenchymatic 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 (1.528%), alcohol-soluble extract (0.564%) and water-soluble extract (4.11%)
- Preliminary phytochemical analysis of ethanolic extract of *J. gendarussa* showed the presence of alkaloid, steroid, flavonoid, phenol, carbohydrate, saponin and quinone
- High-performance thin layer chromatography fingerprinting showed different peaks at different wavelength
- Chemical profiling of medicinal roots of *J. gendarussa* by gas chromatography-mass spectrometry revealed 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.



Abbreviations Used: TLC: Thin layer chromatography, HPTLC: High performance thin layer chromatography, GCMS: Gas chromatography-mass specrtometry, QSIMP: Quality standard of indian medicinal plant, LOD: Loss on drying, TA: Total ash, AIA: Acid insoluble ash, WSA: Water soluble ash, ASE: Alcohol soluble extractive, WSE: Water soluble extractive.

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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 adulsa 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.[4-8] 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.[2,9-13]

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*.[15-18] 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.[19-21] 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-κB 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).[23] After 48 h, very thin transverse sections of root were obtained using sharp blade followed by safranin staining[24] 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 mess 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.[25]

Physicochemical analysis

Physicochemical characterization such as loss on drying (LOD) at 105°C, total ash (TA), acid-insoluble ash (AIA), water-soluble ash (WSA), alcohol-soluble extractive (ASE) value (ASE) 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 µl 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

Figure 1: (a-e) Macroscopic features of Justicia gendarussa. (f-k) Microscopic observations of dried roots of *Justicia gendarussa*. Phloem (Ph); Cork (Ck); Vessel (Ve); Xylem Rays (XR); Phloem fibres (PF); Cortex (CO); Pith (Pi)

anisaldehyde-sulfuric acid reagent,[28] 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.[29,30]

Gas chromatography-mass specrtometry analysis

Gas chromatography-mass specrtometry (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 µm. 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 programed initially at 60°C for 3.5 min and then programed 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. The spectra of the components were compared with the spectral database of known components in the GC-MS library (NIST-11). Measurement of peak areas and data processing were carried out by Turbo-Mass-OCPTVS-Demo SPL software.[31]

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) 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.

Gas chromatography-mass specrtometry analysis

GC-MS of *J. gendarussa* indicated the presence of five constituents. Out of five constituents, one was unidentified. The remaining four constituents

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

Parameter (%w/w)	Mean±SE (*n*=3)
Loss on drying	10.474±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	0.564±0.122
Water-soluble extractive value	4.11±0.005

SE: Standard error

Table 2: Preliminary phytochemical screening

Test	Colour if positive	Inference
Alkaloid		
Dragendroff's test	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 $FeCl_3$	Dark blue or green or brown	−ve
Flavonoids		
Shinoda's test	Red or pink	+ve
Saponins		
With $NaHCO_3$	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 $NaHCO_3$	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

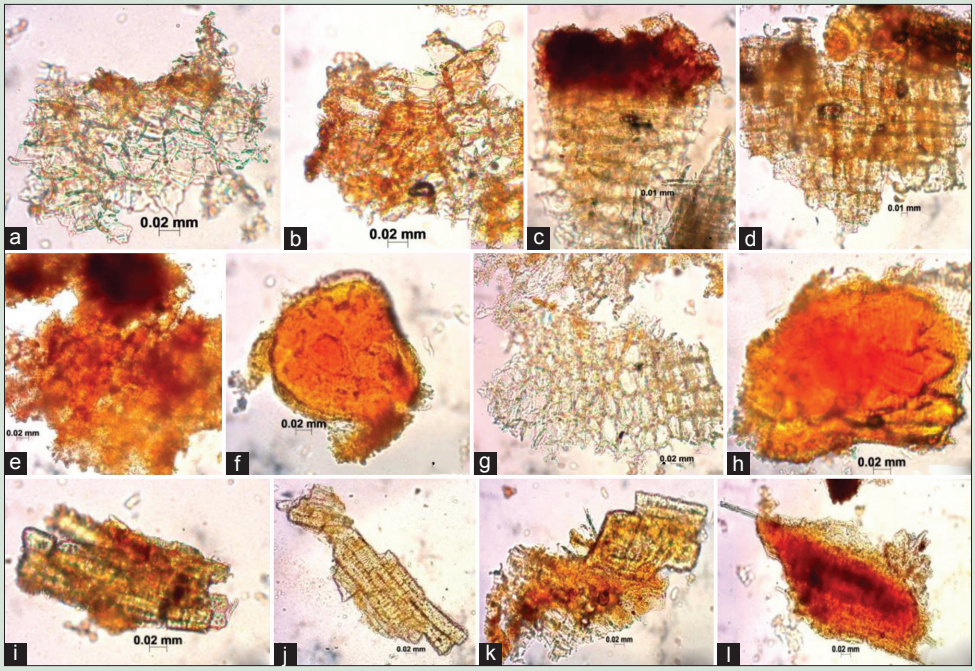


Figure 2: (a-l) Powder microscopy of dried roots of *Justicia gendarussa*

Table 3: R_f values of all the samples

At 254 nm	At 366 nm	After postderivatisation
-	0.07 (FL purple)	-
-	0.10 (FL purple)	-
0.12 (D green)	-	-
0.18 (D green)	0.18 (FD purple)	0.18 (orange)
-	-	0.21 (orange)
-	-	0.24 (orange)
0.30 (L green)	-	-
-	-	0.33 (orange)
-	0.50 (F aqua blue)	-
0.53 (D green)	-	-
-	0.56 (F blue)	-
-	0.64 (F blue)	-
-	0.72 (F blue)	0.71 (orange)
0.78 (L green)	-	-
-	-	0.80 (D purple)
-	0.85 (FD aqua blue)	-
0.87 (D green)	-	-
-	0.96 (F blue)	-

L: Light; D: Dark; F: Fluorescence

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.[32] The physicochemical constants of *J. gendarussa* were standardized to check for purity of drug.[26,33] 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_f at different wavelengths.[34] Values as R_f 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.

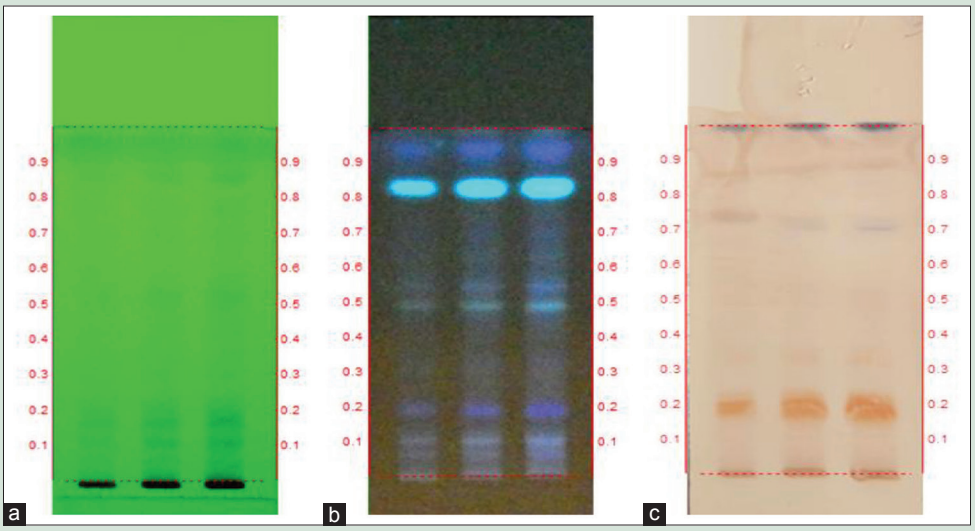
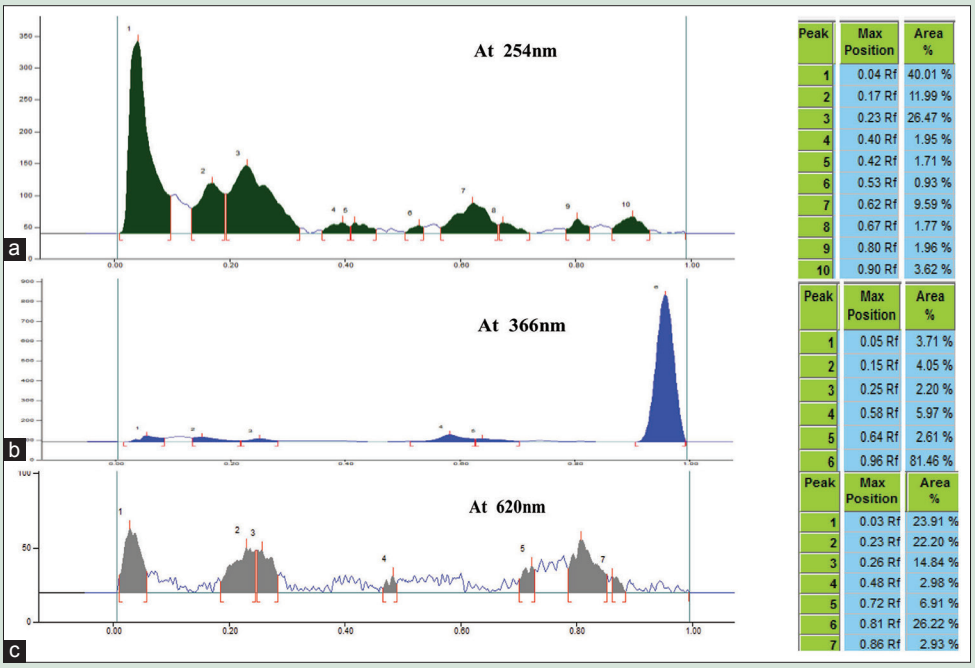


Figure 3: (a-c) High-performance thin layer chromatography photodocumentation of ethanolic extract of *Justicia gendarussa*



Peak	Max Position	Area %
1	0.04 Rf	40.01 %
2	0.17 Rf	11.99 %
3	0.23 Rf	26.47 %
4	0.40 Rf	1.95 %
5	0.42 Rf	1.71 %
6	0.53 Rf	0.93 %
7	0.62 Rf	9.59 %
8	0.67 Rf	1.77 %
9	0.80 Rf	1.96 %
10	0.90 Rf	3.62 %

Peak	Max Position	Area %
1	0.05 Rf	3.71 %
2	0.15 Rf	4.05 %
3	0.25 Rf	2.20 %
4	0.58 Rf	5.97 %
5	0.64 Rf	2.61 %
6	0.96 Rf	81.46 %

Peak	Max Position	Area %
1	0.03 Rf	23.91 %
2	0.23 Rf	22.20 %
3	0.26 Rf	14.84 %
4	0.48 Rf	2.98 %
5	0.72 Rf	6.91 %
6	0.81 Rf	26.22 %
7	0.86 Rf	2.93 %

Figure 4: (a-c) HPTLC densitometric scan of ethanolic extract of *Justicia gendarussa*

Table 4: Details of compounds identified from ethanolic extract of *Justicia gendarussa* Burm F. root

Peak	R_T	Percentage area	Name	Formula	MF	RMF
1	6.099	51.19	Oleic acid	$C_{18}H_{34}O_2$	882	897
2	8.936	35.74	9,12-octadecadienoic acid	$C_{18}H_{32}O_2$	811	847
3	13.27	5.22	6,9,12-octadecatrienoic acid	$C_{19}H_{34}O_2$	770	770
4	14.18		-	-	-	-
5	15.12	5.19	Estra-1,3,5(10)-trein-17-β-ol	$C_{18}H_{34}O$	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.

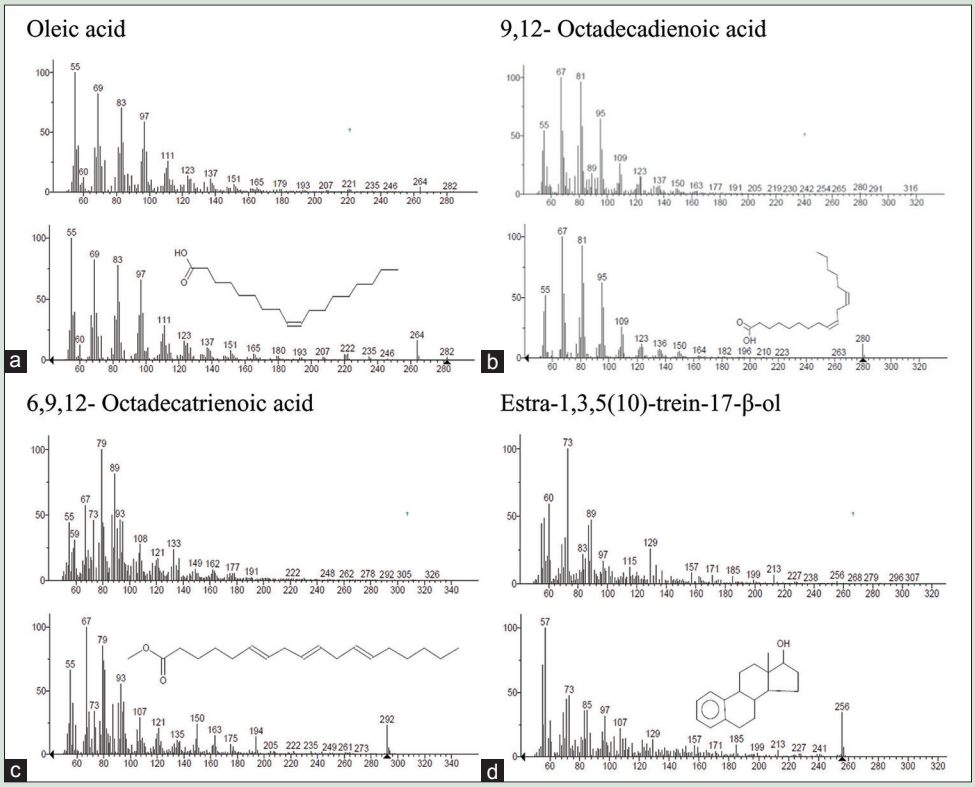


Figure 5: (a-d) Mass specrtometry of compounds identified from ethanolic extract of *Justicia gendarussa* root

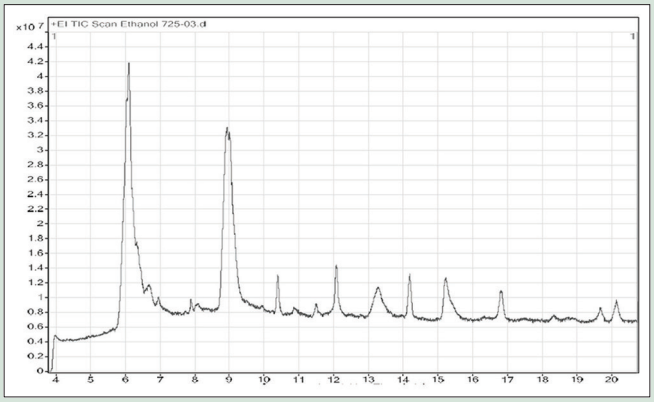


Figure 6: Gas-liquid chromatogram of ethanolic extract of *Justicia gendarussa*

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Conflicts of interest

There are no conflicts of interest.

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