PHYTOCHEMICAL PROFILING, QUANTITATIVE ESTIMATION, ISOLATION AND CHARACTERIZATION OF BIOACTIVE CONSTITUENTS FROM *JUSTICIA BETONICA* (L.) AND *VIGNA TRILOBATA* (L.) VERDC.

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ABSTRACT:
Background: Medicinal plants are important sources of natural antioxidants due to their rich phytochemical composition, which contributes to the prevention and management of oxidative stress-related disorders. *Justicia betonica* (L.) and *Vigna trilobata* (L.) Verdc. are traditionally used medicinal plants; however, comprehensive phytochemical profiling and scientific evaluation of their antioxidant potential remain limited.
Objective: The present study aimed to investigate the phytochemical profile, quantify major bioactive constituents, and evaluate the *in vitro* antioxidant potential of the ethanolic extract of *Justicia betonica* and the aqueous extract of *Vigna trilobata*.
Methods: Plant materials were collected, authenticated, and extracted using Soxhlet extraction. Preliminary phytochemical screening was performed using standard qualitative tests. Total phenolic content (TPC), total flavonoid content (TFC), and total tannin content were determined using Folin–Ciocalteu, aluminium chloride colorimetric, and indigosulphonic acid titrimetric methods, respectively. Bioactive constituents were further characterized by thin-layer chromatography (TLC), Fourier transform infrared spectroscopy (FT-IR), high-performance liquid chromatography (HPLC), and liquid chromatography–high resolution mass spectrometry (LC–HRMS).
Results: Qualitative phytochemical analysis confirmed the presence of alkaloids, flavonoids, phenolics, tannins, glycosides, terpenoids, steroids, and saponins in both extracts. The ethanolic extract of *Justicia betonica* exhibited higher total phenolic (250–280 mg GAE/g extract), total flavonoid (35–60 mg QE/g extract), and total tannin (4.99%) contents than the aqueous extract of *Vigna trilobata* (190–200 mg GAE/g extract, 10–12 mg QE/g extract, and 1.66%, respectively). TLC, FT-IR, HPLC, and LC–HRMS analyses confirmed the presence of diverse phytochemical constituents and established characteristic chemical fingerprints for both plant extracts.
Conclusion: The findings demonstrate that both *Justicia betonica* and *Vigna trilobata* are rich sources of bioactive phytochemicals with significant antioxidant potential. Among the two plants, *Justicia betonica* exhibited comparatively higher phytochemical content, suggesting its potential as a promising natural source of antioxidant compounds for future pharmacological investigations.
Keywords: *Justicia betonica*; *Vigna trilobata*; phytochemical profiling; total phenolic content; total flavonoid content; tannins; antioxidant activity; TLC; HPLC; FT-IR; LC–HRMS.
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INTRODUCTION:

Medicinal plants have served as an indispensable source of therapeutic agents for centuries and continue to play a significant role in modern drug discovery. Owing to their rich diversity of bioactive secondary metabolites, plant-derived products have gained increasing attention as safer alternatives to synthetic drugs for the prevention

and management of various chronic disorders. Among these bioactive compounds, phenolics, flavonoids, tannins, alkaloids, terpenoids, and glycosides are widely recognized for their antioxidant, anti-inflammatory, antimicrobial, antidiabetic, cardioprotective, and anticancer activities. The biological efficacy of medicinal plants is largely attributed to the synergistic action of these phytochemicals, making phytochemical characterization an essential step in the scientific validation of traditional herbal medicines.[1]

Oxidative stress, resulting from an imbalance between reactive oxygen species (ROS) generation and the antioxidant defense system, plays a crucial role in the pathogenesis of several chronic diseases, including cardiovascular diseases, diabetes mellitus, hyperlipidemia, neurodegenerative disorders, and cancer. Natural antioxidants derived from medicinal plants have attracted considerable interest because they effectively neutralize free radicals, inhibit lipid peroxidation, and protect cellular components from oxidative damage. Consequently, the identification of plants rich in antioxidant phytochemicals has become an important area of pharmaceutical and biomedical research.[2]

Justicia betonica (L.), a medicinal plant belonging to the family Acanthaceae, has been traditionally employed for the treatment of various ailments. Previous investigations have demonstrated that the plant contains several biologically active constituents, including phenolic compounds, flavonoids, tannins, alkaloids, terpenoids, steroids, and glycosides, which are associated with antioxidant and therapeutic activities.[3] Similarly, *Vigna trilobata (L.) Verdc*., a member of the family Fabaceae, is an underexplored medicinal legume rich in flavonoids, phenolics, sterols, terpenoids, and other secondary metabolites. Despite its traditional medicinal importance, comprehensive phytochemical characterization and scientific evaluation of its antioxidant potential remain limited.[4]

Phytochemical profiling using chromatographic and spectroscopic techniques such as Thin Layer Chromatography (TLC), High-Performance Liquid Chromatography (HPLC), Fourier Transform Infrared Spectroscopy (FT-IR), and Liquid Chromatography–High Resolution Mass Spectrometry (LC–HRMS) provides valuable information regarding the chemical composition of plant extracts. Furthermore, the quantitative estimation of total phenolic, flavonoid, and tannin contents serves as an important indicator of antioxidant potential, as these compounds are among the primary contributors to free radical scavenging activity.[5-7]

Considering the growing demand for natural antioxidant agents and the limited phytochemical information available for these medicinal plants, the present study aimed to perform a comprehensive phytochemical investigation of the ethanolic extract of *Justicia betonica (L.)* and the aqueous extract of Vigna trilobata *(L.) Verdc*. The study included preliminary phytochemical screening, quantitative estimation of total phenolic, flavonoid, and tannin contents, chromatographic fingerprinting by TLC and HPLC, characterization of bioactive constituents using FT-IR and LC–HRMS, and evaluation of the in vitro antioxidant potential.[8] The findings of this study are expected to provide a scientific basis for the traditional medicinal use of these plants and support their future development as potential natural antioxidant and therapeutic agents.

MATERIALS AND METHODS:

MATERIALS:

Methodology:

Collection and Authentication of Plant Material

Fresh leaves of *Justicia betonica (L.)* and the whole plant of Vigna trilobata *(L.) Verdc.* were collected during the appropriate growing season from different localities of Chhatrapati Sambhajinagar, Maharashtra, India. The collected plant materials were carefully cleaned to remove adhering soil, dust, and other foreign matter. Botanical identification and authentication of both plant species were carried out by Dr. Arvind S. Dhabe, Senior Professor and Head, Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Chhatrapati Sambhajinagar, Maharashtra, India. The authenticated plant materials were used for subsequent phytochemical investigations.

Preparation of Plant Extracts

The collected leaves of *Justicia betonica* and the whole plant of *Vigna trilobata* were washed thoroughly under running tap water followed by rinsing with distilled water to eliminate surface contaminants. The plant materials were shade dried at ambient temperature (25–30°C) with adequate ventilation until a constant weight was obtained. The dried materials were pulverized separately using a mechanical grinder to obtain coarse powder and stored in airtight containers protected from light and moisture.

Approximately 50 g of each powdered plant material was packed in a cellulose extraction thimble and extracted by Soxhlet apparatus using suitable organic solvents of increasing polarity. Each extraction was continued until the siphon tube became colourless, indicating complete extraction of phytoconstituents. The extracts were filtered and concentrated under reduced pressure using a rotary vacuum evaporator at temperatures below 45°C. The concentrated extracts were further dried to obtain semisolid residues, weighed to determine extraction yield, and stored in airtight amber-coloured containers at 4°C until further phytochemical analysis.

Preliminary Phytochemical Screening

The concentrated extracts of *Justicia betonica* and *Vigna trilobata* were subjected to qualitative phytochemical analysis to detect the presence of major classes of secondary metabolites using standard pharmacognostic procedures. The extracts were screened for alkaloids, carbohydrates, glycosides, flavonoids, phenolic compounds, tannins, saponins, steroids, terpenoids, triterpenoids, proteins, amino acids, fixed oils, fats, gums, and mucilage using appropriate chemical tests. The appearance of characteristic colour changes or precipitates was considered indicative of the presence of the respective phytochemical constituents.[9-12]

Determination of Total Phenolic Content (TPC)

The total phenolic content of the plant extracts was estimated using the Folin–Ciocalteu colorimetric method with gallic acid as the reference standard. A series of gallic acid standard solutions (50–250 µg/mL) was prepared to construct the calibration curve. Appropriate aliquots of the standard or plant extract solution were mixed with diluted Folin–Ciocalteu reagent and allowed to react for 5 min at room temperature. Subsequently, sodium carbonate solution (7.5%, w/v) was added to the reaction mixture, and the volume was adjusted with distilled water. The mixtures were incubated for 30 min at room temperature in the dark to allow complete colour development. The absorbance of each solution was measured at 765 nm using a UV–Visible spectrophotometer against a reagent blank. The calibration curve was generated from gallic acid standards, and the total phenolic content of each extract was calculated from the regression equation. The results were expressed as milligrams of gallic acid equivalents (GAE) per gram of dry extract (mg GAE/g extract). All analyses were performed in triplicate, and the results were reported as mean ± standard deviation.[13-14]

Determination of Total Flavonoid Content (TFC)

The total flavonoid content of the extracts was determined by the aluminium chloride colorimetric method using quercetin as the reference compound. Quercetin standard solutions in the concentration range of 20–200 µg/mL were prepared for construction of the calibration curve. Aliquots of plant extract or standard solution were mixed with aluminium chloride reagent and potassium acetate solution, followed by dilution with distilled water. The reaction mixtures were incubated at room temperature for 30 min to allow formation of the flavonoid–aluminium complex. The absorbance was measured at 415 nm using a UV–Visible spectrophotometer against the corresponding blank. The flavonoid concentration in each extract was calculated from the quercetin calibration curve and expressed as milligrams of quercetin equivalents (QE) per gram of dry extract (mg QE/g extract). Each analysis was carried out in triplicate, and the results were expressed as mean ± standard deviation.[15-17]

Determination of Total Tannin Content

The total tannin content of the plant extracts was determined by the indigo carmine (indigosulphonic acid) titrimetric method as described in the Standardization of Botanicals by V. Rajpal with slight modifications. Briefly, 1 g of powdered plant material was extracted with 100 mL of distilled water and filtered. An aliquot of 10 mL of the filtrate was transferred into a 1 L conical flask containing 750 mL of distilled water. Subsequently, 25 mL of indigosulphonic acid indicator solution was added, and the mixture was titrated against 0.1 N potassium permanganate ($KMnO_4$) with continuous stirring until a permanent golden-yellow endpoint was obtained. A blank determination was performed under identical conditions using distilled water in place of the sample extract. The tannin content was calculated using the factor that 1 mL of 0.1 N $KMnO_4$ is equivalent to 0.004157 g of tannic acid, and the results were expressed as percentage (% w/w) of tannins calculated as tannic acid equivalents. All determinations were carried out in triplicate.[18-19]

Isolation of Bioactive Compounds

The ethanolic extract of *Justicia betonica* and the aqueous extract of *Vigna trilobata* exhibiting higher phytochemical content and antioxidant activity were selected for isolation of bioactive constituents. The concentrated extracts were subjected to silica gel column chromatography using gradient solvent systems of increasing polarity. Fractions were collected systematically and monitored by thin-layer chromatography (TLC). Fractions exhibiting similar TLC profiles were pooled and concentrated under reduced pressure to obtain purified compounds.[20-22]

High-Performance Liquid Chromatography (HPLC) Analysis

The phytochemical profiles of the ethanolic extract of *Justicia betonica (L.)* and the aqueous extract of Vigna trilobata *(L.) Verdc*. Were analyzed using High-Performance Liquid Chromatography (HPLC). The extracts were filtered through a 0.45 µm membrane filter before injection into the HPLC system. Chromatographic separation was carried out on a reversed-phase C18 column under optimized operating conditions. Detection was performed at 269 nm for *Justicia betonica* and 276 nm for *Vigna trilobata*. The chromatograms were recorded, and retention times of the resolved peaks were used to establish the phytochemical fingerprint of each extract.[23-24]

Characterization of Isolated Compounds

The isolated compounds were characterized by determining their physicochemical properties, including appearance, melting point, and Rf values obtained by TLC. Structural characterization was performed using spectroscopic techniques such as

Fourier Transform Infrared (FT-IR) spectroscopy, Ultraviolet–Visible (UV–Vis) spectroscopy, Proton Nuclear Magnetic Resonance (^1H NMR), Carbon-13 Nuclear Magnetic Resonance (^13C NMR), and Mass Spectrometry (MS).[25-29]

Liquid Chromatography–High Resolution Mass Spectrometry (LC–HRMS)

The phytochemical profiles of the ethanolic extract of *Justicia betonica (L.)* and the aqueous extract of Vigna trilobata *(L.) Verdc*. were characterized using Liquid Chromatography–High Resolution Mass Spectrometry (LC–HRMS) equipped with an electrospray ionization (ESI) source operating in positive ion mode. The extracts were filtered through 0.22 µm membrane filters, and aliquots were injected into the LC–HRMS system. Chromatographic separation was carried out under optimized analytical conditions, and mass spectra were acquired over an m/z range of 50–1200. The accurate mass-to-charge (m/z) values of the detected ions were recorded to generate characteristic LC–HRMS fingerprints of both plant extracts. The obtained mass spectral data were used for phytochemical profiling and to assess the diversity of bioactive constituents present in the extracts.[30-38]

RESULTS AND DISCUSSIONS:

Preliminary Phytochemical Investigation

The qualitative phytochemical screening of the ethanolic extract of *Justicia betonica (L.)* and the aqueous extract of Vigna trilobata *(L.) Verdc*. revealed the presence of several bioactive secondary metabolites. Both extracts tested positive for alkaloids, carbohydrates, glycosides, flavonoids, phenolic compounds, tannins, steroids, terpenoids, proteins, amino acids, and fixed oils. However, gums and mucilage were absent in both extracts. The ethanolic extract of *Justicia betonica* exhibited comparatively stronger reactions for phenolic compounds, flavonoids, tannins, and triterpenoids than the aqueous extract of *Vigna trilobata*, indicating a richer phytochemical composition.

Table 1. Qualitative phytochemical screening of the ethanolic extract of *Justicia betonica* and the aqueous extract of *Vigna trilobata*

Phytochemical Constituents	***Justicia betonica* (Ethanolic Extract)**	***Vigna trilobata* (Aqueous Extract)**
Alkaloids	+	+
Carbohydrates	+	+
Glycosides	+	++
Flavonoids	+++	+++
Phenolic compounds	+++	++
Tannins	+++	++
Saponins	++	+
Steroids	++	++
Terpenoids	++	++
Triterpenoids	+++	+
Proteins	+	+
Amino acids	+	+
Fixed oils and fats	+	+
Gums	–	–
Mucilage	–	–

***Note:** (+++) Strongly present; (++) Moderately present; (+) Present; (–) Absent.*

The phytochemical screening confirmed that both extracts are rich in biologically active secondary metabolites, particularly phenolic compounds and flavonoids, which are well known for their antioxidant properties. The ethanolic extract of *Justicia betonica* showed a comparatively richer phytochemical profile than the aqueous extract of *Vigna trilobata*, suggesting a higher concentration of bioactive constituents. The presence of tannins, saponins, terpenoids, and steroids further supports the potential therapeutic value of both plants. These phytochemicals may contribute individually or synergistically to the antioxidant activity and provide a scientific basis for their further pharmacological evaluation.

Total Phenolic Content (TPC)

The total phenolic content (TPC) of the ethanolic extract of *Justicia betonica* and the aqueous extract of *Vigna trilobata* was determined using the Folin–Ciocalteu method with gallic acid as the reference standard. The gallic acid calibration curve exhibited good linearity over the concentration range of 50–250 µg/mL with a coefficient of determination (R^2) of 0.9947. The ethanolic extract of *Justicia betonica* showed a higher phenolic content (250–280 mg GAE/g extract) compared to the aqueous extract of *Vigna trilobata* (190–200 mg GAE/g extract).

Table 2. Total Phenolic Content of the Plant Extracts

Sample Code	**Plant Extract**	**Concentration (µg/mL)**	**Absorbance (765 nm)**	**Total Phenolic Content**
01	*Justicia betonica* (Ethanolic extract)	75	0.0578	250–280 mg GAE/g extract
		125	0.1387	
02	*Vigna trilobata* (Aqueous extract)	75	0.0079	190–200 mg GAE/g extract
		125	0.0869	

GAE: *Gallic acid equivalent.*

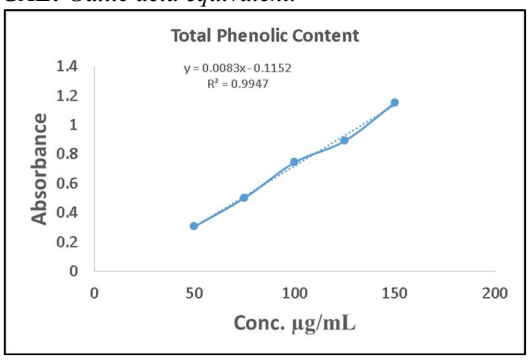

Fig.1. Total Phenolic Content

Phenolic compounds are among the major plant-derived antioxidants and play an important role in scavenging free radicals and protecting biological systems against oxidative stress. In the present study, the ethanolic extract of *Justicia betonica* exhibited a higher total phenolic content than the aqueous extract of *Vigna trilobata*. The greater phenolic concentration in *Justicia betonica* suggests a higher antioxidant potential, which may contribute to its pharmacological activity. These findings indicate that both plant extracts are valuable sources of natural phenolic compounds, with *Justicia betonica* being comparatively richer in phenolic constituents.

Total Flavonoid Content (TFC)

The total flavonoid content (TFC) of the ethanolic extract of *Justicia betonica* and the aqueous extract of *Vigna trilobata* was determined by the aluminium chloride colorimetric method using quercetin as the reference standard. The quercetin calibration curve showed good linearity over the concentration range of 20–200 µg/mL with a coefficient of determination (R^2) of 0.9970. The ethanolic extract of *Justicia betonica* exhibited higher flavonoid content (35–60 mg QE/g extract) than the aqueous extract of *Vigna trilobata* (10–12 mg QE/g extract).

Table 3. Total Flavonoid Content of the Plant Extracts

Sample Code	Plant Extract	Concentration (µg/mL)	Absorbance	Total Flavonoid Content
01	*Justicia betonica* (Ethanolic extract)	1000	0.4876	35–60 mg QE/g extract
		2000	0.5526	
02	*Vigna trilobata* (Aqueous extract)	1000	0.0847	10–12 mg QE/g extract
		2000	0.1841	

QE: *Quercetin equivalent.*

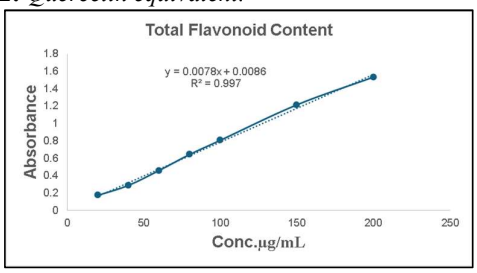

Fig.2. Total Phenolic Content

Flavonoids are important polyphenolic compounds known for their antioxidant, free radical scavenging, and protective biological activities. In the present study, the ethanolic extract of *Justicia betonica* contained markedly higher total flavonoid content than the aqueous extract of *Vigna trilobata*. The higher flavonoid concentration in *Justicia betonica* suggests a greater antioxidant potential, which may contribute to its pharmacological efficacy. The results indicate that both plant extracts are natural sources of flavonoids; however, *Justicia betonica* possesses a comparatively richer flavonoid profile, supporting its potential use in the management of oxidative stress-related disorders.

Total Tannin Content

The total tannin content of the ethanolic extract of *Justicia betonica* and the aqueous extract of *Vigna trilobata* was determined by the indigosulphonic acid titrimetric method. The tannin content was found to be 4.99% w/w for Justicia betonica and 1.66% w/w for Vigna trilobata.

Table 4. Total Tannin Content of the Plant Extracts

Plant Extract	Tannin Content (% w/w)
Justicia betonica (Ethanolic extract)	4.99
Vigna trilobata (Aqueous extract)	1.66

The ethanolic extract of Justicia betonica exhibited higher tannin content than the aqueous extract of *Vigna trilobata*. Since tannins possess well-established antioxidant properties, their higher concentration in *Justicia betonica* may contribute to its enhanced antioxidant activity.

Isolation and Characterization of Bioactive Constituents

The crude extracts of *Justicia betonica* and *Vigna trilobata* were successfully fractionated by silica gel column chromatography. Thin-layer chromatography (TLC) using Hexane: Ethyl acetate: Methanol (13:5:2) as the mobile phase revealed six distinct spots with Rf values of 0.12, 0.23, 0.56, 0.81, 0.91, and 0.97, indicating the

presence of multiple phytoconstituents with different polarities.

The isolated fractions were further characterized by FT-IR spectroscopy. The spectra exhibited characteristic absorption bands corresponding to hydroxyl (O–H), aliphatic C–H, carbonyl (C=O), aromatic C=C, and C–O functional groups, confirming the presence of polyphenolic and other bioactive compounds. The TLC profile demonstrated effective separation of the phytoconstituents present in both plant extracts, indicating a chemically diverse composition. The FT-IR spectra confirmed the presence of characteristic functional groups commonly associated with phenolics, flavonoids, and related phytochemicals. These findings support the results of the preliminary phytochemical screening and the quantitative estimation of total phenolic and flavonoid contents. The abundance of these bioactive constituents suggests that both Justicia betonica and Vigna trilobata possess significant antioxidant potential and may contribute to their reported pharmacological activities.

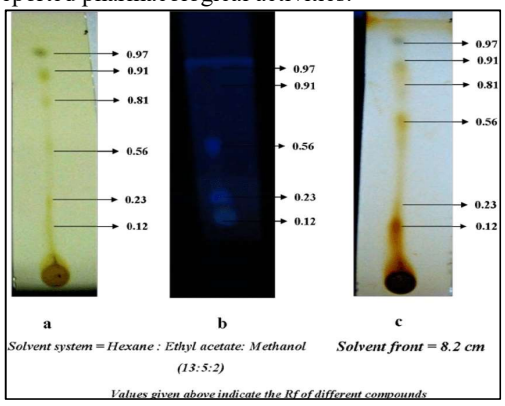

Fig. 3. Thin-layer chromatogram of the isolated phytoconstituents developed in Hexane:Ethyl acetate:Methanol (13:5:2), showing multiple compounds with distinct Rf values

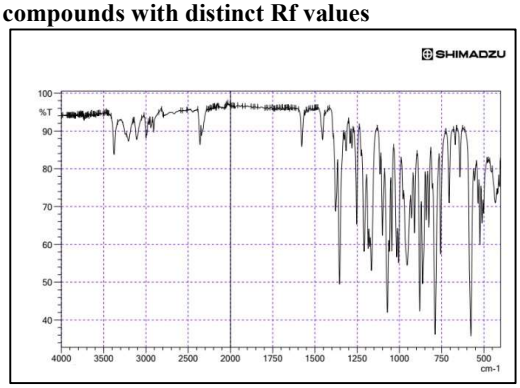

Fig. 4. FT-IR spectrum of the isolated fraction from the ethanolic extract of *Justicia betonica* showing characteristic functional groups

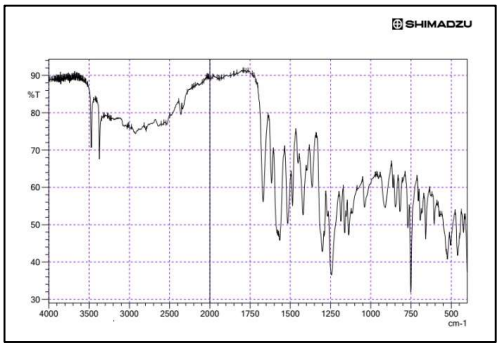

Fig. 5. FT-IR spectrum of the isolated fraction from the aqueous extract of *Vigna trilobata* showing characteristic functional groups

HPLC Analysis

The HPLC chromatogram of the ethanolic extract of *Justicia betonica* recorded at 269 nm showed several well-resolved peaks, indicating the presence of multiple phytochemical constituents. A prominent major peak was observed at a retention time of approximately 3.67 min, suggesting the presence of a major bioactive constituent, while several minor peaks detected before and after the principal peak represent additional phytochemicals. Similarly, the HPLC chromatogram of the aqueous extract of *Vigna trilobata* recorded at 276 nm exhibited a characteristic chromatographic fingerprint with one dominant peak at a retention time of approximately 2.76 min, along with several minor peaks distributed throughout the chromatogram. These peaks indicate the presence of multiple compounds with varying polarities and concentrations. HPLC fingerprinting confirmed the chemical complexity of both plant extracts by resolving several phytochemical constituents. The presence of distinct major peaks along with multiple minor peaks demonstrates that both extracts contain a diverse range of secondary metabolites. The major peak observed at 3.67 min in *Justicia betonica* and 2.76 min in *Vigna trilobata* indicates the predominance of specific phytoconstituents in the respective extracts. The chromatographic profiles are consistent with the results of the preliminary phytochemical screening, total phenolic content, total flavonoid content, and FT-IR analysis, confirming that both plants are rich in polyphenolic compounds. The HPLC fingerprints generated in the present study can serve as a reliable tool for the quality control, authentication, and standardization of Justicia betonica and Vigna trilobata extracts and provide a basis for future isolation and identification of their active constituents.

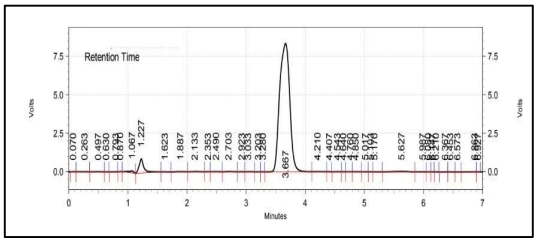

Fig. 6. HPLC Chromatogram of the Ethanolic Extract of *Justicia betonica (L.)* Recorded at 269 nm.

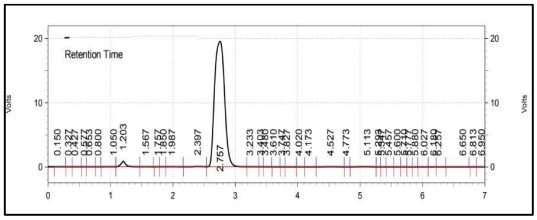

Fig. 7. HPLC Chromatogram of the Aqueous Extract of *Vigna trilobata (L.) Verdc*. Recorded at 276 nm.

LC–HRMS Analysis of *Justicia betonica* (L.)

LC–HRMS analysis of the ethanolic extract of Justicia betonica revealed the presence of several molecular ions, indicating a chemically diverse phytochemical composition. The major ions detected in positive ion mode were observed at m/z 125.0711, 147.0531, 245.1293, 255.0662, 257.1283, 271.1167, 279.1059, 287.1395, 293.0990, 423.1999, 519.2450, 593.2765, 621.3061, 813.5628, 871.5727, and 887.5659. These accurate mass signals indicate the presence of multiple low- and high-molecular-weight phytoconstituents in the extract. The LC–HRMS fingerprint confirmed that the ethanolic extract of Justicia betonica contains a wide range of phytochemicals with different molecular masses. The detection of numerous molecular ions reflects the complexity of the extract and supports the results obtained from preliminary phytochemical screening, TLC, FT-IR, HPLC, and quantitative estimation of phenolics and flavonoids. The abundance of ions within the polyphenolic mass range suggests the presence of phenolic compounds, flavonoids, and other secondary metabolites that may contribute to the antioxidant activity of the plant. The LC–HRMS profile provides a reliable chemical fingerprint for the identification, quality control, and future characterization of bioactive constituents from *Justicia betonica*.

LC–HRMS Analysis of *Vigna trilobata (L.) Verdc*.

LC–HRMS analysis of the aqueous extract of *Vigna trilobata (L.) Verdc*. revealed a complex phytochemical profile with several molecular ions detected in positive ion mode. The major ions were observed at m/z 104.1083, 125.0714, 138.0552, 166.0864, 185.0961, 198.1265, 218.1171, 245.1283, 257.1280, 271.1445, 287.1386, 309.1019, 325.0949, 375.1342, 413.0896, 427.2037, 439.1731, 455.1946, 495.1694, 523.3053, 541.2660, 567.3286, 611.3551, 699.4104, and 832.2480, indicating the presence of numerous low- and high-molecular-weight phytoconstituents in the extract. The LC–HRMS fingerprint demonstrated that the aqueous extract of *Vigna trilobata* possesses a chemically diverse composition containing multiple phytochemical constituents with different molecular masses. The presence of numerous molecular ions supports the qualitative phytochemical screening, HPLC fingerprinting, and the quantitative estimation of phenolic and flavonoid contents. The detected mass signals are consistent with the occurrence of phenolic compounds, flavonoids, and other bioactive secondary metabolites, which may contribute to the antioxidant potential of the plant. The LC–HRMS profile generated in this study provides a characteristic chemical fingerprint that can be used for the authentication, standardization, and future identification of bioactive compounds from *Vigna trilobata*.

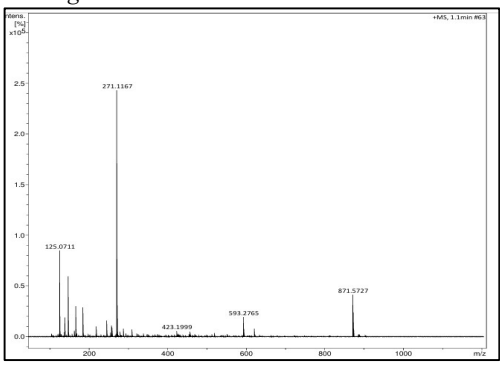

Fig. 8. LC–HRMS positive ion mass spectrum of the ethanolic extract of *Justicia betonica (L.)* showing the characteristic molecular ion peaks.

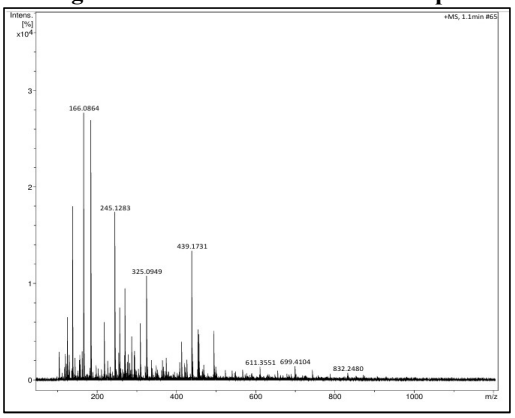

Fig. 9. LC–HRMS positive ion mass spectrum of the aqueous extract of *Vigna trilobata (L.) Verdc*. showing the characteristic molecular ion peaks.

CONCLUSION:

The present study demonstrated that the ethanolic extract of *Justicia betonica* (L.) and the aqueous extract of *Vigna trilobata* (L.) Verdc. are rich sources of bioactive phytochemicals with

promising antioxidant potential. Qualitative phytochemical screening confirmed the presence of important secondary metabolites, including phenolics, flavonoids, tannins, alkaloids, glycosides, terpenoids, and steroids. Quantitative analysis revealed that *Justicia betonica* possessed higher total phenolic, flavonoid, and tannin contents than *Vigna trilobata*, indicating a comparatively greater abundance of antioxidant constituents. Chromatographic and spectroscopic analyses using TLC, HPLC, FT-IR, and LC–HRMS established characteristic phytochemical fingerprints and confirmed the presence of diverse bioactive compounds in both plant extracts. The identified functional groups and molecular ion profiles supported the findings of the preliminary phytochemical investigation and highlighted the chemical complexity of the extracts. Overall, the results suggest that both *Justicia betonica* and *Vigna trilobata* are valuable natural sources of antioxidant phytoconstituents, with *Justicia betonica* exhibiting comparatively higher phytochemical richness and antioxidant potential. These findings provide scientific evidence supporting the traditional medicinal use of both plants and establish a foundation for further isolation, structural characterization, and pharmacological evaluation of their active constituents for the development of plant-based therapeutic agents.

CONCFILCT OF INTREST:

The authors declare that there is no conflict of interest.

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