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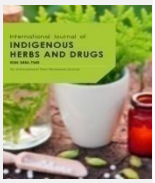

PHYTOCHEMICAL PROFILING AND COMPARATIVE ANTIOXIDANT ACTIVITY FROM THE LEAF, ROOT, AND FLOWER EXTRACTS OF *JUSTICIA BETONICA* L.

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ABSTRACT

Oxidative stress, defined as the imbalance between reactive oxygen species and intrinsic antioxidant defense, is a major contributor to the pathogenesis of many chronic diseases. Medicinal plants are a rich source of bioactive compounds and have immense therapeutic potential due to their antioxidant properties. In the present study, the phytochemical contents and antioxidant properties were assessed from the methanolic extracts of the leaves, roots and flowers from the plant Justicia betonica L. which is traditionally used but scientifically under-explored. Quantitative analysis revealed significant variations in the concentrations of phenolics, flavonoids, tannins, alkaloids and saponin in different parts of the plant. Moreover, flowers had the highest amount of extract yield and saponin while leaves contained high concentration of phenolic and tannins. The antioxidant activity was evaluated by DPPH, ABTS, FRAP, superoxide and nitric oxide scavenging assays. All the extracts showed dose dependent scavenging of radicals. The floral extract showed the maximum antioxidant activity in DPPH and nitric oxide assays with the lowest IC50 values, whereas, the root extract was better in superoxide scavenging activity. The findings suggest the high antioxidant activity of J. betonica, especially in its floral parts, indicating its possible application as a natural source of antioxidants in prevention and control of diseases related to oxidative stress.

Keywords: *Antioxidant activity, Justicia betonica, Methanolic extracts, Phytochemicals, Reactive oxygen species.*

INTRODUCTION

Oxidative stress is a pathological state in which the synthesis of reactive oxygen species (ROS) and other free radicals exceeds the body's endogenous antioxidant defense systems. This imbalance causes molecular and cellular damage, being involved in the start and progression of various chronic diseases in humans [1]. Oxidative stress is detrimental to the aging process and the development of cardiovascular diseases, diabetes, neurological disorders such as Alzheimer's and Parkinson's diseases, cancer, and chronic respiratory ailments [2, 3]. Furthermore, globally, non-communicable diseases (NCDs) that are significantly connected with oxidative stress account for 71% of all fatalities each year, which is more than 41 million deaths globally [4]. Cardiovascular disorders alone kill almost 17.9 million people per year, and cancer accounts for nearly 10 million fatalities every year. The incidence of diabetes and neurological illnesses is also increasing, representing major health and economic burdens to societies [4]. The omnipresence of oxidative stress in human illness and its contribution to global morbidity and mortality make the knowledge of causes and elaboration of effective prevention and therapeutic approaches a priority for improving the global health without side-effects.

A multitude of medicinal plants have been utilized for ages to address conditions associated with inflammation and oxidative stress. The scientific validation of traditional usage via standardized bioactive characteristics not only endorses the continuation of ethnobotanical information but also promotes the incorporation of natural medicines into evidence-based medicine [5]. Moreover, plant-derived antioxidants, specifically polyphenols, flavonoids, phenolic acids, and terpenoids, function by neutralizing free radicals, chelating metal ions, and enhancing endogenous antioxidant enzymes [6]. Consequently, the scientific community has recently exhibited increased interest in the antioxidant capabilities of medicinal plants.

These bioactivities are essential for the therapeutic effectiveness of traditional herbal treatments and are increasingly corroborated by modern research [7, 8]. The identification and quantification of antioxidant activity in plant extracts are essential for discovering novel compounds that can be developed into functional foods, nutraceuticals, or therapeutic agents to prevent or alleviate diseases associated with oxidative stress [9]. Consequently, this study selects *Justicia betonica* owing to its traditional therapeutic applications and the scarcity of scientific research on this species.

Justicia betonica L., referred to as the white shrimp plant or squirrel's tail, is a perennial shrub belonging to the Acanthaceae family, extensively found in tropical and subtropical areas of Asia and Africa [10]. The plant generally attains a height of 1–2 meters and is distinguished by its upright, branching stems and opposing, ovate-lanceolate leaves that can reach lengths of up to 15 cm. The most recognizable characteristic is the inflorescence: dense spikes of overlapping white to pale pink bracts, frequently exhibiting green or purple lines, which encase little tubular flowers. In appropriate temperatures, flowering transpires throughout the year, drawing bees and butterflies for pollination [11]. *Justicia betonica* has been traditionally utilized in several medicinal systems for the treatment of respiratory disorders, wounds, and fevers. Phytochemical investigations have identified flavonoids, alkaloids, and saponins, which may underlie its purported anti-inflammatory and antibacterial properties [10]. The primary aim of this work was to evaluate the phytochemicals and antioxidant characteristics through free radical scavenging activities from the leaf, root, and flower extracts of *J. betonica*.

MATERIALS AND METHODS

Collection of *Justicia betonica* L. Plants

Young and healthy plants of *Justicia betonica* were collected from the Vijinigiripalem village which are situated near the Simhachalam, Visakhapatnam district, Andhra Pradesh, India (Long: 17°47' 12.11" to 17°47' 10.20" N; Lat: 83°14' 49.55" to 83°14' 50.19" E). After being collected, the samples were transferred into zip pouches and brought to the lab. Then the plant parts such as leaves, roots, and flowers were separated and rinsed using tap water, followed by a distilled water.

Methanolic Extract Preparation

The methanolic extract was prepared using the method of Farrukh et al. [12] with few modifications. To prepare methanolic extracts, the collected leaves, roots, and flowers from *J. betonica* were shade dried and macerated into fine powder using mortar and pestle. Then 100 gm of powder from each plant were soaked separately in 500 mL of methanol for 10 days at room temperature in a dark environment, and the mixture was stirred gently every 18 hours using a sterile glass rod. The extract was then filtered through Whatman filter paper No.1 and the extract were concentrated by using a Soxhlet extractor and rotary evaporator. Then, the concentrated extract was allowed to concentrate till dry powder was obtained.

% of Extract yield $=\frac{m2}{m1} \times 100$

Where m1 = weight of the shade dried muscle tissue

m2 = weight of the freeze-dried crude extract

Quantitative Evaluation of Phytochemicals

The evaluation of different phytochemicals from the leaf, root, and flower extracts of *J. betonica* was quantified by the estimation of total phenolics, flavonoids, tannins, alkaloids, and saponins. The total phenolic content from the leaf, root, and flower extracts of *J. betonica*was assessed using the Folin-Ciocalteau method [13]. The colorimetric procedure described by Bao et al. [14] was employed to measure the total flavonoid content. The total tannin content was estimated by the Makkar [15] methodology using Folin-Ciocalteau reagent. The estimation of the total alkaloid content was conducted using the Sreevidya and Mehrotra [16] method. The method of Obadoni and Ochuko [17] was employed for estimating the total saponins concentration.

Quantitative Evaluation of Antioxidant Potentials

The antioxidant potential of leaf, root, and flower methanolic extracts from *J. betonica* were evaluated by the quantitative estimation of free radical scavenging activities including DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging activity, ABTS (2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) scavenging activity, Ferric reducing power assay, Superoxide scavenging (SO) activity, and Nitric oxide (NO) scavenging activity. The antioxidant properties were evaluated at 5 different extract concentrations with increasing manner such as 6.25, 12.5, 25, 50, and 100 mg/mL.

The DPPH scavenging activity of leaf, root, and flower methanolic extracts were determined by Mensor et al. [18] method. Shirwaikar et al. [19] method was used to evaluate the scavenging ability of ABTS radicals. Benzie and Strain [20] method was used to determine the ferric ion reducing ability of leaf, root, and flower extracts. The superoxide scavenging activity was determined by Beauchamp and Fridovich [21] method using nitroblue tetrazolium (NBT). The nitric oxide scavenging activity was determined by the Balakrishnan et al. [22] method using sodium nitroprusside.

Statistical Analysis

The results were expressed as mean ± standard deviation of three individual replicates and the data was assessed by one-way analysis of variance (ANOVA) and subjected to regression-correlation analysis by using SPSS 10.0 software. The 'P' value less than 0.05 was considered as significant difference.

RESULTS AND DISCUSSION

Percentage Yield of Extracts

The yield of methanolic extracts obtained from leaf, root, and floral tissues of *J. betonica* are shown in Table 01. According to these results, it was observed that the flower extract showed the highest yield (18.46%) followed by leaf extract(14.7%), and the lowest yield obtained from roots (9.16%). These studies have shown that the leaf and flower tissues exhibited higher concentrations of phytochemicals than the root tissues. Terblanche et al. [23] have been reported that the yield of extract varies according to different conditions, which includes selection of solvent, sample solvent ratio, pH, temperature, time, and the extraction method.

Table 01:Percentage of extract yield from the leaf, root, and floral tissues of *J. betonica*.

S. No	Type of Tissue Extract	Amount Recovered m2/m1(gm)	Percentage Yield (%)
1	Leaf	7.35/50	14.7
2	Root	4.58/50	9.16
3	Flower	9.23/50	18.46

Evaluation of Phytochemicals

Total Phenolics

The concentrations of total phenolics exhibited substantial variation in the leaf, root, and flower extracts of *J. betonica*. Figure 01 display the total phenolic content in the leaf, root, and flower extracts of *J. betonica*. From these results, it was observed that the leaf extracts exhibited greater quantity (15.3±0.96 mg GAE/gm) of phenolics, followed by flower extracts (12.54±1.21 mg GAE/gm), and root extracts (9.78±1.08 mg GAE/gm). These findings suggest that the leaf extracts may contribute higher phenolic compounds.

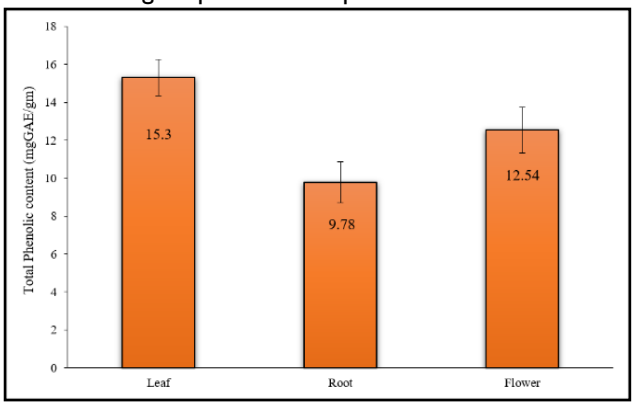

Figure 01: Total phenolic content in leaf, root, and flower extracts of *J. betonica.*

Total Flavonoids

Figure 02 display the total flavonoids content in the leaf, root, and flower extracts of *J. betonica*. The results showed that the flower extracts had a higher concentration of flavonoids, whereas the root extracts had lowest quantity of flavonoids. The flavonoids contents in the leaf, root, and flower extracts of *J. betonica* were found to be 18.31±2.6, 8.61±1.3, and 24.64±3.7 mg QE/gm respectively. From these results, it was found that the concentration of total flavonoids exhibited substantial variation in the leaf, root, and flower extracts of *J. betonica*.

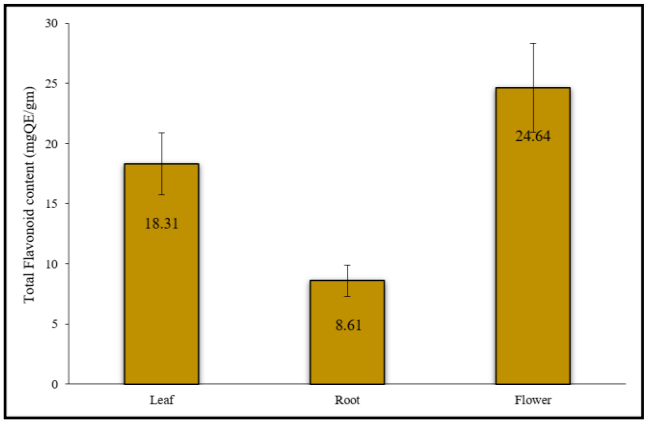

Figure 02: Total flavonoid content in leaf, root, and flower extracts of *J. betonica.*

Total Tannin Content

Figure 03 presents the total tannin content in the leaf, root, and flower extracts of *J. betonica*.The results showed that the leaf extracts exhibited a greater concentration of tannins, whereas the flower extracts showed lowest quantity of tannins. The total tannin contents in the leaf, root, and flower extracts of *J. betonica* were determined as 34.56±4.2, 29.33±3.4, and 17.26±2.5 mg TAE/gm respectively. From these results, it was found that the concentration of total tannins exhibited substantial variation among the leaf, root, and flower extracts of *J. betonica*.

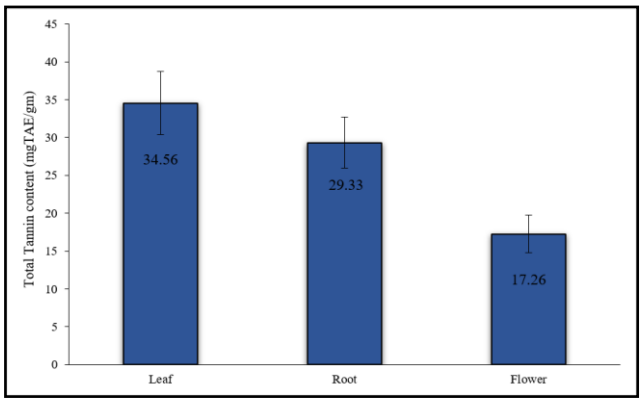

Figure 03: Total tannin content in leaf, root, and flower extracts of *J. betonica.*

Total Alkaloids

Figure 04 display the total alkaloid content in the leaf, root, and flower extracts of *J. betonica*. From these results, it was observed that the flower extracts exhibited greater quantity of alkaloids, while lowest quantity of alkaloids were observed in root extracts. The total alkaloid content in the leaf, root, and flower extracts of *J. betonica* were found to be 21.04±2.6, 10.53±1.8, and 22.36±2.9 mg BE/gm respectively. These findings suggest that the leaf and flower extracts didn't exhibit significant variation, but root extracts show significant variation when compared to leaf and flower extracts.

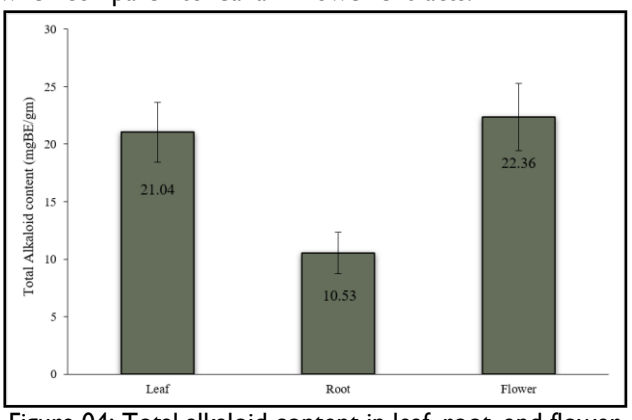

Figure 04: Total alkaloid content in leaf, root, and flower extracts of *J. betonica.*

Total Saponins

Figure 05 illustrates the total tannin content in the leaf, root, and flower extracts of *J. betonica*. The results indicated that the flower extracts contain highest saponin content, while leaf extracts had lowest saponin content. The total saponin content from the leaf, root, and flower extracts were found to be 6.31±1.2, 7.83±0.8, and 12.24±2.3 mg/gm respectively. These findings demonstrate a substantial variation in saponin concentration among the leaf, root, and flower extracts.

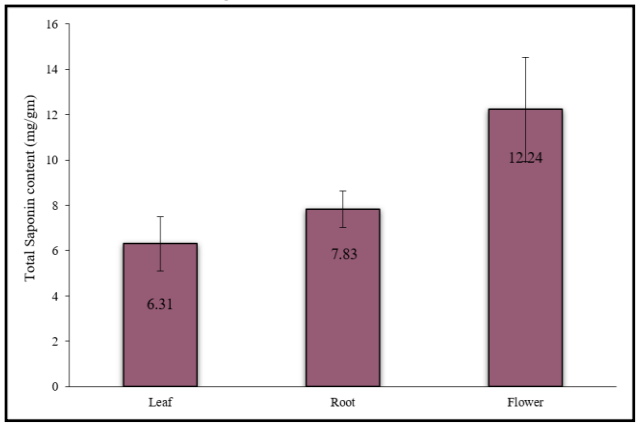

Figure 05: Total saponin content in leaf, root, and flower extracts of *J. betonica.*

The presence of numerous phenol rings distinguishes phenols, a diverse group of chemical compounds that exist naturally across the plant kingdom [24]. Nychas et al. [25] proposed that phenols function as reducing agents, hydrogen donors, and singlet oxygen quenchers. The current findings align with the results reported by John et al. [26] who observed that the leaf extracts of *J. adhatoda* (38.75 mg GAE) exhibited greater phenolic content, whereas root extracts of *J. beddomei* (22.65 mg GAE) showed least quantity of total phenolic compounds. In contrast to the present findings, Sini et al. [27] reported that the total phenolic content in the aqueous root, stem, and leaf extracts of *J. betonica* as 9.31, 2.11, and 2.57 mg GAE/gm respectively. Kiran et al. [28] reported that the total phenolic content from the leaf extracts of eight tested mangrove plants were ranged between 0.72±0.012 and 0.51±0.017 mg GAE/gm.

Plants synthesise flavonoids in their tissues as a defence mechanism against harmful impacts. Furthermore, flavonoids work as scavengers of free radicals, especially because of the hydroxyl groups present in their structure [29]. The current results are similar to those from Franz et al. [30] who reported that the methanolic extracts of roots and leaves from *Betonica officinalis* L. exhibited total flavonoid content in range of 9.63 and 39.70 mg Catechin/g dm. John et al. [26] found that the leaf extracts of *J. betonica* (2.86 mgQE) exhibited greater flavonoid content, whereas root extracts of *J. adhatoda* (1.25 mg QE) showed least flavonoid content.

Tannins are a heterogenous collection of polyphenolic chemicals present in plants, recognised for their capacity to bind and precipitate proteins [31]. In accordance to the present findings, Sharma et al. [32] found that the leaf extracts of *Byttneria herbacea* exhibited greater tannin content (8.14%), followed by whole plant (3.886%), and root (1.553%). Similarly, Sarvade [33] observed that the leaf methanolic extract from *Leea macrophylla* Roxb. Ex Hornem exhibited greater quantity of tannins (3.67%), followed by root (2.01%), and stem (1.23%).

Alkaloids are a diverse group of naturally occurring organic compounds that mostly contain basic nitrogen atoms, and are found in plants [34]. In the present study, there is no significant variation of alkaloid content between leaf and flower extracts. These results are supported by the findings of Sarvade [33] who observed greater alkaloid content in the leaf extracts (0.52%) of *Leea macrophylla*, whereas root and stem extracts exhibited 0.37 and 0.50% of total alkaloids respectively. Similarly, Sharma et al. [32] found that the leaf extracts of *Byttneria herbacea* had greater alkaloid content (2.306%), while total plant and root extracts exhibited a total alkaloid content of 1.319 and 0.814%. Rackova et al. [35] proposed that the antioxidant effects of alkaloids mostly rely on parameters that affect the stereochemical structure and electrical characteristics.

Saponins exhibiting diverse structural and biological characteristics [36] and they have antidiabetic [37], antioxidant [38], antidiabetic, antimicrobial, and antihyperlipidemic activities [39]. The saponin content in the present study is higher than the findings of Ajuru et al. [40] who found that the leaf extracts of *J. carnea* exhibited greater quantity of saponins (6.75 mg/kg), whereas the leaf extracts of *J. secunda* had least saponin content (0.8 mg/kg). Contrastingly, the saponin content in the present study is lower than the studies of Almahy and Nasir [41] and Belewu et al. [42] who found the total saponin content as 7.36 and 5% in *Acacia nilotica* and *Leucaena leucocephala* extracts.

Determination of Antioxidant Potential

DPPH Radical Scavenging Activity

The DPPH radical scavenging activity of leaf, root and flower extracts of *J. betonica* at different concentrations like 6.25, 12.5, 25, 50 and 100 mg/mL are shown in Table 02. Results show the dose dependent increase in % of inhibition for all the three extracts. The methanolic extract of leaves showed the highest inhibition (22.19±1.49%) at the concentration of 6.25mg/ml followed by the methanolic extract of flowers (21.47±3.62%) and roots (20.72±0.51%). The antioxidant activity of all the extracts increased significantly with increasing concentration. The highest percentage of inhibition (55.37±1.72%) was observed in flower extracts 100 mg/mL which was significantly different from leaf (43.86±1.83%) and root (40.27±0.89%). The IC_{50} values representing extract concentration required to block 50% of DPPH radicals indicated that the flower extracts (85.12 mg/mL) exhibited lowest IC_{50} value which in turn indicated the best antioxidant activity followed by leaves (119.02 mg/mL) and roots (136.12 mg/mL). The concentrations of extracts from leaf, root, flower and DPPH scavenging activity indicated a good positive linear relationship with a correlation value of 0.9511, 0.8906 and 0.9933 respectively (Table 03).

Table 02: DPPHradical scavenging percentages with increasing concentrations of methanolic extracts from the leaf, root, and flowers of *J. betonica.*

S. No	Conc. of Extracts (mg/mL)	% Inhibition (Mean ± SD)		
		Leaf	Root	Flower
1	6.25	22.19±1.49	20.72±0.51	21.47±3.62
2	12.5	26.35±0.93	25.17±1.01	27.03±2.25
3	25	31.39±1.05	31.60±0.86	30.74±0.83
4	50	38.32±0.85	37.99±1.43	36.74±1.51
5	100	43.86±1.83	40.27±0.89	55.37±1.72
6	IC_{50}	119.02 mg/mL	136.12 mg/mL	85.12 mg/mL
P<0.05 was considered as significant difference.				

Table 03: Correlation analysis of the DPPH antioxidant activity of extracts with increasing concentration.

Type of Extract	Extract Conc.	*Leaf*	*Root*	*Flower*
Leaf	0.9511	1		
Root	0.8906	0.9871	1	
Flower	0.9933	0.9489	0.8897	1

DPPH (2,4-diphenyl-picryl-hydrazyl) is a free radical that can take an electron or hydrogen radical, resulting in the formation of a stable diamagnetic molecule [43]. Priyanka et al. [44] reported that the greater the bleaching of DPPH solution, the higher the antioxidant property. In the present study, methanolic extracts of leaf, root, and flower tissue display a significant increase in DPPH radical scavenging activity with increasing concentration. These results are in agreement with previous studies of Paulsamy and Karthika [45] who found that the increasing concentrations (50 to 250 µg/mL) of methanolic leaf and root extracts from *Hypochaeris radicata* L. exhibited increased DPPH radical scavenging activity ranging between 54.21 and 97.99% and 23.4 and 96.44%. Similarly, Pillai et al. [46] reported that the leaf extracts of *Rhamnus prinoides* showed DPPH scavenging activity ranging between 3.33±0.89 and 55.03±3.40%, whereas stem-bark extracts exhibited relatively stronger DPPH radical scavenging activity ranging between 3.65±1.02 and 59.55±2.27%. Kumar et al. [47] found that the leaf extracts of *Myriostachya wightiana* had 14.5±0.75% of DPPH radical scavenging activity.

ABTS Radical Scavenging Activity

Table 04 depicts the percentages of ABTS scavenging activity from the leaf, root, and flower extracts of *J. betonica*. From these results, it was observed that the root and flower extracts exhibited almost similar activities at all tested concentrations and comparatively greater than the leaf extracts. At 6.25 mg/mL concentration, the ABTS radical scavenging activities from leaf, root, and flower extracts of *J. betonica* were found to be 53.13±2.02, 61.88±1.72, and 62.57±0.87% respectively. As well as, at 100 mg/mL concentration, the extracts exhibited 90.53±1.45, 94.49±0.82, and 96.58±0.75% of ABTS scavenging activities correspondingly. Furthermore, the IC_{50} value for leaf, root, and flower extracts were measured as 8.08, 5.81, and 5.72 mg/mL. The ABTS radical scavenging activity of all the tested extracts was significantly (<.0005) increased with increasing extract concentrations. In addition, the ABTS radical scavenging activities of leaf, root, and flower extracts show strong positive correlations of r =0.9111, r =0.8166, and r =0.9229 with respect to the increasing concentration (Table 05).

Table 04: ABTSradical scavenging percentages with increasing concentrations of methanolic extracts from the leaf, root, and flowers of *J. betonica.*

S. No	Conc. of Extracts (mg/mL)	% Inhibition (Mean ± SD)		
		Leaf	Root	Flower
1	6.25	53.13±2.02	61.88±1.72	62.57±0.87
2	12.5	62.86±1.51	76.51±0.64	71.37±1.24
3	25	74.35±1.39	85.17±1.05	80.73±0.82
4	50	81.98±1.19	90.52±1.09	88.34±0.90
5	100	90.53±1.45	94.49±0.82	96.58±0.75
6	IC_{50}	8.08 mg/mL	5.81 mg/mL	5.72 mg/mL
P<0.05 was considered as significant difference.				

Table 05: Correlation analysis of the ABTS antioxidant activity of extracts with increasing concentration.

Type of Extract	Extract Conc.	*Leaf*	*Root*	*Flower*
Leaf	0.9111	1		
Root	0.8166	0.9768	1	
Flower	0.9229	0.9994	0.9723	1

The $ABTS^{\cdot 1}$ cation radical is a derivative of 3-ethylbenzthiazoline-6-sulfonic acid, which is also known as 2,2[1]-azinobis [48]. The current findings revealed that the methanolic extracts from the leaf, root, and flowers of *J. betonica*effectively eliminated proton radicals, and their scavenging ability depended on the extract concentration. Furthermore, the leaf methanolic extracts of *J. betonica* exhibited greater ABTS scavenging activity. These results are evident from previous reports of Kiran et al. [49] who observed that the ethanolic extracts of leaf, root, flower, and seed from *Clitoria ternatea* exhibited dose-dependent ABTS scavenging activity. Furthermore, the inhibition was greater with flower extract (96.7 mg GAE/gFW) at 1 mg/mL concentration, followed by leaf extract (85.76 mg GAE/gFW), root extract (68.73 mg GAE/gFW), and seed extract (68.5 mg GAE/gFW). Hariharan and Vasan [50] reported that the ethanolic extracts of *Andrographis paniculata* whole plant powder showed ABTS scavenging activity of 2.46, 5.36, 12.64, 24.52, and 41.85% with increasing concentrations such as 5, 10, 25, 50, and 100 µg/mL respectively.

Ferric Reducing Antioxidant Power Assay (FRAP)

The percentages of FRAP activity from the leaf, root, and flower extracts of *J. betonica* are shown in Table 06. From these results, it was observed that the leaf extracts exhibited greatest ferric ion reducing ability at 100, 50, and 25 mg/mL extract concentrations, while at 12.5 and 6.25 mg/mL extract concentration, root extracts showed greater FRAP activity. Flower extracts showed comparatively lowest FRAP activity than the leaf and root extracts at all tested concentrations. At 6.25 mg/mL extract concentration, the ferric reducing activities from leaf, root, and flower extracts of *J. betonica* were found to be 48.53±1.39, 53.55±0.62, and 12.72±1.18% respectively. As well as, at 100 mg/ml concentration, the respective methanolic extracts exhibited 82.12±1.12, 78.49±1.29, and 69.53±0.59% of ferric reducing activities correspondingly. Furthermore, the EC_{50} values for leaf, root, and flower extracts were measured as 7.01, 5.92, and 23.9 mg/mL.The ferric reducing abilities from the leaf, root, and flower extracts of *J. betonica* were significantly (<.0005) increased with increasing extract concentrations. In addition, the FRAP activities of leaf, root, and flower extracts show strong positive correlations of r = 0.8717, r = 0.9548, and r = 0.7537 with respect to the increasing concentration (Table 07).

Table 06: FRAP activity with increasing concentrations of methanolic extracts from the leaf, root, and flowers of *J. betonica.*

S. No	Conc. of Extracts (mg/mL)	% FRAP activity (Mean ± SD)		
		Leaf	Root	Flower
1	6.25	48.53±1.39	53.55±0.62	12.72±1.18
2	12.5	54.87±1.82	58.6±1.32	45.59±0.63
3	25	70.08±1.6	65.8±1.19	56.89±0.59
4	50	77.44±1.22	68.66±0.7	63.22±1.11
5	100	82.12±1.12	78.49±1.29	69.53±0.59
6	IC_{50}	7.01 mg/mL	5.92 mg/mL	23.9 mg/mL
P<0.05 was considered as significant difference.				

Table 07: Correlation analysis of the FRAP activity of extracts with increasing concentration.

Type of Extract	Extract Conc.	*Leaf*	*Root*	*Flower*
Leaf	0.8717	1		
Root	0.9548	0.9631	1	
Flower	0.7537	0.9228	0.8976	1

For the determination of antioxidant abilities, the ferric reducing antioxidant power assay is one of the extensively used method and by using FRAP assay, the ferric ion reducing capability of the sample is quantified [51]. According to the Halvorsen et al. [52] most of the metabolites are redox active compounds that will be detected by the FRAP assay. The present study has shown that the leaf, root, and flower extracts of *J. betonica* exhibit significant ferric reduction properties. In agreement with the present results, Oran et al. [53] observed that the methanolic extracts of *Alcea chrysantha* whole plant showed greater FRAP activity (3.86±0.06 TE mg/gDW), than the aqueous extract (1.23±0.01 TE mg/gDW). Similarly, Paulsamy and Karthika [45] reported that the methanolic leaf and root extracts of *H. radicata* exhibited significantly increased ferric reducing potentials with increased extract concentration from 200 to 600 µg. Furthermore, leaf extract exhibited higher reducing activity than the root extract. Priyanka et al. [44] has been proposed that the stronger FRAP activities correlates with the enhanced antioxidant activities.

Superoxide Scavenging Activity

Table 08 depicts the percentages of superoxide scavenging activity from the leaf, root, and flower extracts of *J. betonica*.The results demonstrated that the all the tested extracts exhibited significant superoxide scavenging activity. However, the root extracts of *J. betonica* exhibited greatest superoxide scavenging activity with low IC_{50} value. Whereas, the lowest activity was found in flower extracts.At 6.25 mg/mL extract concentration, the superoxide scavenging activities from the leaf, root, and flower extracts of *J. betonica* were observed as 31.62±0.89, 38.4±1.31, and 34.38 % respectively. As well as, at 100 mg/mL concentration, the respective methanolic extracts exhibited 72.65±1.14, 68.03±1.82, and 61.29±1.27% of superoxide scavenging activities correspondingly. Furthermore, the IC_{50} value for leaf, root, and flower extracts were measured as 39.85, 20.69, and 46.69 mg/mL.These results also revealed that the superoxide scavenging activity from the leaf, root, and flower extracts of *J. betonica* were significantly (<.0005) increased with increasing extract concentrations. In

addition, the superoxide scavenging activities of leaf, root, and flower extracts show strong positive correlations of r =0.9778, r =0.8705, and r =0.9063 with respect to the increasing concentration (Table 09).

Table 08: Superoxide scavenging percentages with increasing concentrations of methanolic extracts from the leaf, root, and flowers of *J. betonica.*

S. No	Conc. of Extracts (mg/mL)	% Inhibition (Mean ± SD)		
		Leaf	Root	Flower
1	6.25	31.62±0.89	38.4±1.31	34.38±1.16
2	12.5	40.37±1.18	47.8±1.21	37.68±1.43
3	25	47.62±1.14	55.77±1.55	47.16±1.09
4	50	55.52±1.17	65.36±1.01	58.19±0.37
5	100	72.65±1.14	68.03±1.82	61.29±1.27
6	IC_{50}	39.85 mg/mL	20.69 mg/mL	46.69 mg/mL
P<0.05 was considered as significant difference.				

Table 09: Correlation analysis of the Superoxide scavenging activity of extracts with increasing concentration.

Type of Extract	Extract Conc.	*Leaf*	*Root*	*Flower*
Leaf	0.9778	1		
Root	0.8705	0.9422	1	
Flower	0.9063	0.9461	0.9828	1

Superoxide scavenging assay is an in vitro approach to determine the ability of extract to remove the reactive oxygen species such as O^{-}, OH^{-}, and H_2O_2 and thereby, screen for the possible antioxidant activity [54]. In the present study, the results of superoxide scavenging activity from the leaf, root, and flower extracts of *J. betonica* clearly demonstrate that the extracts have an increasing superoxide scavenging activity with increasing extract concentrations. These results show similarity the observations of Kavitha and Ponne [55] who reported that the methanolic seed extracts of *O. sanctum* exhibited 31±0.9 to 67±0.5% superoxide scavenging activity with increasing extract concentrations ranged between 50 and 350 µg/mL. Etim et al. [56] found that the methanolic leaf extract of *Napoleona imperialis* had significant superoxide scavenging activity with the IC_{50} of 20.23 µg/mL which is comparable to the root extracts of present study. This pattern of results might be occurring due to the presence of flavonoids, which are considered the most efficient antioxidants since they can easily scavenge superoxides [57].

Nitric Oxide Scavenging Activity

Table 10 presents the nitric oxide scavenging activities from the leaf, root, and flower extracts of *J. betonica* at various concentrations. From these results, it was observed that the flower extracts exhibited greater nitric oxide scavenging activity, whereas leaf and root extracts exhibited almost similar nitric oxide inhibition at all tested extract concentrations. At the lowest concentration (6.25 mg/mL), the methanolic extracts of leaf, root, and flowers from *J. betonica* showed 37.66±0.47, 39.32±0.43, and 54.21±0.63% of NO scavenging activities respectively. As the extract concentration increases to 100 mg/mL, the nitric oxide scavenging activity of leaf, root, and flowers from rises to 73.87±0.65, 74.62±0.46, and 85.64±0.57% respectively. Furthermore, lowest IC_{50} values were measured for flower extract (1.38 mg/mL), followed by root extract (19.38 mg/mL), and highest for leaf extract (25.14 mg/mL). This suggests that flower extract is the most potent among the three, requiring the lowest concentration to reach half maximal inhibition, whereas leaf extract is the least potent. The nitric oxide scavenging activities of leaf, root, and flower extracts show strong positive correlations of r =0.9993, r =0.9956, and r =0.9691 with respect to the increasing concentration (Table 11).

Table 10: Nitric oxidescavenging percentages with increasing concentrations of methanolic extracts from the leaf, root, and flowers of *J. betonica.*

S. No	Conc. of Extracts (mg/mL)	% Inhibition (Mean ± SD)		
		Leaf	Root	Flower
1	6.25	37.66±0.47	39.32±0.43	54.21±0.63
2	12.5	43.68±0.49	47.84±0.42	66.81±0.45
3	25	54.86±0.72	56.99±0.74	72.75±0.53
4	50	64.86±0.75	64.13±0.61	80.39±0.48
5	100	73.87±0.65	74.62±0.46	85.64±0.57
6	IC_{50}	25.14 mg/mL	19.38 mg/mL	1.38 mg/mL
P<0.05 was considered as significant difference.				

Table 11: Correlation analysis of the Nitric oxidescavenging activity of extracts with increasing concentration.

Type of Extract	Extract Conc.	*Leaf*	*Root*	*Flower*
Leaf	0.9993	1		
Root	0.9956	0.9949	1	
Flower	0.9691	0.9718	0.9822	1

Reactive nitrogen species, such as nitric oxide, are free radicals created by the reaction of NO with other reactive oxygen species and molecular oxygen [58]. Nitric oxide is a free radical because of its unpaired electrons and it is highly reactive with certain proteins and other free radicals such as superoxide [59]. The results of the present study align with those of Mishra et al. [60] who indicated that flower extracts from various medicinal plants exhibited enhanced nitric oxide scavenging capabilities. Kaur et al. [61] similarly noted that floral extracts from various plant species exhibited greater nitric oxide scavenging activity compared to leaves and roots. Patel et al. [62] indicated that the leaf and root extracts demonstrated the lowest antioxidant capacity in comparison to the flower extracts, potentially attributable to the reduced presence of bioactive phytochemicals in the former.

CONCLUSION

The present study revealed that *Justicia betonica* L. exhibited significant antioxidant activity and a wide range of phytochemicals with significant differences in leaves, roots and flowers. The methanolic flower extract exhibited potent DPPH and nitric oxide radical scavenging abilities and the highest saponin concentration, whereas the leaves had more phenolics and tannins. The roots exhibited a very significant superoxide scavenging activity. The antioxidant capacity increased with the dose for all the extracts, pointing to their medicinal importance in combating oxidative stress. These findings provide the scientific basis for the traditional medical use of *J. betonica* and point to the potential of floral extracts of *J. betonica* in particular to act as important natural antioxidants.

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AUTHOR CONTRIBUTIONS

All authors contributed to the conception and design of the study, laboratory experiments, data analysis, manuscript preparation, and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ETHICS STATEMENT

This study involved only plant materials and laboratory-based *in vitro* experiments. Therefore, ethical approval was not required.

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INFORMED CONSENT STATEMENT

Not applicable.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article.

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