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Research Article

HARNESSING *JUSTICIA BETONICA* L. FOR HEALTH: ANTIMICROBIAL AND ANTIDIABETIC ACTIVITIES ACROSS DIFFERENT PLANT PARTS

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*CORRESPONDING AUTHOR Jyothsna Lakshman L	The growing incidence of antimicrobial resistance and metabolic disorders such as diabetes, has led to an urgent search for novel therapeutic agents from plants. The objective of the present study was to explore the antimicrobial and antidiabetic potential of methanolic extracts of leaf, root and flower of <i>Justicia betonica</i> L., a plant with traditional medicinal use. The antimicrobial activity of the extracts was determined against gram-positive and gram-negative bacteria and fungi by the agar well diffusion method at five different concentrations (6.25, 12.5, 25, 50 and 100 mg/mL). The results showed significant dose dependent antimicrobial activity with highest inhibition zones of leaf extract especially on <i>Aspergillus niger</i> (28 mm) and <i>Bacillus subtilis</i> (20 mm) at 100 mg/mL. In vitro antidiabetic activity was evaluated for carbohydrate binding capacity, glucose diffusion inhibition and alpha-amylase inhibition assays. The highest positive correlation of the concentration of extract with adsorption of glucose and retardation of glucose diffusion was reported for the root extracts. However, leaf extract showed the highest α-amylase inhibitory activity (IC_{50} = 51.54 mg/mL). The findings suggested that <i>J. betonica</i> could be a valuable source of natural products for managing microbial infections and hyperglycemia and further studies are needed to explore its medicinal or nutraceutical applications.

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INTRODUCTION

The intensive utilization of natural resources leads to irreversible changes and the depletion of conventional food sources [1]. Consequently, it is essential to observe the environment and investigate the feasibility of utilizing biologically active substances as alternative food sources. Moreover, the proliferation of pathogenic bacteria pollution has emerged as a significant global public health issue, leading to serious human diseases and considerable economic losses [2]. About 80% of pathogenic infections are challenging to eradicate and markedly increase the resistance of pathogenic bacteria to standard antibiotics and disinfectants [3]. The development of antimicrobial resistance (AMR) constitutes a significant worldwide health crisis, compromising the efficacy of infectious disease therapies and increases mortality [4]. Traditional antibiotics

are becoming less effective due to overuse and misuse, resulting in multidrug-resistant bacterial strains [5]. Furthermore, oxidative stress, resulting reactive oxygen species (ROS) is linked to the onset of numerous chronic and degenerative diseases, including cancer, cardiovascular diseases, diabetes, and neurodegenerative disorders [6]. The escalating peril of antimicrobial resistance and oxidative stress has heightened the quest for novel therapeutic agents derived from natural sources, particularly plants, which serve as a promising reservoir of bioactive chemicals exhibiting antimicrobial, antidiabetic, and anticancer effects.

Plant-based substances persist in their significant importance in primary health care as therapeutic medicines in numerous underdeveloped nations. Plants remain the predominant source of pharmaceuticals for most of the global population. In traditional medicine,

which is practiced by an estimated 80% of the global population, a large portion of the treatments, mostly comprised of plant extracts or active components, is utilised [7]. Numerous studies have been conducted globally to identify secondary metabolites in natural resources [8]. Recently, much attention has been focused on the use of plant products to manage various microbial infestations [9]. The identification and quantification of antimicrobial and antidiabetic properties 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 [10]. Consequently, this study selects *Justicia betonica* owing to its traditional therapeutic applications and the scarcity of scientific research on this species.

Justicia betonica L., commonly known as the white shrimp plant or squirrel's tail, is a perennial shrub in the Acanthaceae family, prevalent in tropical and subtropical regions of Asia and Africa [11]. The plant typically reaches a height of 1–2 meters and is characterized by its erect, branching stems and opposite, ovate-lanceolate leaves that may extend up to 15 cm in length. The most distinguishing feature is the inflorescence: compact spikes of overlapping white to pale pink bracts, often displaying green or purple stripes, which enclose small tubular flowers. Under suitable temperatures, flowering occurs year-round, attracting bees and butterflies for pollination [12]. *Justicia betonica* has been conventionally employed in several pharmaceutical systems for the management of respiratory ailments, wounds, and febrile conditions. Phytochemical analyses have revealed the presence of flavonoids, alkaloids, and saponins, which may contribute to its alleged anti-inflammatory and antibacterial effects [11]. Therefore, the present study focused on the evaluation of antimicrobial and antidiabetic properties from the leaf, root, and flower extracts of *Justicia betonica*.

MATERIALS AND METHODS

Collection of *Justicia betonica* L. Plants

Young and healthy plants of *Justicia betonica* were collected from the Vijnigiripalem 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. [13] 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.

Screening of Antimicrobial Activity

The antimicrobial potential of methanolic extracts from the leaf, root, and flowers of *J. betonica* were evaluated by the agar well diffusion method at 5 different extract concentrations with increasing manner such as 6.25, 12.5, 25, 50, and 100 mg/mL. The bactericidal activity was assessed against gram positive bacteria such as *Bacillus subtilis* (MTCC-441), *Micrococcus luteus* (MTCC-1809) and gram-negative bacteria such as *Escherichia coli* (MTCC-293), *Pseudomonas aeruginosa* (MTCC-1034). For antibacterial activity erythromycin was used as positive control. The antifungal activity was assessed against *Aspergillus niger* (MTCC-872) and *Candida albicans* (MTCC-3017). For antifungal activity nystatin was used as positive control. DMSO was used as negative control for both antibacterial and antifungal activity. For antibacterial and antifungal activity, Nutrient agar plates and potato dextrose agar plates were prepared. Then the bacterial strains were spread on the nutrient agar plates and the fungal strains were spread on the PDA plates by using L-shape glass rod. After culture spreading, 0.5 mm wells were prepared and the wells were loaded with test extracts, positive control, and negative controls. Then, the plates were sealed with parafilm and incubated for 24 hours at 37°C in a bacteriological incubator. After incubation, zone of inhibition for extracts were measured in millimetres using zone scale.

Screening of In Vitro Antidiabetic Activity

The antidiabetic potential of methanolic extracts from the leaf, root, and flower extracts of *J. betonica* were evaluated at five increasing concentrations (6.25, 12.5, 25, 50, and 100 mg/mL) by assessing their carbohydrate binding capacity, effect on in-vitro glucose diffusion, and alpha amylase inhibitory activity. Glucose adsorption capacity of methanolic extracts from leaf, root, and flowers of *J. betonica* were determined by the methodology of Ou et al. [14]. The effect of methanolic extracts on in-vitro glucose diffusion was determined by Ahmed et al. [15] methodology. The α -amylase inhibitory assay for the methanolic extracts was evaluated by the Malik and Singh [16] method.

Statistical Analysis

The results were expressed as mean $\pm$ 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

Antimicrobial Activity of *J. betonica* Leaf Extracts

Table 01 and Figure 01 depict the results of antimicrobial activity of leaf extracts with increasing concentra-

tions. From the results, it was observed that the antimicrobial potentials of leaf extracts show significant increase with increasing extract concentration. Among all the tested gram-positive, gram-negative bacteria, and fungi, the leaf extract showed maximum antimicrobial activity against *A. niger* (28 mm), followed by *B. subtilis* (20 mm), *M. luteus* (20 mm), *E. coli* (14 mm), *P. aeruginosa* (18 mm), and *C. albicans* (16 mm) at 100 mg/mL concentration. While at 6.25 mg/mL concentration, the highest antimicrobial activity was found against

A. niger (14 mm) followed by *E. coli* (10 mm), *P. aeruginosa* (10 mm), *B. subtilis* (8 mm), *M. luteus* (8 mm), and *C. albicans* (6 mm). Furthermore, the antimicrobial potentials of leaf extracts with increasing extract concentration show very strong positive correlations such as $r = 0.9868$, $r = 0.9683$, $r = 0.9669$, $r = 0.9225$, $r = 0.9562$, and $r = 0.9862$ against to the *B. subtilis*, *M. luteus*, *E. coli*, *P. aeruginosa*, *A. niger*, and *C. albicans* respectively (Table 02).

Table 01: Antimicrobial activity of leaf methanolic extract from *J. betonica* with increasing concentration.

Name of the Organism		Diameter of the Inhibition Zone (mm)						
		Conc. of Extract(mg/mL)					+ve Control	-ve Control
		6.25	12.5	25	50	100		
Gram Positive Bacteria	Bacillus subtilis	8	9	12	15	20	33	-
	Micrococcus luteus	8	8	12	16	20	32	-
Gram Negative Bacteria	Escherichia coli	10	12	14	15	19	33	-
	Pseudomonas aeruginosa	10	11	13	17	18	26	-
Fungi	Aspergillus niger	14	16	20	24	28	28	-
	Candida albicans	6	8	9	12	16	25	-

Table 02: Correlation analysis of antimicrobial activity from the leaf methanolic extract of *J. betonica* with increasing concentration.

Microbial Species	Extract. conc.	<i>B. subtilis</i>	<i>M. luteus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>A. niger</i>	<i>C. albicans</i>
<i>B. subtilis</i>	0.9868	1					
<i>M. luteus</i>	0.9683	0.9925	1				
<i>E. coli</i>	0.9669	0.9843	0.9612	1			
<i>P. aeruginosa</i>	0.9225	0.9626	0.9792	0.9309	1		
<i>A. niger</i>	0.9562	0.9899	0.9909	0.9783	0.9848	1	
<i>C. albicans</i>	0.9862	0.9905	0.9738	0.9833	0.9573	0.9808	1

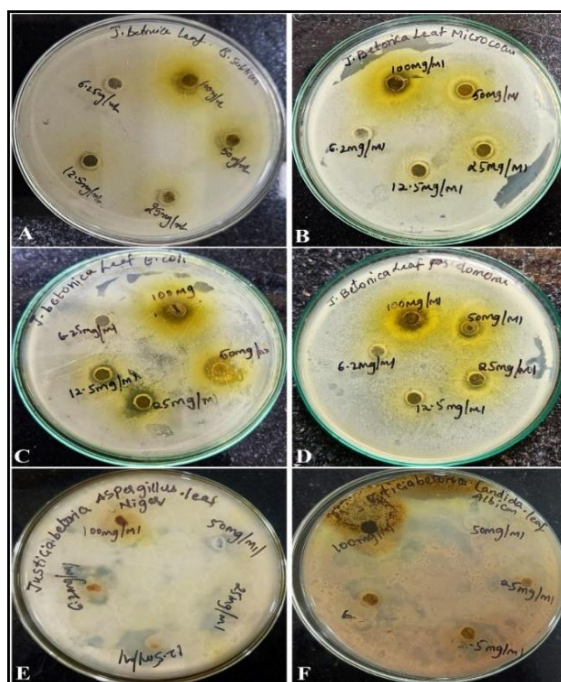


Figure 01: Antimicrobial activity of *J. betonica* leaf methanolic extracts. Inhibitory zones against A) *B. Subtilis*, B) *M. Luteus*, C) *E. coli*, D) *P. aeruginosa*, E) *A. niger*, F) *C. albicans*.

Antimicrobial Activity of *J. betonica* Root Extracts

Table 03 and Figure 02 depict the results of antimicrobial activity of root extracts with increasing concentrations. From the results, it was observed that the antimicrobial potentials of root extracts show significant increase with increasing extract concentration. Among all the tested gram-positive, gram-negative bacteria, and fungi, the root extract showed maximum antimicrobial activity against *M. luteus* (23 mm) followed by *E. coli* (17 mm), *P. aeruginosa* (16 mm), *B. subtilis* (15 mm), *A. niger* (10 mm), and *C. albicans* (8 mm) at 100 mg/mL concen-

tration. While at 6.25 mg/mL concentration only *M. luteus* (6 mm) and *P. aeruginosa* (6 mm) showed zone of inhibition. The fungal strains *A. niger* and *C. albicans* exhibited zone of inhibition only at 100 and 50 mg/mL extract concentration. Furthermore, the antimicrobial potentials of root extracts with increasing extract concentration show very strong positive correlations such as $r = 0.9578$, $r = 0.9809$, $r = 0.9331$, $r = 0.9811$, $r = 0.9378$, and $r = 0.9378$ against to the *B. subtilis*, *M. luteus*, *E. coli*, *P. aeruginosa*, *A. niger*, and *C. albicans* respectively (Table 04).

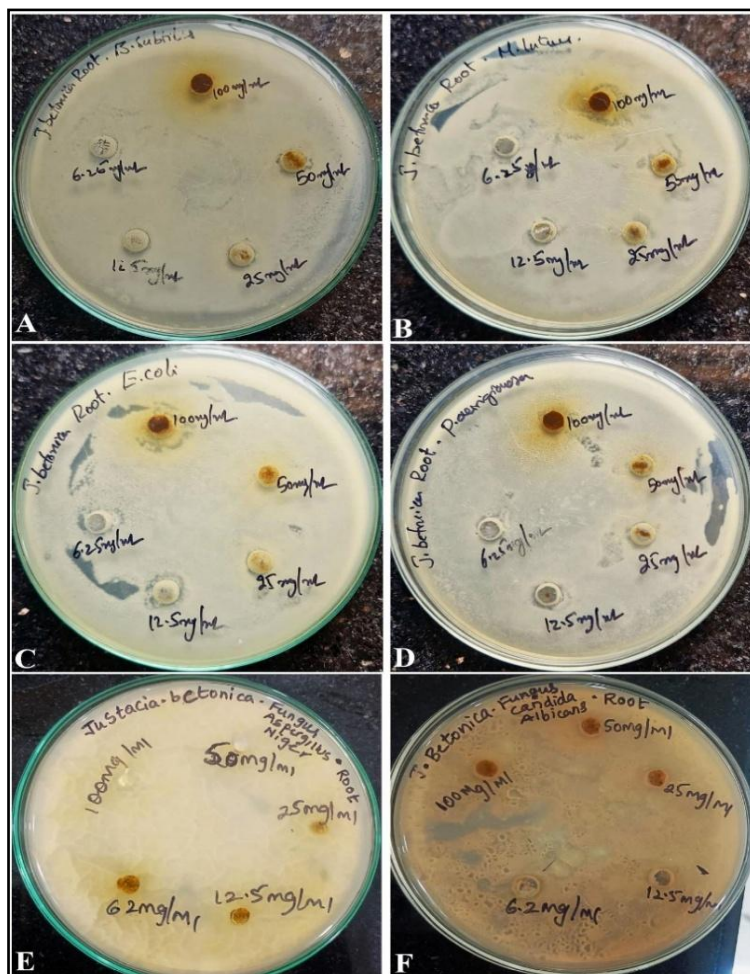


Figure 02: Antimicrobial activity of *J. betonica* root methanolic extracts. Inhibitory zones against A) *B. Subtilis*, B) *M. Luteus*, C) *E. coli*, D) *P. aeruginosa*, E) *A. niger*, F) *C. albicans*.

Table 03: Antimicrobial activity of root methanolic extract from *J. betonica* with increasing concentration.

Name of the Organism		Diameter of the Inhibition Zone (mm)						
		Conc. of Extract(mg/mL)					+ve Control	-ve Control
		6.25	12.5	25	50	100		
Gram Positive Bacteria	<i>Bacillus subtilis</i>	0	0	6	10	15	33	-
	<i>Micrococcus luteus</i>	6	8	9	17	23	32	-
Gram Negative Bacteria	<i>Escherichia coli</i>	0	6	8	12	17	33	-
	<i>Pseudomonas aerugi- nosa</i>	6	8	9	10	16	26	-
Fungi	<i>Aspergillus niger</i>	0	0	0	6	10	28	-
	<i>Candida albicans</i>	0	0	0	6	8	25	-

Table 04: Correlation analysis of antimicrobial activity from the root methanolic extract of *J. betonica* with increasing concentration.

Microbial Species	Extract. conc.	<i>B. subtilis</i>	<i>M. luteus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>A. niger</i>	<i>C. albicans</i>
<i>B. subtilis</i>	0.9578	1					
<i>M. luteus</i>	0.9809	0.9586	1				
<i>E. coli</i>	0.9331	0.9423	0.9465	1			
<i>P. aeruginosa</i>	0.9811	0.9212	0.9411	0.9410	1		
<i>A. niger</i>	0.9378	0.9199	0.9812	0.8794	0.8641	1	
<i>C. albicans</i>	0.9378	0.9199	0.9812	0.8794	0.8641	1	1

Antimicrobial Activity of *J. betonica* Flower Extracts

Table 05 and Figure 03 depict the results of antimicrobial activity of flower extracts with increasing concentrations. From the results, it was observed that the flower extracts showed significant antimicrobial potentials only at 100 and 50 mg/mL concentration against tested microorganisms. Furthermore, *Candida albicans* didn't exhibit any zone of inhibition at all the tested concentrations. Among all the tested gram-positive, gram-negative bacteria, and fungi, the flower extract

showed maximum antimicrobial activity against *M. luteus* (28 mm) followed by *B. subtilis* (18 mm), *P. aeruginosa* (16 mm), *E. coli* (15 mm), and *A. niger* (14 mm) at 100 mg/mL concentration. At 6.25 mg/mL concentration no zone inhibition was observed for all the tested microorganisms. Furthermore, the antimicrobial potentials of flower extracts with increasing extract concentration show strong positive correlations such as $r = 0.9007$, $r = 0.9303$, $r = 0.9702$, $r = 0.9379$, and $r = 0.9728$ against to the *B. subtilis*, *M. luteus*, *E. coli*, *P. aeruginosa*, and *A. niger* respectively (Table 06).

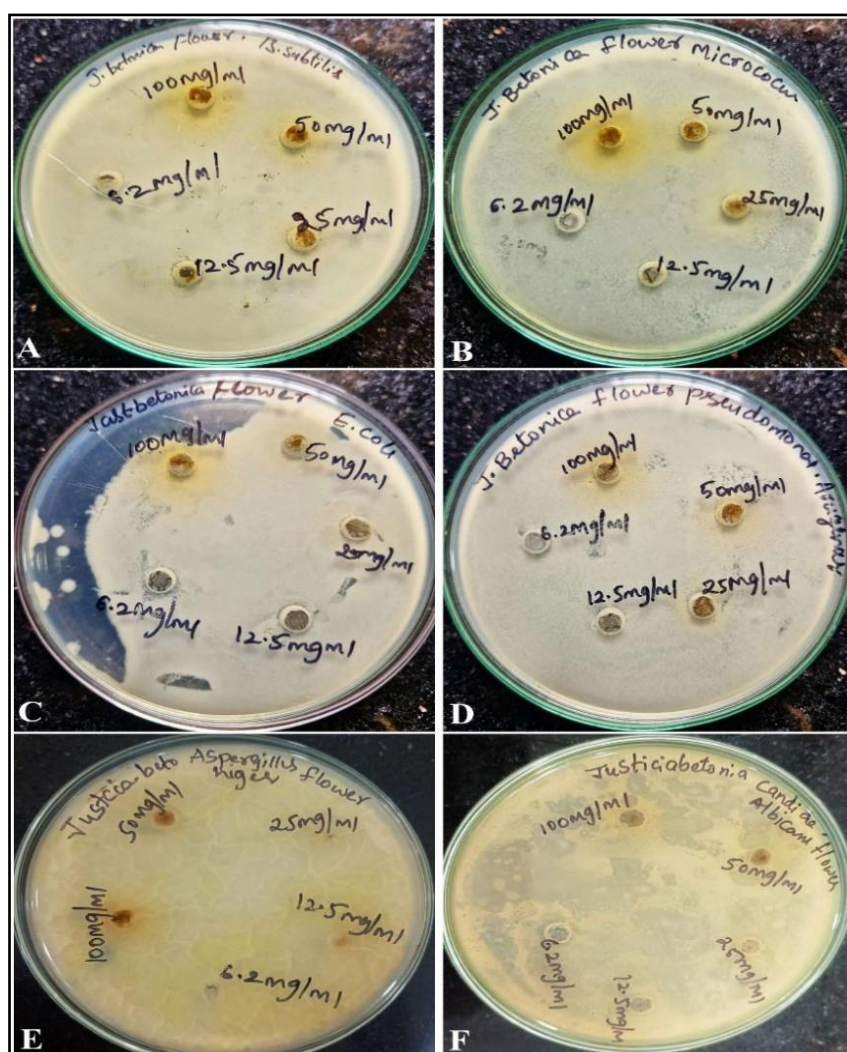


Figure 03: Antimicrobial activity of *J. betonica* flower methanolic extracts. Inhibitory zones against A) *B. Subtilis*, B) *M. Luteus*, C) *E. coli*, D) *P. aeruginosa*, E) *A. niger*, F) *C. albicans*.

Table 05: Antimicrobial activity of flower methanolic extract from *J. betonica* with increasing concentration.

Name of the Organism		Diameter of the Inhibition Zone (mm)						
		Conc. of Extract(mg/mL)					+ve Control	-ve Control
		6.25	12.5	25	50	100		
Gram Positive Bacteria	Bacillus subtilis	0	0	10	14	18	33	-
	Micrococcus luteus	0	8	14	21	28	32	-
Gram Negative Bacteria	Escherichia coli	0	0	6	8	15	33	-
	Pseudomonas aerugi- nosa	0	0	8	10	16	26	-
Fungi	Aspergillus niger	0	0	0	8	14	28	-
	Candida albicans	0	0	0	0	0	25	-

Table 06: Correlation analysis of antimicrobial activity from the flower methanolic extract of *J. betonica* with increasing concentration.

Microbial Species	Extract. conc.	<i>B. subtilis</i>	<i>M. luteus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>A. niger</i>	<i>C. albicans</i>
<i>B. subtilis</i>	0.9007	1					
<i>M. luteus</i>	0.9303	0.9570	1				
<i>E. coli</i>	0.9702	0.9693	0.9516	1			
<i>P. aeruginosa</i>	0.9379	0.9902	0.9572	0.9927	1		
<i>A. niger</i>	0.9728	0.8581	0.8876	0.9152	0.8796	1	
<i>C. albicans</i>	0	0	0	0	0	1	1

In the present study leaf extracts exhibited more antimicrobial activity than root and flower extracts. Furthermore, all the extracts demonstrated increased zone of inhibition against the tested microbes with increase in concentration of the extracts. The findings align with those of Patel et al. [17] who noted a dose-dependent antimicrobial activity, as the concentration of the extract increases, the zone of inhibition against tested bacteria also increases, while the minimum inhibitory concentration decreases. The present results are in agreement with the findings of Rasool et al. [18] who also observed increase in antibacterial activity of ethanolic leaf extracts of *Azadirachta indica* against *Staphylococcus aureus* and *Escherichia coli* with increase in concentrations from 50 - 200 mg/mL. Similarly, Mahek et al. [19] reported that flower extracts of *Hibiscus rosa-sinensis* showed better inhibition of *Pseudomonas aeruginosa* at concentration more than 100 mg/mL. Recent studies have shown that the most important antimicrobial mechanisms of phytochemicals are related to the destruction of the integrity of the microbial cell wall and cell membrane, leading to the release of cellular components and cell death [20].

Glucose Adsorption Capacity

The results of glucose adsorption abilities from the leaf, root, and flower extracts of *J. betonica* are shown in Table 07. From these results, it was observed that the root extracts exhibited greatest glucose adsorption ability at all tested concentrations. At 6.25 mg/mL extract concentration, the glucose adsorption ability of extracts from leaf, root, and flowers of *J. betonica* were observed as 30.69 ± 0.73 , 31.4 ± 0.56 , and 23.91 ± 0.59 % respectively. As well as, at 100 mg/mL concentration, the respective extracts exhibited 35.72 ± 0.62 , 40.49 ± 0.51 , and 33.02 ± 0.61 % of glucose adsorption abilities correspondingly. Furthermore, the EC_{50} value for leaf, root, and flower extracts were measured as 338.92, 201.69, and 273.59 mg/mL. The present results revealed that the extracts exhibited a dose-dependent glucose adsorption ability and the highest activity was shown at high concentration. However, the increasing abilities with concentrations were not significant in all tested extracts. In addition, the glucose adsorption abilities of leaf, root, and flower extracts show strong positive correlations of $r = 0.9574$, $r = 0.9897$, and $r = 0.9909$ with respect to the increasing concentration (Table 08).

Table 07: Glucose adsorption capacity of leaf, root, and flower extracts from *J. betonica* with increasing concentrations.

S. No.	Extract Conc. (mg/ml)	Glucose adsorption capacity (%)		
		Leaf	Root	Flower
1	6.25	30.69 ± 0.73	31.4 ± 0.56	23.91 ± 0.59
2	12.5	31.22 ± 0.46	34.23 ± 0.26	26.06 ± 0.46
3	25	31.93 ± 0.54	35.96 ± 0.34	28.21 ± 0.35
4	50	35.33 ± 0.69	38.26 ± 0.49	31.71 ± 0.55
5	100	35.72 ± 0.62	40.49 ± 0.51	33.02 ± 0.61
6	EC_{50}	338.92 mg/mL	201.69 mg/mL	273.59 mg/mL
P<0.05 was considered as significant difference.				

Table 08: Correlation of Glucose adsorption capacity from the leaf, root, and flower extracts with increasing concentrations.

Type of Extract	Extract conc.	Leaf	Root	Flower
Leaf	0.9574	I		
Root	0.9897	0.9382	I	
Flower	0.9909	0.9721	0.9898	I

The results of current study clearly demonstrated that there are strong positive correlations between the extract concentrations and the glucose adsorption ability of leaf, root, and flower extracts of *J. betonica*. In evident with the present findings, Rehman et al. [21] reported that the ethanolic extracts of *Cassia nemophila* pod showed significantly increasing glucose adsorption capabilities with increasing extract concentrations. Furthermore, the greatest and lowest glucose adsorption was found at the 5 and 30 mM concentration of extract. The studies Bhutkar et al. [22] demonstrated that the extracts of *C. bonducella*, *B. aristata*, and *M. pruriens* showed glucose adsorption capacities of 73.96, 71.44, and 71.25% respectively from 100 mM glucose solution. Numerous studies have demonstrated that there is an association between dietary fiber and glucose adsorption activity. Later, it was identified that the glucose adsorption ability also depends on the presence of secondary metabolites, especially phenolic compounds [21].

Effect of Extracts on In-Vitro Glucose Diffusion

Table 09 displays the effects of different doses of leaf, root, and flower extracts on in-vitro glucose diffusion. From these results, it was observed that the root extracts of *J. betonica* greatly decreased the transport of glucose across dialysis membrane and increases the % of GDRI at all tested concentrations. Whereas, the lowest % of GDRI was observed with leaf extracts. At 6.25 mg/mL extract concentration, the leaf, root, and flower extracts exhibited 46.87 ± 0.63 , 63.15 ± 0.95 , and 55.83 ± 0.57 % of GDRI respectively. As well as, at 100 mg/mL concentration, the percentage of GDRI for the respective extracts were found to be 62.34 ± 0.58 , 77.81 ± 0.76 , and 70.09 ± 0.52 %. Furthermore, the EC_{50} value for leaf, root, and flower extracts were measured as 2.39, 0.73, 0.94, 2.74, and 6.89 mg/mL. The present results revealed that the extracts increase % of GDRI in a dose-dependent manner and the highest activity was shown at high concentration. In addition, the % of GDRI from leaf, root, and flower extracts of *J. betonica* show strong positive correlations of $r=0.9935$, $r=0.9802$, and $r=0.99$ with respect to the increasing concentration (Table 10).

Table 09: Percentage of In-vitro Glucose Diffusion Activity with increasing concentrations of leaf, root, and flower extracts from *J. betonica*.

S. No.	Extract Conc. (mg/mL)	% GDRI		
		Leaf	Root	Flower
1	6.25	46.87 ± 0.63	63.15 ± 0.95	55.83 ± 0.57
2	12.5	51.24 ± 0.55	68.01 ± 0.82	60.25 ± 0.59
3	25	54.24 ± 0.58	71.96 ± 0.45	62.77 ± 0.46
4	50	58.29 ± 0.52	74.99 ± 0.94	66.69 ± 1.15
5	100	62.34 ± 0.58	77.81 ± 0.76	70.09 ± 0.52
6	EC_{50}	7.68 mg/mL	2.42 mg/mL	4.58 mg/mL
P<0.05 was considered as significant difference.				

Table 10: Correlation analysis of In-vitro Glucose Diffusion Activity from leaf, root, and flower methanolic extracts with increasing concentration.

Type of Extract	Extract conc.	Leaf	Root	Flower
Leaf	0.9935	I		
Root	0.9802	0.9912	I	
Flower	0.99	0.9995	0.9927	I

Results of the present study suggest that methanolic extracts of leaves, roots and flowers of *J. betonica* increase GDRI in a dose dependent manner thereby indicating their ability to inhibit glucose transport through dialysis membrane. The root extract showed highest GDRI in all the tested doses with EC_{50} value 2.42 mg/mL. These results are consistent with Hannan et al. [23] who reported that ethanolic extract of *O.*

sanctum significantly reduced glucose diffusion in a dose-dependent manner at doses of 100-25,000 μ g/mL. Likewise, Wang et al. [24] showed that proanthocyanidins extracted from Chinese bayberry leaves inhibited glucose transport through Caco-2 cells significantly under simulated fasting and simulated feeding conditions. The present findings are corroborated by Rane et al. [25] which showed increased inhibition on glu-

cose diffusion with increased concentrations of the plant extracts.

α -Amylase Inhibitory Activity

The percentages of α -amylase inhibitory activity from the leaf, root, and flower extracts from *J. betonica* are shown in Table 11. From these results, it was observed that the leaf extracts exhibited greatest α -amylase inhibitory activity at all tested concentrations. Whereas, the lowest activity was found in root extracts. At 6.25 mg/mL extract concentration, the α -amylase inhibitory activities of extracts from leaf, root, and flowers were observed as 36.24 ± 0.41 , 12.31 ± 0.78 , and 28.08 ± 0.82 % respectively. As well as, at 100 mg/mL concentration,

the respective extracts exhibited 57.26 ± 0.62 , 32.73 ± 0.74 , and 49.24 ± 0.51 % of α -amylase inhibitory activities correspondingly. The IC_{50} value for leaf, root, and flower extracts was measured as 51.54, 174.49, and 92.98 mg/mL. Furthermore, these results revealed that the extracts exhibited α -amylase inhibitory activity in a dose-dependent manner and the highest activity was shown at high concentration. In addition, the α -amylase inhibitory activities of leaf, root, and flower extracts show strong positive correlations of $r = 0.8686$, $r = 0.9384$, and $r = 0.9102$ with respect to the increasing concentration (Table 12).

Table 11: α -amylase inhibitory activity of leaf, root, and flower extracts from *J. betonica* with increasing concentrations.

S. No.	Extract Conc. (mg/mL)	α -Amylase Inhibitory Activity (%)		
		Leaf	Root	Flower
1	6.25	36.24 ± 0.41	12.31 ± 0.78	28.08 ± 0.82
2	12.5	38.71 ± 0.58	16.94 ± 0.67	33.31 ± 0.78
3	25	49.7 ± 0.45	21.32 ± 0.85	40.23 ± 0.72
4	50	54.43 ± 0.54	28.32 ± 0.38	44.34 ± 0.47
5	100	57.26 ± 0.62	32.73 ± 0.74	49.24 ± 0.51
6	IC_{50}	51.54 mg/mL	174.49 mg/mL	92.98 mg/mL

P<0.05 was considered as significant difference.

Table 12: Correlation of α -amylase inhibitory activity of extracts with increasing concentrations.

Type of Extract	Extract conc.	Leaf	Root	Flower
Leaf	0.8686	1		
Root	0.9384	0.9684	1	
Flower	0.9102	0.9847	0.9889	1

α -amylase is an enzyme that catalyses the breakdown of complex polysaccharides like starch into a group of simpler oligosaccharides, which can subsequently hydrolyse into an absorbable sugar in the intestine. Hence, limiting carbohydrate hydrolysis by inhibiting enzymes such as α -amylase is important in diabetes management. Sim et al. [26] proposed that limiting oligosaccharide hydrolysis by inhibiting pancreatic α -amylase is one of the significant therapeutic strategies to decrease absorbable monosaccharides in the intestine, thereby reducing postprandial hyperglycemia. According to the present findings, the α -amylase inhibitory activity of leaf, root, and flower extracts from *J. betonica* increased with increasing extract concentrations, suggesting a dose-dependent impact. Furthermore, the leaf extract showed highest α -amylase inhibitory activities in all the tested doses with IC_{50} value of 51.54 mg/mL. These results are consistent with the findings of Oluwagunwa et al. [27] who found that the aqueous leaf extracts of *Amaranthus viridis*, *Solanum macrocarpon*, and *Telfairia occidentalis* showed significant dose-dependent α -amylase inhibitory activity. Similarly, Sachan et al. [28] demonstrated dose-dependent α -amylase inhibitory effects of organic extracts from *Alhagi pseudalhagi*, *Caesalpinia bonduc*, and *Pluchea lanceolata*.

CONCLUSION

The results of the present study revealed that the methanolic extracts of leaf, root and flower of *Justicia betonica* showed a significant dose dependent antimicrobial and anti-diabetic activities. The leaf extract exhibited better antimicrobial and α -amylase inhibitory effects while the root extract showed better glucose adsorption and diffusion inhibition. The observed activities are attributed to the presence of bioactive secondary metabolites and suggest that the plant could be a source of functional food ingredients or therapeutic agents.

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AUTHOR CONTRIBUTIONS

All authors contributed to the study conception, experimental work, 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. No human participants or animals were involved; 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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