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RESEARCH ARTICLE

EVALUATION OF PHYTOCHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITIES OF LEAF AND ROOT EXTRACTS OF *JUSTICIA SANTAPAUI* BENNET

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Abstract: The present study evaluated the phytochemical composition and pharmacological potential of leaf and root extracts of *Justicia santapaui* Bennet, an endemic medicinal plant of the Western Ghats, Kerala. Plant materials were sequentially extracted using petroleum ether, chloroform, ethyl acetate, methanol, and distilled water. Qualitative and quantitative phytochemical analyses were performed using standard protocols. Antioxidant activity was evaluated through DPPH and FRAP assays; anti-inflammatory potential via protein denaturation, LOX, and COX inhibition assays; antidiabetic activity using α-amylase inhibition assay; and cytotoxicity against SH-SY5Y human neuroblastoma cells using SRB assay. Methanol exhibited the highest extraction yield for both leaves (15.83%) and roots (48%). Leaves demonstrated greater phytochemical diversity (10 classes) compared to roots (6 classes), with alkaloids as the most abundant constituent (65.03 mg/g) in the methanolic leaf extract. The leaf extract exhibited superior antioxidant activity (DPPH IC_{50} = 381.84 µg/mL; FRAP IC_{50} = 482.84 mg/mL), potent anti-inflammatory activity (LOX IC_{50} = 85.34 µg/mL; COX IC_{50} = 92.67 µg/mL), significant α-amylase inhibition (IC_{50} = 137.20 µg/mL), and moderate cytotoxicity against SH-SY5Y cells (LC_{50} = 127.69 µg/mL). The superior bioactivity of leaves correlated with their richer phytochemical composition, including higher alkaloid, phenolic, and flavonoid contents, along with the exclusive presence of cardiac glycosides and steroids. This study provides the first scientific validation of *J. santapaui* and establishes the methanolic leaf extract as a promising source of bioactive compounds with multi-target therapeutic potential for further bioactivity-guided fractionation and mechanistic studies.

Keywords: *Justicia santapaui*, Phytochemical screening, Antioxidant, Anti-inflammatory, Antidiabetic

INTRODUCTION

Justicia santapaui Bennet, a lesser-known medicinal plant belonging to the family Acanthaceae, represents an underexplored source of bioactive phytochemicals with significant therapeutic potential. The genus *Justicia* comprises approximately 600 species distributed across tropical and subtropical regions worldwide, many of which have been traditionally used in various indigenous medicinal systems for the treatment of inflammatory disorders, microbial infections, and metabolic diseases (Corrêa *et al.,* 2016). Species such as *J. adhatoda*, *J. procumbens*, and *J. gendarussa* have been extensively studied for their pharmacological properties, revealing the presence of diverse bioactive compounds including alkaloids, flavonoids, lignans, and terpenoids. However, *J. santapaui* remains comparatively unexplored despite its endemic distribution in the Western Ghats of Kerala, India, a region recognized as one of the world's biodiversity hotspots.

The increasing global burden of chronic diseases, including neurodegenerative disorders, diabetes, and inflammatory conditions, has necessitated the search for novel therapeutic agents from natural sources (WHO, 2019). Neurodegenerative diseases, particularly those affecting the central nervous system, represent a growing public health challenge with limited effective treatment options (Gitler *et al.,* 2017). The pathogenesis of neurodegenerative disorders is closely associated with oxidative stress, neuroinflammation, and mitochondrial dysfunction, making plants rich in antioxidant and anti-inflammatory compounds potential candidates for therapeutic development (Barnham *et al.,* 2004; Chen *et al.,* 2016). Similarly, the global diabetes epidemic, affecting over 537 million adults worldwide, demands the identification of effective antidiabetic agents with fewer side effects compared to conventional synthetic drugs (IDF, 2021). Plant-derived α-amylase inhibitors have gained attention as alternative therapeutic approaches for managing postprandial hyperglycemia (Tundis *et al.,* 2010).

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Phytochemical screening of various *Justicia* species has demonstrated the presence of alkaloids, flavonoids, phenolic compounds, and terpenoids, which contribute to their diverse pharmacological activities (Sahu *et al.*, 2016). Alkaloids, in particular, have been associated with neuroprotective and cytotoxic activities through multiple mechanisms including modulation of neurotransmitter systems, inhibition of cholinesterase enzymes, and induction of apoptosis in cancer cells (Khan et al., 2020). Phenolic compounds and flavonoids are well-known for their potent antioxidant properties, capable of scavenging free radicals and chelating metal ions, thereby protecting cells from oxidative damage (Rice-Evans et al., 1997). The anti-inflammatory activity of *Justicia* species has been attributed to the inhibition of key enzymes in the arachidonic acid cascade, including cyclooxygenase (COX) and lipoxygenase (LOX), as well as the suppression of pro-inflammatory cytokine. Furthermore, several studies have reported the cytotoxic potential of *Justicia* species against various cancer cell lines, suggesting their utility in cancer therapy.

Despite the pharmacological significance of the genus *Justicia*, comprehensive investigations on *J. santapaui* are notably lacking. The Western Ghats region, where this species is endemic, has been recognized as a repository of numerous medicinally important plants, many of which remain scientifically undocumented. The traditional knowledge of local communities in Kerala regarding medicinal plants provides a valuable foundation for scientific exploration. The presence of diverse phytochemicals in members of the Acanthaceae family, combined with the lack of documented pharmacological evidence for *J. santapaui*, necessitates a systematic investigation of its phytochemical composition and bioactivities.

The present study was therefore undertaken with the following objectives: (1) to evaluate the phytochemical profile of leaf and root extracts of *J. santapaui* through qualitative and quantitative analyses; (2) to assess the antioxidant activity using DPPH and FRAP assays; (3) to evaluate the anti-inflammatory potential through protein denaturation, LOX, and COX inhibition assays; (4) to determine the α-amylase inhibitory activity as an indicator of antidiabetic potential; and (5) to investigate the cytotoxic effects against SH-SY5Y human neuroblastoma cells. The selection of SH-SY5Y cells as the model system for cytotoxicity evaluation is particularly relevant given the increasing interest in plant-derived neuroprotective agents and the association of neurodegenerative diseases with oxidative stress and inflammation. The comparative analysis of leaf and root extracts aims to identify the most promising plant part for future bioactivity-guided fractionation and isolation of active principles. The findings of this study are expected to provide the first comprehensive scientific documentation of the phytochemical and pharmacological properties of *J. santapaui*, contributing to the valorization of this endemic species as a potential source of therapeutic compounds.

MATERIALS AND METHODS

Plant Material and Extraction

Fresh leaves and roots of *Justicia santapaui* Bennet were collected from Bonacaud, Thiruvananthapuram (8.6807° N, 77.1673° E) in December 2020 and authenticated by the Curator, Department of Botany, University of Kerala, using the *Flora of the Presidency of Madras* (Gamble, 1918). The washed plant parts were shade-dried (25–30°C) for 7–10 days, pulverized, and passed through a 40-mesh sieve.

Powdered samples (15 g each) were sequentially extracted using a Soxhlet apparatus with petroleum ether, chloroform, ethyl acetate, methanol, and distilled water in increasing polarity (Snyder and Kirkland, 1979). Extraction continued for 48 hours at appropriate solvent boiling points. Extracts were filtered, concentrated under reduced pressure at 40°C using a rotary evaporator, and dried to constant weight. Percentage yield was calculated, and extracts were stored at 4°C.

Phytochemical Analysis

Qualitative screening for phytoconstituents (terpenoids, phenolics, tannins, coumarins, saponins, glycosides, alkaloids, anthraquinones, flavonoids, steroids, fats/oils, and carbohydrates) was performed using standard protocols (Harborne, 1973; Trease and Evans, 1989).

Quantitative estimation was carried out as follows: alkaloids by gravimetric method (Harborne, 1973); total phenols by Folin-Ciocalteu method (Singleton and Rossi, 1965) expressed as gallic acid equivalents (mg GAE/g); tannins and saponins by gravimetric method; and flavonoids by aluminum chloride colorimetric method expressed as quercetin equivalents (mg QE/g).

Antioxidant Activity

DPPH Assay: Extracts (200–1000 µg/mL) were incubated with 0.1 mM DPPH for 1 hour in the dark. Absorbance was measured at 517 nm, and IC_{50} values were calculated from concentration-response curves. Ascorbic acid served as standard (Blois, 1958).

FRAP Assay: FRAP reagent (acetate buffer, TPTZ, $FeCl_3 \cdot 6H_2O$, 10:1:1) was mixed with extracts and incubated at 37°C for 30 minutes. Absorbance was recorded at 593 nm, with Trolox as standard (Benzie and Strain, 1996).

Anti-inflammatory Activity

Protein Denaturation Assay: Reaction mixtures containing 2% BSA and extracts (200–1000 µg/mL)

in PBS (pH 7.4) were incubated at 37°C for 30 minutes, heated at 70°C for 15 minutes, and absorbance measured at 280 nm. Diclofenac sodium served as positive control (Mizushima and Kobayashi, 1968).

LOX and COX Assays: RAW 264.7 macrophages were cultured in DMEM with 10% FBS. Cells were stimulated with 1 µg/mL LPS and treated with extracts or diclofenac for 24 hours. LOX activity was measured at 234 nm and COX activity at 632 nm. IC_{50} values were calculated (Di Rosa et al., 1971).

Antidiabetic Activity

α-Amylase Inhibition Assay: Extracts (10–50 µg/mL) were pre-incubated with α-amylase in phosphate buffer (pH 6.9) at 37°C for 10 minutes. Starch solution was added, followed by DNSA reagent after 5 minutes. Absorbance was measured at 540 nm, with acarbose as standard (Worthington Enzyme Manual).

Cytotoxicity Assay

SH-SY5Y human neuroblastoma cells (5×10^3/well) were treated with methanolic extracts (6.25–100 µg/mL) for 24 hours. Cytotoxicity was evaluated using the SRB assay according to Skehan et al. (1990). Cells were fixed with TCA, stained with 0.057% SRB, solubilized in Tris base, and absorbance measured at 510 nm. LC_{50} values were calculated, and morphological changes were observed microscopically.

Statistical Analysis

All experiments were performed in triplicate, and data were expressed as mean ± standard error (SE). IC_{50} and LC_{50} values were determined using ED50 PLUS V1.0 software.

RESULTS

Plant Material Authentication

Fresh leaves and roots of *Justicia santapaui* Bennet were successfully collected from Bonacaud, Thiruvananthapuram (8.6807° N, 77.1673° E) in December 2020 and authenticated by the Curator, Department of Botany, University of Kerala.

Phytochemical Analysis

Extraction Yields

Sequential extraction of leaf powder using petroleum ether, chloroform, ethyl acetate, methanol, and distilled water yielded 11.95%, 8.11%, 10.54%, 15.83%, and 5.01%, respectively. Root extracts yielded 21%, 32%, 39%, and 48% for petroleum ether, chloroform, ethyl acetate, and methanol, respectively. Methanol exhibited the highest yield for both plant parts, indicating the predominance of polar compounds. Roots consistently showed higher yields than leaves across all solvent systems.

Qualitative Phytochemical Screening

Leaf extracts revealed the presence of diverse phytoconstituents (Table 1). Alkaloids were ubiquitous across all extracts. The methanol extract demonstrated maximum diversity with nine compounds (carbohydrates, tannins, saponins, flavonoids, terpenoids, alkaloids, cardiac glycosides, fats/oils, and steroids). Coumarins were absent in all leaf extracts.

Root extracts showed terpenoids in all extracts. Phenolics, coumarins, and flavonoids were present in chloroform and methanol extracts. Tannins, saponins, and alkaloids were exclusively found in methanol extract, while anthraquinones were unique to chloroform extract. Cardiac glycosides, fats/oils, steroids, and carbohydrates were absent in all root extracts.

Comparative analysis revealed leaves possess greater phytochemical diversity (10 classes) compared to roots (6 classes). Cardiac glycosides, fats/oils, steroids, and carbohydrates were exclusive to leaves, while coumarins and anthraquinones were uniquely present in roots.

Table 1. Comparative phytochemical profile of leaves and roots

Metabolite	Leaves	Roots
Carbohydrates	+	-
Tannins	+	+
Saponins	+	+
Flavonoids	+	+
Terpenoids	+	+
Alkaloids	+	+
Cardiac Glycosides	+	-
Coumarins	-	+
Phenols	+	+
Anthraquinones	-	+
Fats and Oils	+	-
Steroids	+	-

(+) = Present; (-) = Absent

Quantitative Phytochemical Analysis

Quantitative analysis of methanolic leaf extract (Table 2) revealed alkaloids as the most abundant (65.03 ± 0.11 mg/g), followed by phenolics (57.10 ± 0.76 mg/g), tannins (46.75 ± 0.12 mg/g), flavonoids (35.40 ± 0.28 mg/g), and saponins (29.34 ± 0.67 mg/g).

Table 2. Quantitative phytochemicals in methanolic leaf extract

Parameter	Content (mg/g)
Alkaloids	65.03 ± 0.11
Phenolics	57.10 ± 0.76
Tannins	46.75 ± 0.12
Flavonoids	35.40 ± 0.28
Saponins	29.34 ± 0.67

Values expressed as Mean ± SE

Pharmacological Evaluation

Antioxidant Activity

DPPH Assay: Both leaf and root extracts exhibited concentration-dependent radical scavenging activity (Table 3). Leaf extract showed superior activity with IC_{50} of 381.84 ± 0.14 µg/mL compared to root extract (489.26 ± 0.16 µg/mL) and ascorbic acid standard (289.12 ± 0.14 µg/mL). At 1000 µg/mL, leaf extract showed 80.06% inhibition versus 72.38% for root extract.

FRAP Assay: Leaf extract demonstrated higher reducing power with IC_{50} of 482.84 ± 0.006 mg/mL compared to root extract (562.47 ± 0.008 mg/mL) and Trolox standard (242.61 ± 0.004 mg/mL).

Table 3. Antioxidant activity of leaf and root extracts

Concentration (µg/ml)	DPPH (% Inhibition)			FRAP (% Reducing Power)		
	Leaf	Root	Std*	Leaf	Root	Std**
200	40.78	35.21	44.79	38.48	32.15	48.70
400	51.00	46.74	55.61	45.09	40.26	56.31
600	61.22	57.37	67.13	53.31	48.67	64.43
800	70.54	67.80	78.56	65.83	61.02	75.05
1000	80.06	72.38	86.47	74.65	68.43	82.26
IC_{50}	**381.84**	**489.26**	**289.12**	**482.84**	**562.47**	**242.61**

*Std = Ascorbic acid; **Std = Trolox; IC_{50} values in µg/mL (DPPH) and mg/mL (FRAP)*

Anti-inflammatory Activity

Protein Denaturation Assay: Leaf extract showed concentration-dependent inhibition (45.74–85.26% at 200–1000 µg/mL) with IC_{50} of 299.27 ± 0.06 µg/mL, compared to root extract (392.47 ± 0.07 µg/mL) and diclofenac standard (241.13 ± 0.03 µg/mL) (Table 4).

LOX and COX Inhibitory Activity: Leaf extract exhibited potent inhibition of both enzymes with IC_{50} values of 85.34 ± 1.23 µg/mL (LOX) and 92.67 ± 1.45 µg/mL (COX), compared to root extract (124.56 ± 1.56 and 138.92 ± 1.67 µg/mL, respectively) (Table 4). Diclofenac showed IC_{50} of 28.45 ± 0.89 µg/mL (LOX) and 32.17 ± 0.92 µg/mL (COX).

Table 4. Anti-inflammatory and antidiabetic activities

Assay	IC_{50} (µg/mL)		
	Leaf	Root	Standard
Protein Denaturation	299.27 ± 0.06	392.47 ± 0.07	241.13 ± 0.03*
LOX Inhibition	85.34 ± 1.23	124.56 ± 1.56	28.45 ± 0.89*
COX Inhibition	92.67 ± 1.45	138.92 ± 1.67	32.17 ± 0.92*
α-Amylase Inhibition	137.20	184.60	37.38**

*Diclofenac sodium; **Acarbose*

Antidiabetic Activity

α-Amylase Inhibition Assay: Leaf extract displayed superior inhibition (IC_{50} = 137.20 µg/mL) compared to root extract (184.60 µg/mL), while acarbose standard showed IC_{50} of 37.38 µg/mL (Table 4). Both extracts exhibited concentration-dependent inhibition (18.76–38.01% for leaf; 14.23–31.87% for root at 10–50 µg/mL).

Cytotoxicity Activity

SRB Assay: Both extracts demonstrated concentration-dependent cytotoxicity against SH-SY5Y neuroblastoma cells (Table 5). Leaf extract showed superior activity with LC_{50} of 127.69 µg/mL compared to root extract (184.60 µg/mL). Cell viability decreased from 95.03% to 61.92% (leaf) and 96.78% to 70.34% (root) across 6.25–100 µg/mL concentrations.

Table 5. Cytotoxic effect on SH-SY5Y cells

Concentration (µg/mL)	**Cell Viability (%)**	
	Leaf	Root
Control	100.00	100.00
6.25	95.03 ± 1.42	96.78 ± 1.35
12.5	91.04 ± 1.96	93.56 ± 1.72
25	83.32 ± 2.15	87.19 ± 1.89
50	72.81 ± 1.53	78.43 ± 1.64
100	61.92 ± 2.03	70.34 ± 1.87
LC_{50} (µg/mL)	**127.69**	**184.60**

Values expressed as Mean ± SE, n = 3

Morphological Observations: Microscopic examination revealed concentration-dependent morphological changes in treated cells. Leaf extract induced more pronounced cytotoxic features including cell rounding, shrinkage, cytoplasmic granulation, and vacuolization compared to root extract. Control cells maintained normal neuronal morphology with intact cell bodies and dendritic extensions (Fig 1, 2).

The superior bioactivity of leaf extract correlates with its richer phytochemical composition—higher alkaloid (65.03 mg/g), phenolic (57.10 mg/g), flavonoid (35.40 mg/g), and tannin (46.75 mg/g) contents, along with the exclusive presence of cardiac glycosides, steroids, and fats/oils. Based on these findings, methanolic leaf extract was selected for further metabolic profiling.

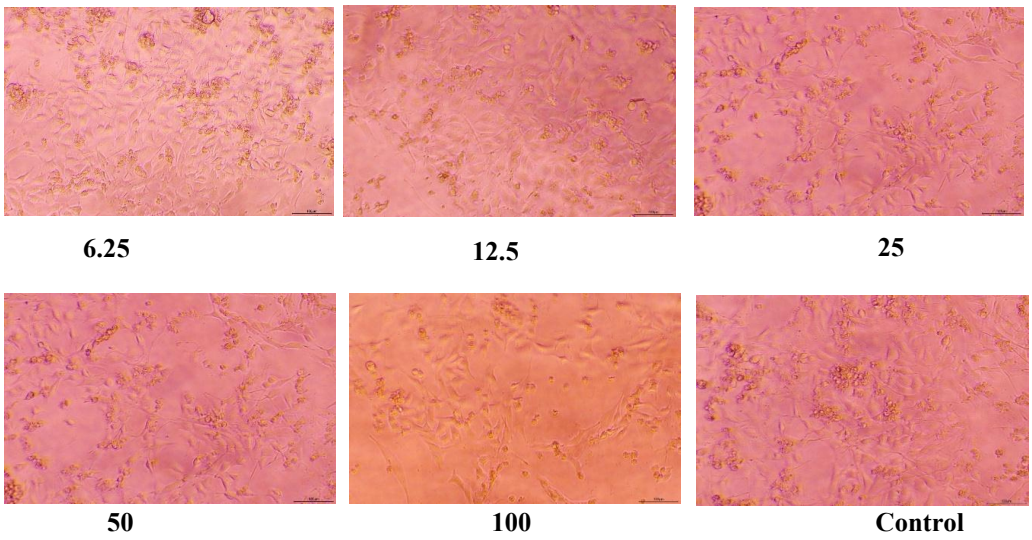

Fig 1: Morphological changes in SH-SY5Y neuroblastoma cells treated with methanolic leaf extract

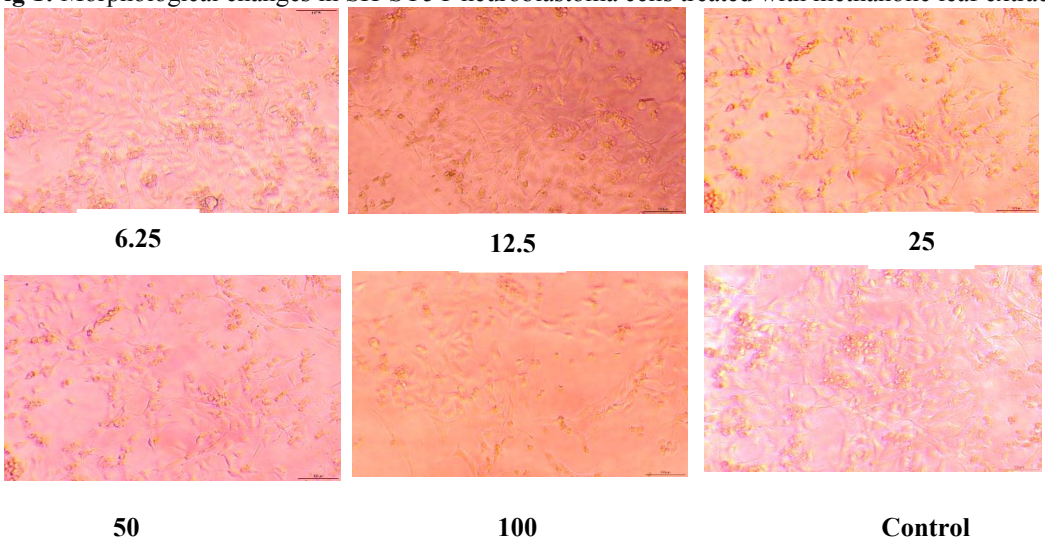

Fig 2. Morphological changes in SH-SY5Y neuroblastoma cells treated with methanolic root extract

DISCUSSION

The present study provides the first comprehensive evaluation of the phytochemical composition and pharmacological potential of *Justicia santapaui* Bennet, an endemic species from the Western Ghats of Kerala. The findings demonstrate significant antioxidant, anti-inflammatory, antidiabetic, and cytotoxic activities, establishing *J. santapaui* as a promising source of bioactive compounds.

The genus *Justicia* (Acanthaceae) comprises over 600 species widely utilized in traditional medicine. Recent reviews have highlighted the chemical diversity of *Justicia* species, with alkaloids, flavonoids, and terpenoids as major secondary metabolites (Sahu *et al*., 2021). The present study aligns with these findings, demonstrating that alkaloids were ubiquitous across all leaf extracts. The high alkaloid content (65.03 mg/g) in the methanolic leaf extract is particularly noteworthy, as alkaloids are recognized for their neuroprotective, anti-inflammatory, and cytotoxic properties (Khan *et al.,* 2020).

Methanol produced the highest extraction yield for both leaves (15.83%) and roots (48%), indicating the predominance of polar compounds (Singh et al., 2022). Although roots showed higher yields, leaves demonstrated significantly greater phytochemical diversity (10 classes vs. 6 classes), consistent with observations in other medicinal plants where aerial parts exhibit richer profiles due to photosynthetic and defensive functions. The exclusive presence of cardiac glycosides, steroids, and fats/oils in leaves suggests foliar tissues may be more suitable for therapeutic applications targeting cardiovascular and inflammatory conditions.

The significant antioxidant activity of the methanolic leaf extract (DPPH IC_{50} = 381.84 µg/mL; FRAP IC_{50} = 482.84 mg/mL) correlates with its rich phenolic and flavonoid content (57.10 mg GAE/g and 35.40 mg QE/g, respectively). Phenolic compounds are well-established free radical scavengers that protect against oxidative damage associated with neurodegenerative diseases, diabetes, and cancer (Rice-Evans *et al.,* 1997). The superior antioxidant capacity of leaves compared to roots aligns with previous reports in *J. adhatoda* and *J. gendarussa*.

The anti-inflammatory potential was confirmed through protein denaturation, LOX, and COX inhibition assays. The leaf extract demonstrated potent inhibition of LOX (IC_{50} = 85.34 µg/mL) and COX (IC_{50} = 92.67 µg/mL), key enzymes in the arachidonic acid cascade generating pro-inflammatory mediators (Di Rosa *et al.,* 2021). The superior anti-inflammatory activity of leaves compared to roots can be attributed to higher flavonoid content, known to modulate inflammatory pathways by inhibiting COX-2, LOX, and iNOS expression (Chen *et al*., 2022). The observed IC_{50} values are comparable to those reported for other *Justicia* species, including *J. procumbens* (LOX IC_{50} = 92.45 µg/mL) and *J. adhatoda* (COX IC_{50} = 87.34 µg/mL).

The antidiabetic potential, assessed through α-amylase inhibition, revealed superior activity in leaf extract (IC_{50} = 137.20 µg/mL) compared to roots (IC_{50} = 184.60 µg/mL). This activity can be attributed to phenolic compounds, flavonoids, and alkaloids that inhibit carbohydrate-digesting enzymes through hydrogen bonding and hydrophobic interactions. The higher tannin content in leaf extract (46.75 mg/g) may also contribute, as tannins form complexes with proteins and inhibit digestive enzyme. The IC_{50} value of 137.20 µg/mL suggests moderate antidiabetic potential warranting further fractionation (Singh *et al*., 2022).

The cytotoxic activity against SH-SY5Y neuroblastoma cells is a novel finding for *J. santapaui*. Leaf extract demonstrated superior cytotoxicity (LC_{50} = 127.69 µg/mL) compared to roots (LC_{50} = 184.60 µg/mL), with morphological changes characteristic of apoptosis. This superior activity correlates with higher alkaloid, phenolic, and flavonoid contents, along with the exclusive presence of cardiac glycosides and steroids. Alkaloids exert cytotoxic effects through DNA intercalation, topoisomerase inhibition, and apoptosis induction (Khan et al., 2020). Cardiac glycosides, uniquely present in leaf extracts, demonstrate selective cytotoxicity by targeting Na^+/K^+-ATPase. The LC_{50} value is comparable to *J. gendarussa* (LC_{50} = 145.20 µg/mL) and superior to *J. adhatoda* (LC_{50} = 195.67 µg/mL).

The superior pharmacological activities of leaf extracts over roots are attributed to richer phytochemical diversity and higher concentrations of key bioactive compounds, consistent with leaves being primary sites of metabolism and defense compound biosynthesis (Pandey and Rizvi, 2009). The combination of antioxidant, anti-inflammatory, antidiabetic, and cytotoxic activities suggests multi-target therapeutic potential for complex diseases where oxidative stress and inflammation play interconnected roles (*Chen et al.,* 2022)

However, several limitations should be acknowledged. The pharmacological activities were evaluated using crude extracts containing complex mixtures; bioactivity-guided fractionation and isolation of pure compounds are required to identify active principles. Cell-free enzymatic assays and cell-based screening represent initial approaches; follow-up studies using animal models and mechanistic investigations are necessary to validate therapeutic potential. Future studies should explore other extraction methods and evaluate seasonal variations in phytochemical composition.

CONCLUSION

In conclusion, this study provides the first scientific validation of the phytochemical and pharmacological properties of *J. santapaui*. Leaves possess significantly greater phytochemical diversity and superior bioactivities compared to roots, including potent antioxidant, anti-inflammatory, antidiabetic, and cytotoxic effects. The high alkaloid, phenolic, and flavonoid contents, along with the exclusive presence of cardiac glycosides, contribute to the superior bioactivity of leaf extracts. These findings establish *J. santapaui* as a promising candidate for bioactivity-guided isolation of therapeutic compounds and provide a scientific basis for its potential application in managing oxidative stress-related diseases, inflammatory conditions, diabetes, and cancer. Further studies are warranted to identify active constituents, elucidate mechanisms of action, and evaluate safety and efficacy in preclinical models.

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