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Evaluation of antioxidant and antidiabetic potential of different plants of *Justicia adhatoda* L

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Abstract
Medicinal plants are a valuable source of bioactive compounds with therapeutic potential against oxidative stress–related disorders and metabolic diseases such as diabetes mellitus. *Justicia adhatoda* L. is a well-known medicinal plant traditionally used for the treatment of respiratory ailments, inflammation, and metabolic disorders. The present study aimed to evaluate the antioxidant and antidiabetic potential of different parts (stem, leaves, and flowers) of *J. adhatoda* extracted using solvents of varying polarity, namely water, ethanol, and n-hexane. Antioxidant activity was assessed using the DPPH free radical scavenging assay, while antidiabetic potential was evaluated through α-amylase inhibition using the DNSA method. All extracts exhibited dose-dependent activity in both assays. Among the solvents tested, ethanolic extracts generally demonstrated superior antioxidant and antidiabetic potential, as indicated by lower IC_{50} values, followed by aqueous and hexane extracts. Variation in bioactivity among different plant parts highlights the influence of solvent polarity and phytochemical composition on biological efficacy. The findings of the present study substantiate the traditional use of *J. adhatoda* and suggest its potential as a natural source of antioxidant and antidiabetic agents for further pharmacological and clinical investigations.

Keywords: *Justicia adhatoda*, antioxidant activity, antidiabetic activity, dpph assay, α-amylase inhibition etc

Introduction
Oxidative stress is a major contributor to the pathogenesis of several chronic diseases, including diabetes mellitus, cardiovascular disorders, cancer, and neurodegenerative conditions. It arises due to an imbalance between the generation of reactive oxygen species (ROS) and the antioxidant defense system of the body (Halliwell & Gutteridge, 2015) [4]. Antioxidants play a crucial role in neutralizing free radicals and preventing oxidative damage to biomolecules such as lipids, proteins, and DNA. In recent years, attention has shifted toward natural antioxidants derived from medicinal plants due to their safety, affordability, and multifaceted biological activities (Shahidi & Ambigaipalan, 2015) [14].

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia resulting from impaired insulin secretion, insulin action, or both. One of the therapeutic strategies for managing postprandial hyperglycemia involves inhibiting carbohydrate-digesting enzymes such as α-amylase and α-glucosidase, thereby reducing glucose absorption (Krentz *et al*., 2017) [7]. Synthetic antidiabetic drugs are effective but often associated with adverse side effects, prompting the search for safer plant-based alternatives (Modak *et al*., 2007) [10].

Medicinal plants constitute a rich reservoir of bioactive secondary metabolites, including alkaloids, flavonoids, phenolics, tannins, and terpenoids, which exhibit strong antioxidant and antidiabetic properties (Pandey & Rizvi, 2009) [11]. The biological activity of plant extracts largely depends on the polarity of the extraction solvent, which influences the solubility and yield of phytoconstituents (Do *et al*., 2014) [3]. Therefore, comparative evaluation of different solvent extracts is essential to identify the most bioactive fractions.

Justicia adhatoda L. (family Acanthaceae), commonly known as Vasaka, is widely distributed in India and has been extensively used in traditional Ayurvedic and Unani medicine. The plant is known to contain bioactive alkaloids such as vasicine and vasicinone, along with flavonoids, phenols, and essential oils (Kumar *et al*., 2013) [8]. Traditionally, *J. adhatoda* has been used to treat asthma, bronchitis, inflammation, fever, and microbial infections, and recent studies have reported its antioxidant, antidiabetic, anti-inflammatory, and antimicrobial activities (Sikri *et al*., 2018; Konar *et al*., 2022) [6, 16].

Despite several reports on the pharmacological properties of *J. adhatoda*, comparative evaluation of antioxidant and antidiabetic potential across different plant parts and solvent systems remains limited. The present study was therefore undertaken to systematically investigate the antioxidant and α-amylase inhibitory activities of stem, leaf, and flower extracts of *J. adhatoda* using aqueous, ethanolic, and hexane solvents, thereby providing scientific validation for its traditional medicinal use.

Materials and Methods
Collection of Plant Materials
Plant material (stem, leaves and flowers) of the selected plants were collected. The collected plant was identified at Herbarium, Department of Botany, University of Rajasthan, Jaipur. Plant parts were washed with running tap water and then distilled water to remove dust particles. After that, plant parts were air dried and grinded into mixer grinder to make coarse powder. Powdered form of plant parts was stored for further work.

Extraction of the Collected Plant Parts
Plant materials were extracted in different polar and non-polar solvents. Selected solvents were- were water (highly polar), ethanol (mid-polar) and n-Hexane (Non-polar). For this purpose, 1 gm of each plant part (root, stem, leaves and flowers) were dipped into 10 ml of the solvent individually. Total 12 tubes were prepared for this purpose. Those were kept at shaker at room temperature for overnight. After that,

tube was centrifuged at 10000 rpm for 10 minutes to collect supernatant. Supernatant were taken into pre-weighed petri-dishes and were left for evaporation of solvent. After complete drying, petri-plates were weighed again and weight of extract per gram plant material was calculated. Extracts were collected in glass vials for further use.

Determination of antioxidant potential

For determination of DPPH radical scavenging potential of the extracted samples 1,1-diphenyl 2-picryl-hydrazil (DPPH) method was applied. The mixing of 100 µl sample was done in 3.9 ml taken from 0.1 mM DPPH (methanolic) solution. Then blend was subjected to vortex and left for incubation in the dark for 30 min. Its OD was calculated at 515 nm while methanol was used as blank.

The radical scavenging activity was determined by the ratio = (Abcontrol – Absample / Abcontrol) × 100

Where Abcontrol is presenting the absorbance of the DPPH solution and absorbance of the DPPH solution with sample is denoted by Absample.

Linear plot of concentration versus % inhibition was plotted and by this IC50 values were determined. The antioxidant potential of each extract was showed in form of IC50 (stated as the quantity of concentration necessary to prevent DPPH radical development by 50%), find out with the help of inhibition curve.

Determination of antidiabetic potential

Chromogenic DNSA approach was used to perform Inhibition assay. Total assay mixture is made up of 500 µl of sodium phosphate buffer 0.02 M (pH 6.9 with 6 mM NaCl), 1 ml of salivary amylase and 400 µl samples of concentrations were incubated for 10 min at 37°C. After this pre-incubation process, 580 µl of starch solution (1% w/v) was mixed to each tube and left for incubation at 37°C for 15 min. Now for the termination of reaction addition of DNSA reagent (1.0 ml) was done, then it is kept in boiling water bath for about 5 min, cooling was done at RT and the OD were calculated at 540 nm. The control having no plant extracts resulted in 100% enzymatic activity. For the elimination of the absorbance developed by plant extract, suitable controls with the sample in the reaction mixture without the enzyme were also included (negative control).

% Inhibition of alpha amylase can be calculated as follows:

Percent relative enzyme activity = (AbControl- Abtest/AbC) * 100

Results and Discussion

Antioxidant Activity of The Selected Plant Parts

DPPH Assay was performed to evaluate the antioxidant activity of the selected extracts. Different concentrations were used for the assay, i.e. 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml. Regression equation was also calculated.

As shown in Table 1, water extracts of *J. adhatoda* stem showed an increase in values with rise in concentration, i.e. 10.77 for 20 µg/ml, 13.16 for 40 µg/ml, 16.37 for 60 µg/ml, 21.11 for 80 µg/ml and 24.18 for 100 µg/ml. The IC_{50} value was recorded as 249.0684301. For ethanolic extracts, again there was a rise with increasing concentration; 16.22, 18.16, 23.16, 27.22 and 29.25 for 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml concentration, respectively. IC_{50} value was observed high as 214.8861048. In hexane extracts the values were 4.287 (20 µg/ml), 8.11 (40 µg/ml), 10.13 (60 µg/ml), 13.25 (80 µg/ml) and 16.37 (100 µg/ml). IC_{50} value was recorded as least, i.e. 38.821843, showing highest antioxidant potential of hexane extracts. Similarly, water extract of leaves was also recorded with rise in values as per concentration. The values were 3.17, 4.18, 8.38, 11.36 and 14.27 for 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml, respectively. IC_{50} value was 336.6780123. For ethanolic extracts the values was 6.87 (20 µg/ml), 8.16 (40 µg/ml), 12.13 (60 µg/ml), 16.74 (80 µg/ml) and 20.88 (100 µg/ml) and IC50 value was 232.3426573. Hexane extracts showed values as 2.65 for 20 µg/ml, 6.17 for 40 µg/ml, 9.62 for 60 µg/ml, 10.36 for 80 µg/ml and 11.15 for 100 µg/ml; with IC50 value 456.2924528. The least IC50 value of ethanolic extract showed its highest antioxidant potential among other solvent extracts. For flowers, water extracts were observed to have values 1.33, 2.16, 4.22, 7.73 and 8.52 for 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml concentrations, respectively. IC50 value recorded was 255.7092907. For ethanolic extracts values were 8.15 (20 µg/ml), 11.27 (40 µg/ml), 14.62 (60 µg/ml), 19.26 (80 µg/ml) and 24.17 (100 µg/ml) with least IC50 value, 232.3426573, indicating its highest antioxidant potential among other flower extracts. Hexane extracts were found to have values 2.77 for 20 µg/ml, 4.17 for 40 µg/ml, 5.11 for 60 µg/ml, 7.82 for 80 µg/ml and 10.36 for 100 µg/ml, with highest IC50 value 526.5711253.

Based on this, the study showed that solvent type plays a significant role in extracting antioxidants from different plant parts, with ethanol generally yielding the best results. Furthermore, different plant parts exhibit varying levels of antioxidant activity, with stems, leaves, and flowers contributing differently depending on the plant.

A number of previously published studies have shown antioxidant activity of the plant species, namely, *Justicia adhatoda* (Sikri *et al.,* 2018; De Sarker *et al.,* 2015; Konar *et al.,* 2022; Martins *et al.,* 2016; Panda *et al.,* 2016; Rattan., 2023) [2, 6, 9, 12, 13, 16].

Antidiabetic activity of the selected plant parts

Glucose DNSA method was performed to evaluate the antidiabetic potential of the selected extracts. Different concentrations were used for the assay, i.e. 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml. Regression equation and IC50 value was also calculated.

All the extracts from different plant parts of selected plants, observed to have increase in values with rising concentrations. As shown in Table 2, for *J. adhatoda* plant, the values for water extract of stem was recorded as 12.67, 15.19, 18.11, 24.91 and 29.51 for 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml, respectively. IC50 value was 197.8894009. For ethanolic extracts of stem, the values were 12.91 (20 µg/ml), 17.88 (40 µg/ml), 18.11 (60 µg/ml), 24.91 (80 µg/ml) and 29.51 (100 µg/ml); with lowest IC50 value i.e. 178.6917486, showing its highest antioxidant potential. Hexane extract of stem was reported to have values as 4.28 for 20 µg/ml, 6.71 for 40 µg/ml, 9.22 for 60 µg/ml, 10.13 for 80 µg/ml, 14.28 for 100 µg/ml and highest IC50 value (410.7771136), indicating its least antioxidant potential. For the water extracts of leaves, the values were found as 8.56 (20 µg/ml), 10.39 (40 µg/ml), 14.27 (60 µg/ml), 16.88 (80 µg/ml), 19.41 (100 µg/ml) and IC50 value was 315.9929078. Ethanolic extract observed with values as 10.78 for 20 µg/ml, 16.38 for 40 µg/ml, 21.77 for 60 µg/ml, 25.68 for 80 µg/ml, 28.83 for 100 µg/ml and least IC50

value 189.1277533, showing highest potential. The values for hexane extract were recorded as 2.81, 3.79, 5.29, 6.82 and 8.15 for 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml, respectively. IC50 value was observed highest as 710.5102041, indicating least antioxidant potential. In case of flowers, water extracts were reported with values as 6.74 for 20 µg/ml, 8.54 for 40 µg/ml, 11.64 for 60 µg/ml, 14.75 for 80 µg/ml, 15.43 for 100 µg/ml and IC50 value 386.9237288. The values for ethanolic extract were recorded as 10.76, 11.76, 15.43, 18.56 and 20.76 for 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml, respectively. IC50 value was observed least as 317.8059701, indicating highest antioxidant potential. For the hexane extracts of flowers, the values were found as 4.86 (20 µg/ml), 5.86 (40 µg/ml), 6.54 (60 µg/ml), 7.43 (80 µg/ml), 8.33 (100 µg/ml) and IC50 value was highest i.e. 1081.152941, showing least potential as antioxidant.

A number of previously published studies have shown antidiabetic activity of the plant species, namely, *Justicia adhatoda* (Khan *et al.*, 2017; Sharma *et al.*, 2016; Afreen *et al.*, 2021; Talukdar *et al.*, 2025; Sidhu *et al.*, 2013) [1, 5, 15, 17, 18].

Table 1: Free radical scavenging potential of various parts of *Justicia adhatoda*

	% Free radical scavenging activity at different concentrations (µg/ml)						Regression equation	IC50 (µg/ml)
		20	40	60	80	100		
Stem	Water	10.77	13.16	16.37	21.11	24.18	y = 0.1739x + 6.687	249.0684301
	Ethanol	16.22	18.16	23.16	27.22	29.25	y = 0.1756x + 12.266	214.8861048
	Hexane	4.287	8.11	10.13	13.25	16.37	y = 0.1465x + 1.6376	38.821843
Leaves	Water	3.17	4.18	8.38	11.36	14.27	y = 0.1469x - 0.542	336.6780123
	Ethanol	6.87	8.16	12.13	16.74	20.88	y = 0.2002x + 3.485	232.3426573
	Hexane	2.65	6.17	9.62	10.36	11.15	y = 0.106x + 1.633	456.2924528
Flowers	Water	1.33	2.16	4.22	7.73	8.52	y = 0.0998x - 1.193	255.7092907
	Ethanol	8.15	11.27	14.62	19.26	24.17	y = 0.2002x + 3.485	232.3426573
	Hexane	2.77	4.17	5.11	7.82	10.36	y = 0.0942x + 0.397	526.5711253

Table 2: Alpha amylase inhibitory potential of different extracts of *Justicia adhatoda*

Plant part	Solvent name	% Inhibition at various concentrations (µg/ml)					Regression equation	IC50 (µg/ml)
		20	40	60	80	100		
Stem	Water	12.67	15.19	18.11	24.91	29.51	y = 0.217x + 7.058	197.8894009
	Ethanol	12.91	17.88	21.34	27.64	31.42	y = 0.2339x + 8.204	178.6917486
	Hexane	4.28	6.71	9.22	10.13	14.28	y = 0.1171x + 1.898	410.7771136
Leaves	Water	8.56	10.39	14.27	16.88	19.41	y = 0.141x + 5.445	315.9929078
	Ethanol	10.78	16.38	21.77	25.68	28.83	y = 0.227x + 7.068	189.1277533
	Hexane	2.81	3.79	5.29	6.82	8.15	y = 0.0686x + 1.259	710.5102041
Flowers	Water	6.74	8.54	11.64	14.75	15.43	y = 0.118x + 4.343	386.9237288
	Ethanol	10.76	11.76	15.43	18.56	20.76	y = 0.134x + 7.414	317.8059701
	Hexane	4.86	5.86	6.54	7.43	8.33	y = 0.0425x + 4.051	1081.152941

Conclusion

The present investigation demonstrated that different parts of *Justicia adhatoda* possess significant antioxidant and antidiabetic potential, as evidenced by DPPH free radical scavenging activity and α-amylase inhibition assays. All extracts showed dose-dependent biological activity, indicating the presence of bioactive phytoconstituents capable of modulating oxidative stress and carbohydrate metabolism. Among the solvents used, ethanolic extracts consistently exhibited superior activity, suggesting that ethanol is more efficient in extracting phenolic and flavonoid compounds responsible for these effects.

Variation in activity among stem, leaf, and flower extracts highlights the importance of plant part selection in pharmacological studies. The results corroborate earlier findings and support the traditional medicinal claims associated with *J. adhatoda*. Overall, the study suggests that *J. adhatoda* may serve as a promising natural source of antioxidant and antidiabetic agents. Further studies involving phytochemical characterization, mechanism-based assays, and *in vivo* validation are recommended to explore its therapeutic potential and facilitate the development of plant-based nutraceuticals or herbal formulations.

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