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Phytochemical Profiling, Antibacterial Efficacy, and Anti-Diabetic Potential of Humboldtia unijuga Leaf Extracts: A Comprehensive in Vitro Study

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Abstract

Humboldtia unijuga is a rare tree species native to the Western Ghats and has been traditionally used for treating various ailments. Despite its medicinal importance, scientific studies on this plant remain limited. The present research was undertaken to examine the phytochemical composition, antibacterial activity, and anti-diabetic potential of *H. unijuga* leaf extracts under laboratory conditions. Leaves were extracted successively using hexane, ethyl acetate, and methanol to obtain fractions with different polarities. Preliminary phytochemical screening revealed the presence of biologically important compounds such as alkaloids, flavonoids, tannins, phenolics, saponins, and terpenoids in all extracts. Among them, the methanolic extract showed the highest concentration of phenolic and flavonoid compounds. Antibacterial activity was evaluated against selected Gram-positive and Gram-negative bacteria using the agar well diffusion method. All extracts exhibited concentration-dependent inhibitory effects, with the ethyl acetate extract showing the most pronounced and broad-spectrum activity, particularly against Staphylococcus aureus. The anti-diabetic potential of the extracts was assessed through α-amylase and α-glucosidase inhibition assays. The methanolic extract demonstrated strong inhibitory activity against both enzymes, with IC_{50} values comparable to the standard drug acarbose. A positive correlation was observed between phenolic content and biological activity, indicating the major role of phenolic compounds in mediating these effects. Overall, this study provides scientific evidence supporting the traditional use of *H. unijuga* and highlights its potential as a natural source of bioactive compounds for managing bacterial

infections and type 2 diabetes. Further studies are required to isolate active constituents and validate these findings through in vivo experiments.

Keywords: Humboldtia unijuga, antibacterial activity, anti-diabetic potential, type 2 diabetes mellitus.

I.INTRODUCTION

The renewed global interest in medicinal plants as sources of new therapeutic agents has been fueled by the rising prevalence of infectious and metabolic diseases, including diabetes, along with growing concerns over antibiotic resistance and the adverse effects associated with synthetic drugs. In this context, endemic plant species from biodiversity-rich regions such as the Western Ghats of India represent valuable yet largely unexplored resources for pharmaceutical research. *Humboldtia unijuga*, a rare endemic tree traditionally used by local communities to treat various ailments, remains scientifically underinvestigated despite its ethnomedicinal significance. Although limited studies on related species suggest the presence of biologically active compounds, comprehensive research focusing specifically on *H. unijuga* is notably lacking.

Addressing this gap, the present study undertakes a systematic evaluation to validate the plant's traditional medicinal claims. The research was designed with three main objectives: to perform detailed phytochemical profiling of *H. unijuga* leaf extracts, to assess their in vitro antibacterial activity against clinically important bacterial strains, and to evaluate their antidiabetic potential through key enzyme inhibition assays. Using sequential solvent extraction, this study seeks to link solvent polarity with chemical composition and corresponding biological effects, thereby establishing a scientific foundation for the potential integration of *H. unijuga* into modern phytotherapeutic applications.

Materials and Methods

Plant Material and Extraction:

Fresh leaves of *Humboldtia unijuga* were collected from the Agasthyamalai region and authenticated, and a voucher specimen was deposited in a recognized herbarium. The collected leaves were shade-dried, finely powdered, and subjected to successive Soxhlet extraction using hexane, ethyl acetate, and methanol as solvents. Approximately 500 g of powdered material was processed. The resulting extracts were concentrated under reduced pressure and dried to obtain residues for further investigation.

Phytochemical Analysis:

Preliminary qualitative screening was conducted to identify major classes of phytochemical constituents using established chemical assays. Quantitative estimation of total phenolic content was performed using the Folin–Ciocalteu method and expressed as milligrams of gallic acid equivalents per gram of extract.

Total flavonoid content was determined by the aluminium chloride colorimetric method and reported as milligrams of quercetin equivalents per gram of extract.

Antibacterial Assay:

The antibacterial activity of the extracts was evaluated against two Gram-positive bacteria (*Staphylococcus aureus* MTCC 96 and *Bacillus subtilis* MTCC 121) and two Gram-negative bacteria (*Escherichia coli* MTCC 1687 and *Pseudomonas aeruginosa* MTCC 1688) using the agar well diffusion technique. Wells were loaded with extract concentrations of 25, 50, and 100 mg/mL. Ciprofloxacin (5 µg/well) was used as a positive control, while dimethyl sulfoxide (DMSO) served as the negative control. After incubation at 37°C for 24 hours, the diameters of inhibition zones were measured in millimetres. The minimum inhibitory concentration (MIC) of the most active extract was subsequently determined using the broth microdilution method.

Anti-Diabetic Assay:

The inhibitory effects of the extracts on α-amylase were evaluated using the DNSA method with starch as the substrate, whereas α-glucosidase inhibition was assessed using *p*-nitrophenyl-α-D-glucopyranoside. Extracts at concentrations ranging from 12.5 to 200 µg/mL were incubated with the respective enzymes prior to substrate addition. Acarbose was employed as the reference inhibitor. Enzyme inhibition percentages and IC_{50} values, representing the concentration required to inhibit 50% of enzyme activity, were calculated.

Statistical Analysis:

All experiments were carried out in triplicate, and results are expressed as mean ± standard deviation. Statistical comparisons were performed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test, with significance set at $p < 0.05$. Correlation coefficients (R^2) were also determined to evaluate the relationships between phytochemical content and biological activities.

Results and Discussion

Phytochemical Profiling

Sequential extraction produced yields of 4.2% for hexane, 6.8% for ethyl acetate, and 12.5% for methanol, indicating a greater abundance of polar compounds in the leaf material. Qualitative screening revealed the presence of a wide range of secondary metabolites (Table 1). Among the extracts, the methanolic fraction exhibited the highest concentrations of total phenolics and flavonoids (Table 2), followed by the ethyl acetate and hexane fractions. This pattern aligns with the well-established ability of solvents with moderate to high polarity to efficiently solubilize hydroxyl-rich antioxidant compounds. The extensive phytochemical composition, particularly the elevated phenolic content, provides a strong chemical foundation for the observed and predicted biological activities.

Table 1

Preliminary Qualitative Phytochemical Screening of H. unijuga Leaf Extracts

Phytoconstituent	Hexane Extract	Ethyl Acetate Extract	Methanol Extract
Alkaloids	+	++	+++
Flavonoids	+	+++	+++
Tannins & Phenolics	-	++	+++
Saponins	+	+	++
Terpenoids	++	+	+
Glycosides	-	+	++
Key: (-) Absent; (+) Present; (++) Moderately Present; (+++) Abundantly Present			

Table 2

Quantitative Analysis of Total Phenolic and Flavonoid Content in H. unijuga Leaf Extracts

Extract	Yield (% w/w)	Total Phenolic Content (mg GAE/g extract)	Total Flavonoid Content (mg QE/g extract)
Hexane	4.2 ± 0.3^{a}	32.5 ± 1.8^{a}	18.7 ± 1.2^{a}
Ethyl Acetate	6.8 ± 0.5^{b}	89.4 ± 2.7^{b}	54.9 ± 2.1^{b}
Methanol	12.5 ± 0.9^{c}	148.7 ± 3.2^{c}	86.4 ± 2.1^{c}
Values are mean ± SD of triplicates. Different superscript letters (^{a},^{b},^{c}) in the same column indicate significant difference ($p < 0.05$). GAE: Gallic Acid Equivalent; QE: Quercetin Equivalent.			

Antibacterial Activity:

All extracts demonstrated antibacterial activity that increased with concentration (Table 3). Among them, the ethyl acetate extract exhibited the widest and strongest antimicrobial effect, producing notable inhibition zones against S. aureus (16.2 ± 0.8 mm at 100 mg/mL) and B. subtilis (14.5 ± 0.6 mm). The minimum inhibitory concentration (MIC) of this extract against S. aureus was 1.56 mg/mL. The methanolic extract showed moderate effectiveness, mainly against Gram-positive bacteria, whereas the hexane extract displayed minimal antibacterial activity. As indicated in Table 3, the superior efficacy of the ethyl acetate extract is likely related to its suitable polarity, which enables efficient extraction of moderately polar bioactive compounds, such as flavonoid aglycones and phenolic acids. These compounds are known to disrupt bacterial cell membranes and inhibit key enzymatic processes. Furthermore, the greater sensitivity of Gram-positive bacteria observed in this study is consistent with previous findings. Their relatively simple cell wall structure, consisting of a single peptidoglycan layer, allows easier penetration of

phytochemicals compared with the more complex outer membrane found in Gram-negative bacteria.

Table 3

Antibacterial Activity of *H. unijuga* Extracts (Zone of Inhibition in mm)

Test Microorganism	Hexane (100 mg/mL)	Ethyl Acetate (100 mg/mL)	Methanol (100 mg/mL)	Ciprofloxacin (5 µg/well)
S. aureus	8.5 ± 0.4^{a}	16.2 ± 0.8^{b}	13.8 ± 0.6^{c}	24.5 ± 0.5^{d}
B. subtilis	7.2 ± 0.5^{a}	14.5 ± 0.6^{b}	12.1 ± 0.5^{c}	26.1 ± 0.7^{d}
E. coli	NI	11.3 ± 0.5^{a}	9.8 ± 0.4^{b}	22.8 ± 0.6^{c}
P. aeruginosa	NI	9.1 ± 0.3^{a}	8.2 ± 0.3^{a}	21.4 ± 0.5^{b}
Values are mean ± SD of triplicates. Different superscript letters (^{a},^{b},^{c},^{d}) in the same row indicate significant difference ($p < 0.05$). NI: No Inhibition (<6 mm).				

Anti-Diabetic Potential:

In the enzymatic inhibition assays, the methanolic extract demonstrated the highest inhibitory potential against both α-amylase and α-glucosidase when compared with the other solvent extracts (Table 4). The IC_{50} values were determined to be 42.8 ± 1.7 µg/mL for α-amylase and 28.4 ± 1.2 µg/mL for α-glucosidase, reflecting strong inhibitory activity. These values were comparable to those of the standard antidiabetic drug acarbose, and notably, the methanolic extract exhibited even greater effectiveness than acarbose in suppressing α-glucosidase activity. This finding highlights the potential of the methanolic extract as a promising natural source of enzyme inhibitors. The ethyl acetate extracts also showed moderate to strong inhibitory effects on both enzymes, suggesting the presence of bioactive constituents capable of interfering with carbohydrate digestion. In contrast, the hexane extract displayed minimal inhibitory activity, indicating that non-polar compounds may contribute less significantly to enzyme inhibition in this system. These differences among extracts emphasize the importance of solvent polarity in extracting compounds with antidiabetic potential.

Furthermore, statistical analysis revealed a strong positive correlation ($R^2 > 0.9$) between total phenolic content (Table 2) and enzyme inhibitory activity (Table 4). This close relationship suggests that phenolic compounds are major contributors to the observed biological effects. Polyphenols are known to possess multiple hydroxyl groups that enable them to form hydrogen bonds and other interactions with the active sites of digestive enzymes, thereby reducing enzymatic activity. By inhibiting α-amylase and α-glucosidase, these polyphenolic compounds slow the breakdown of complex carbohydrates into absorbable sugars. As a result, glucose absorption in the intestine is delayed, leading to a more gradual rise in blood glucose levels after meals. This mechanism helps to prevent sharp postprandial glucose

spikes, which are a major risk factor in the progression and complications of type 2 diabetes. Consequently, the strong enzyme inhibitory activity observed in the methanolic extract supports its potential therapeutic relevance in the development of natural antidiabetic agents.

Table 4

In Vitro Anti-Diabetic Activity (IC_{50} Values) of *H. unijuga* Extracts

Extract / Standard	**α-Amylase Inhibition IC_{50} (µg/mL)**	**α-Glucosidase Inhibition IC_{50} (µg/mL)**
Hexane	$>200^{a}$	185.3 ± 6.5^{a}
Ethyl Acetate	78.6 ± 2.4^{b}	45.2 ± 1.9^{b}
Methanol	42.8 ± 1.7^{c}	28.4 ± 1.2^{c}
Acarbose (Standard)	35.1 ± 1.5^{d}	32.6 ± 1.4^{d}
Values are mean ± SD of triplicates. Different superscript letters (^{a},^{b},^{c},^{d}) in the same column indicate significant difference ($p < 0.05$). Lower IC_{50} indicates higher potency.		

II.CONCLUSION

The present in vitro study provides clear scientific evidence supporting the medicinal value of Humboldtia unijuga leaves. The results demonstrate that the plant is rich in bioactive phenolic and flavonoid compounds, particularly in the ethyl acetate and methanolic extracts. The strong antibacterial activity of the ethyl acetate extract highlights its potential application in controlling bacterial infections. In addition, the potent inhibition of α-amylase and α-glucosidase enzymes by the methanolic extract indicates promising anti-diabetic properties. These biological activities are largely associated with the high phenolic content of the extracts. Overall, the findings validate the traditional use of *H. unijuga* and suggest that it may serve as a valuable natural source for developing novel therapeutic agents. Future research should focus on isolating individual active compounds, evaluating their safety profiles, and confirming their efficacy through in vivo studies. Moreover, conservation efforts are essential to ensure the sustainable utilization of this endemic plant species for medicinal purposes.

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