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Scientific assessment besides HPTLC study profile of *Premna barbata Wall. Ex Schauer* plant stem bark extract with *in- vitro* antidiabetic potential

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KEYWORDS

Premna Barbata Wall. Ex Schauer, alpha-glucosidase, Phytochemical, HPTLC investigation.

ABSTRACT:

Premna Barbata Wall. Ex Schauer (family Verbenaceae) is found in the jungle provinces of the eastern and northern parts of India, Pakistan, Nepal, and Myanmar, in addition to in tropical and subtropical areas of Asia, Africa, Australia, and the Pacific Islands. It can be found in the lower Himalayas and the Shivalik tract in India. The range of raises is 800–1800 meters. Plant bark is 2 to 5 cm long and 1 to 2 cm wide, with a little quilled shape. The superficial surface is grey, while the inner surface is white. The analysis of the literature divulges that the plant has been the subject of very few actual investigations and studies. The present study is based on an initial phytochemical screening examination and an assessment of the in vitro potential for the inhibition of the enzyme's alpha-glucosidase and alpha-amylase. The research indicates that the hydro-alcoholic extract comprises a range of phytochemical constituents present in diverse quantities, such as loss on drying (11.4%), total starch (0.92%), total sugar (0.66%), flavonoids (0.24%), tannins (1.24%), phenolic compounds (3.10%), proanthocyanidins (2.18%), and flavanols (0.23%), among others.

Introduction

Premna Barbata Wall. Ex Schauer plant is fitting to family Verbenaceae.[1] In humid and semitropical Asia, Africa, Australia, and the Pacific Islands, it can be found in the arboreal regions of eastern and north India, Pakistan, Nepal, and Myanmar. In India generally found in the lower Himalaya and the Shivalik territory. The raise range is between 800 and 1800 meters.[2] Expressly native to lower altitudes of the Himalaya area, West Bengal, Kashmir, Uttarakhand, and Arunachal Pradesh. The plant is broadly discrete in Nepal, Western China, Bhutan, and India.[3,4] Premna barbata usually also known as Bearded Premna. It is a deciduous shrub or minor tree fit in to the family Lamiaceae (formerly Verbenaceae). Traditionally, the plant is exceedingly valued in ethnomedicine, especially amid tribal communities in the Himalayan regions, Uttarakhand, and other parts of India, where it is known as "Aganyo" in folk language. The plant is used in numerous forms, such as powders, juices, pastes, & decoctions, for the treatment of a wide range of ailments[5,6]

Fig. 1: Whole plant of Premna Barbata

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Table 1: Key Features of Premna barbata bark [7-11]

Feature	*Details*
Botanical Family	*Lamiaceae (Mint family)*
Common Names	*Bearded Premna, Lammar, Karadi, Bakaar, Gineri, Vasuka, Ganhila*
Habit and Size	*Deciduous shrub or small tree, 3–5 meters tall; bark greyish brown, rough, corky outside*
Distribution	*Himalayas, India, Nepal, Pakistan, Myanmar, Australia, Africa, Pacific*
Bark Morphology	*Outer surface greyish brown with grooves and furrows; inner surface wrinkled, yellowish white; astringent taste, no Odor.*
Root System	*Fibrous roots, 1–2 feet deep, spread horizontally up to 3–4 feet*
Major Uses	*Anti-inflammatory, antibacterial, wound healing, fever, stomach issues*
Key Phytochemicals	*Iridoid glycosides, diterpenoids, flavonoids, alkaloids, terpenoids*
Pharmacological Action	*Antibacterial, anti-inflammatory, antioxidant, hepatoprotective*
Microscopic Features	*Cork cells, phelloderm, multiseriate medullary rays, fibres, secondary phloem*
Pharmacological Activities	*Antibacterial, anti-inflammatory, wound healing, febrifuge, analgesic*
Cultivation & Lifespan	*Perennial, moderate growth rate, lives 10–15 years; prefers well-drained soil and regular care.*
Conservation Status	*At risk in some regions; needs sustainable management*

Table 2: Reported Hypoglycaemic bioactivity of Iridoid present in Premna Barbata Bark 12-14

Iridoid Name	*Reported Hypoglycaemic Activity*
Premnosidic acid	Reported in Premna barbata, potential activity
Diterpenoid iridoid glycosides	General antihyperglycemic activity in Premna spp.
Acyclic monoterpenediols (Premnaodorosides D–G)	Isolated from Premna genus, potential activity
Flavonoid–iridoid Conjugates	General hypoglycaemic activity in Premna spp.
Geniposidic acid	Reported in Premna spp., potential activity
Lamiide	Present in Premna spp., potential activity
6-O-trans-p-coumaroyl-geniposidic acid	Isolated from Premna spp., potential activity
8-epi-kingiside	Isolated from Premna spp., potential activity
Premnacorymboside	Isolated from Premna spp., potential activity
Premnacorymboside B	Isolated from Premna spp., potential activity

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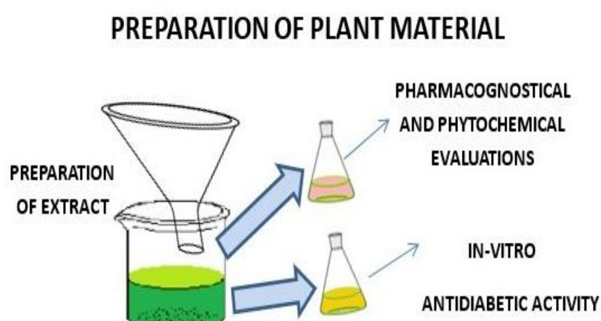

Fig. 2. Display of the study's graphical abstract.

MATERIAL AND METHODS

PLANT MATERIAL

The plant that was taken from a wild-growing area of the Kumaon Region (Almora, Uttarakhand, India) was verified for its antidiabetic properties by the Forest Research Institute (FRI), Dehradun, Uttarakhand, India.

EXTRACT PREPARATION

After being harvested, the plant was let to dry in the shade. To obtain a uniform powdered drug, it must be pounded into a powder and then sieved. After being macerated in ethanol for four days, the 15g of powdered plant material was filtered through muslin cloth and dried by evaporation. The extract was kept in a desiccator.

SCIENTIFIC STUDIES[15,16]

By using powdered drug after shade-dried plant bark, various Pharmacognostical and phytochemical analyses were done. By using dextrose and starch (soluble), respectively, as a reference solution, the total sugar and total starch of plant bark were projected. Tannic acid was used as the standard to measure total tannins, while gallic acid was used to measure total phenolics. Rutin (Standard) was used as a reference to determine the total amount of flavonoids and flavanols. Catechin was used as a benchmark for the assessment of proanthocyanidins. Furthermore, extractive values and fluorescence analyses were also carried.

HPTLC PROFILE ANALYSIS [17-22]

Sample preparation: Stationary phase: 0.2 mm uniformly thick pre-coated silica gel 60 F254 plate (E. Merck)

Sample applicator: The CAMAG Linomate-5 applicator applies samples in narrow bands. It ensures optimal resolution for a given chromatographic system. The CAMAG Linomate-5 applies samples onto the chromatogram layer using the spry-on technique, which permits the application of a larger amount of sample. A 500 µl syringe is used in place of the typical 100 µl dosage syringe when a larger volume is needed, particularly in preparative applications.

Sample application: An extract having 10 mg/ml was made. By applying 20 µl of the solution, a standard marker solution containing 1 mg/ml was prepared in 10 µg, 20 µg, and 30 µg.

Chromatography: In CAMAG twin through chambers, a corresponding mobile phase saturation plate (E. Merck) with a uniform thickness of 0.2 mm was used to fill the chamber.

Video documentation: Done under CAMAG Reproster-3, the eluted plate was examined for UV visualization at 254 nm and 366 nm.

Scanning of tracks: Different elute tracks on the eluted plate are densitometrical scanned using the CAMAG Scanner-3 at the appropriate wavelengths or at multiple wavelengths for the crude extract to get area under the curve for each component present in the extract.

ANTIDIABETIC BIOACTIVITY IN LABORATORY SETTINGS [23-29]

Inhibition of alpha-amylase enzyme - The enzyme solution was made carefully weighing 27.5 mg of alpha-amylase per 100 ml volumetric flask and filled up to distilled water. Weigh 0.1g of potato starch into 100ml of 16mM sodium acetate buffer and stirring continuously. For preparing the colorimetric reagent with 96 mM solutions of sodium potassium tartrate and 3,5-dinitro salicylic acid. The control group was given regular acarbose. The starch solution was kept at 25 oC in an alkaline environment to react with the alpha-amylase solution after the addition of the synthesized compound or compounds and the control group (acarbose). The reaction mixture was continuously observed for three minutes. Maltose production was quantified by reducing both 3,5-dinitro salicylic acid and 3-amino-5-nitro salicylic acid. The experimental reaction's absorbance was quantified at 540 nm.

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Inhibition of alpha-glucosidase enzyme – By mixing 1 mm of starch substrate solution (2% w/v maltose or sucrose), 0.2 M Tris buffer (pH 8.0), the synthesized compound or compounds, and the control group (Acarbose) at different concentrations, the enzyme inhibitory bioactivity was evaluated. After that, the solution mixture was maintained at 37°C for five minutes. The reaction is initiated by adding 1 ml of alpha glucosidase enzyme (1U/ml) to the solution mixture. The mixture is then incubated at 35 °C for 40 minutes. Two mm of 6N HCl solution were added to stop the reaction, and the color intensity absorbance was quantified at 540 nm.

Calculation of 50% Inhibitory Concentration (IC 50) [30]

Using the extract's % scavenging capabilities at five distinct concentrations, the 50% scavenging of radicals (IC50) needed by various quantities of produced compounds was computed. Using the formula Percentage inhibition(I%) = (Ac-As)/Ac X 100, the percentage inhibition of various concentrations (I%) was computed.

Were,

Ac is the control group's absorbance.

As = Absorbance at various concentrations of the sample.

RESULTS AND DISCUSSION

Table 2: **Observation of Fluorescence Analysis of powdered of Urtica parviflora Roxb.**

S.N.	Experiments	Daylight	UV-light	
			254nm	365nm
1	Powder & water	Amber green	Light green	Amber green
2	Powder & aqueous NaOH	Green	Brown	Dark green
3	Powder & alcoholic NaOH	Light green	Brick red	Green
4	Powder & conc. H_2SO_4	Black	Black	Black
5	Powder & conc. HCl	Dark brown	Black	Black
6	Powder & Nitric acid	Yellow	Yellow	Yellowish green
7	Powder & 5% ferric chloride	Brown	Brown	Amber green

Table 3: Percent of different components in hydro-alcoholic extract of plant.

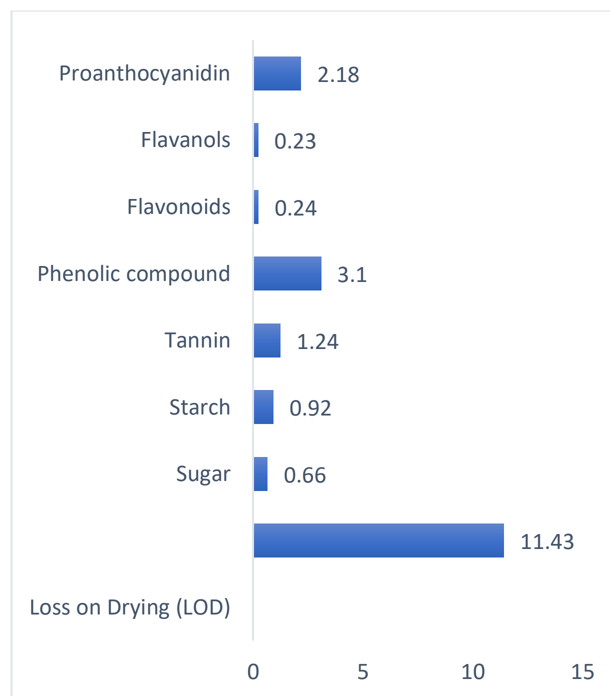

Table 4. When the successive fraction from Soxhlet is screened phytochemically, (Positive) suggests the presence of stem content and (Negative) suggests its absence.

S.N.	Compound	Test	n-hexane extract	Chloroform extract	Ethyl acetate extract	Methanol extract	Water extract
1	Carbohydrates	Molish's	-	-	+	+	+
		Fehling's	-	+	+	+	+
		Benedict's	-	+	+	-	-
		Caramelisatin	+	+	+	+	+
2	Alkaloids	Dragendroff's	+	+	-	+	+
		Mayer's	+	-	+	-	+
		Wagner's	-	+	+	-	+
		Hager's	+	+	+	+	+
3	Tannin	Ferric chloride	+	+	+	-	-
		Vanillin HCl test	-	-	-	-	-
		Alkaline reagent	+	+	+	-	+
4	Test for protein and amino acid	Biuret	+	+	-	+	-
		Millon's	+	+	+	-	-
		Ninhydrin	+	+	-	+	-
5	Flavonoids	Alkaline reagent	-	+	-	+	+
		Zinc hydrochloride	+	-	+	+	+
6	Glycosides	General test	+	+	+	-	+
		Froth test	+	+	+	-	-

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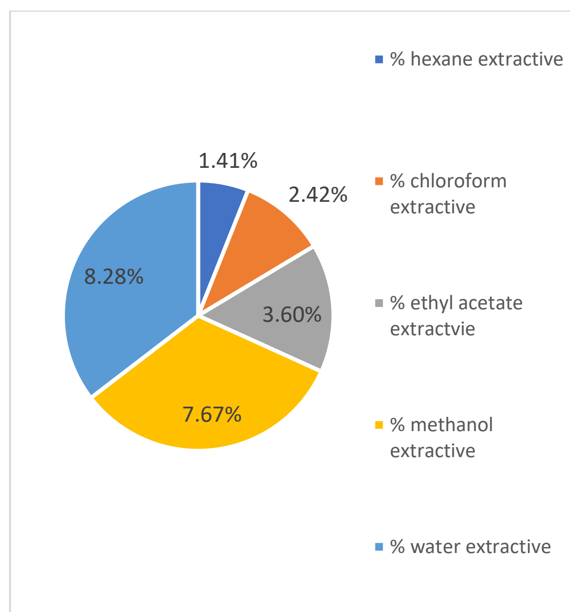
Table 5. Total extractive value of hexane, chloroform, ethyl acetate, methanol, and water extractive.



Using High Performance Thin Layer Chromatography (HPTLC) to identify different components in plant extract:

The present investigation's results include the HPTLC profile of a plant extract of the stem using the CAMAG HPTLC System with Wincats-3 programming software, and the video documentation of plates using the CAMAG Reproster-3 under UV light at 254 nm, 366 nm, and in visible light following post-derivatization with the anisaldehyde sulfuric acid reagent.

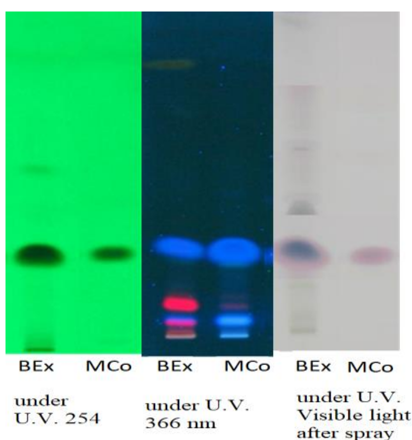


Fig. 3. HPTLC PROFILE of Plant extract (Stem Part) (BEX- Bark Extract ; MCo- Marker Compound Geniposidic acid)

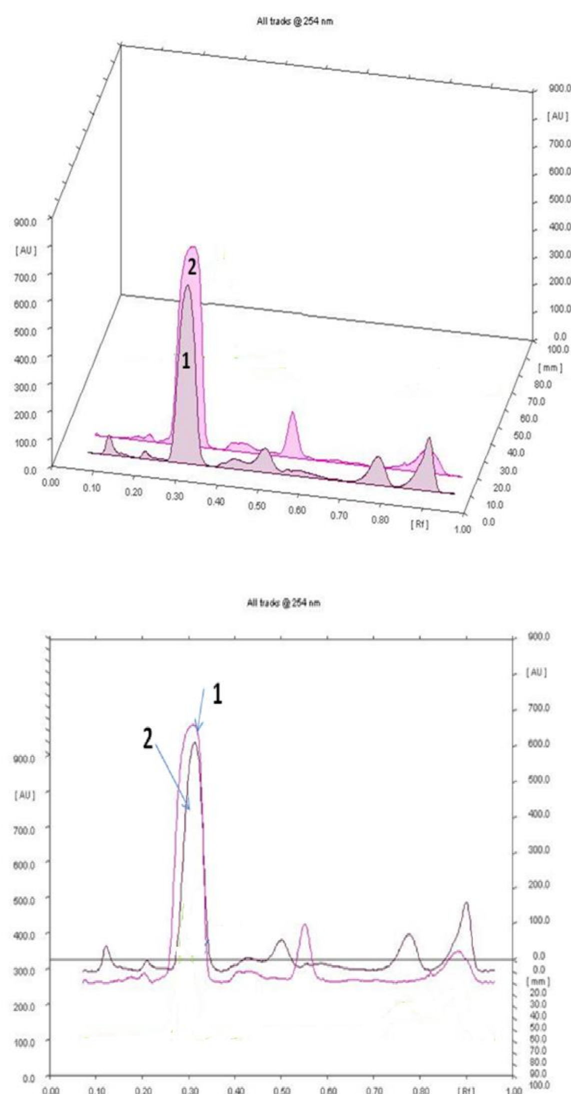
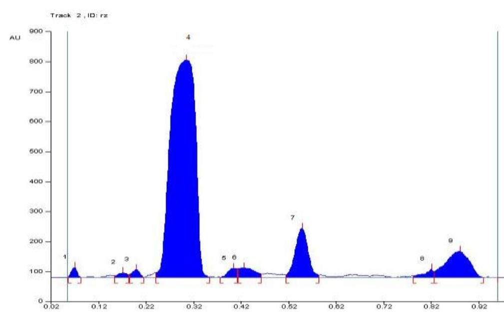


Fig. 4. For plant samples and markers at 254 nm, the UV scan profile is both filled and line.



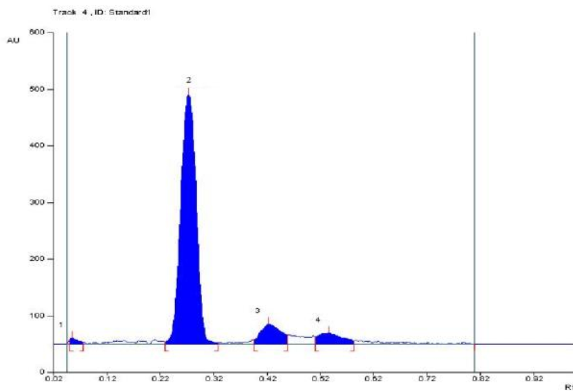
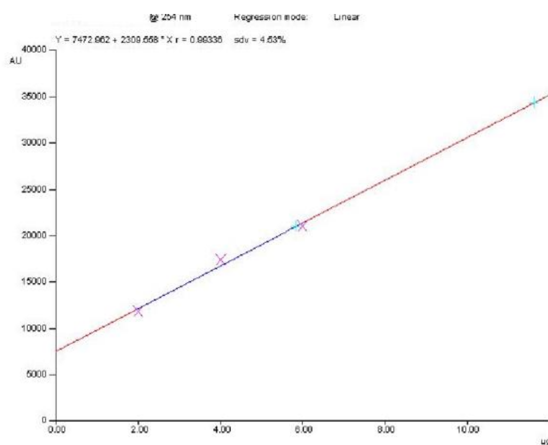


Fig. 5. Densitometric scan profiles at 254 nm for the extract and the marker compound (Geniposidic acid).



Tab.5 Results of HPTLC Profile of Plant

Sample	Amount applicated from 10mg/ml sample solution (µl)	Area under curve (AUC)	Amount present	% content in plant
Bark extract	10	34312.61	11.62 µg	1.162

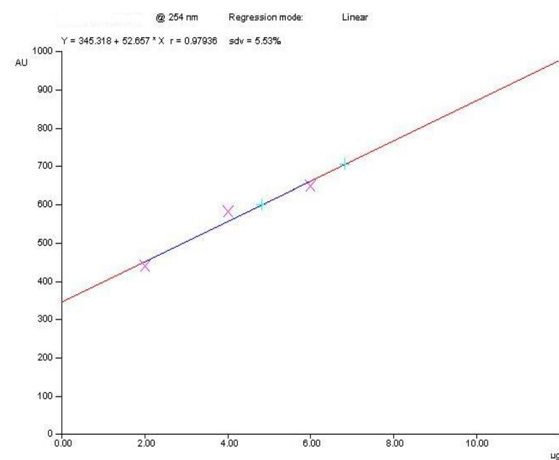


Fig. 6. This graph displays linearity (a) in relation to the peak's area (b) in relation to the peak's height.

ANTIDIABETIC BIOACTIVITY

S.NO	EXTRACTS	% INHIBITION OF ALPHA-AMYLASE ENZYME					% INHIBITION OF ALPHA GLUCOCIDASE ENZYME				
		PERCENTAGE OF THE SAMPLE'S INHIBITION CONCENTRATION (MG/ML)									
		0.2	0.4	0.6	0.8	1	0.2	0.4	0.6	0.8	1
1	HYDRO-ALCOHOLIC	28.34	48.16	63.19	82.62	32.84	54.43	66.26	78.81	84.17	84.53
2	STANDARD	40.83	68.45	72.66	88.67	41.77	69.35	75.33	88.89	97.96	96.34

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Conclusion

The current work aims to examine hydro-alcoholic extract from bark of plant Premna Barbata Wall. Ex Schauer (Family- Verbenaceae). Plant's phytochemical estimate, HPTLC fingerprinting and In-vitro anti-diabetic efficacy were estimated and reported. Findings revealed that this species majorly contains glycosides like iridoids and other Phyto constituents. According to the current study, extracts effectively and dose-dependently inhibit alpha glucosidase and alpha amylase enzymes in laboratory settings. The extracts demonstrate strong inhibitory effect, according to the results obtained. The findings can thus be used as a reference for next in-vivo study on plant potential.

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