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Phytochemical Fingerprinting and Activity of Premna barbata Wall. ex-Schauer Stem Bark Extract

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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 flavonols (0.23%), among others..

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

1. 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 ailments5,6. ,

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

Fig. 1: Whole plant of Premna Barbata

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

Table 3: Aspects and Metabolic Syndromes of Type 2 Diabetes [10-12]

Aspect	Type 2 Diabetes	Metabolic Syndrome/Other Disorders
Onset	Predominantly after 40 years, but increasingly seen in youth	Typically, adulthood increases with age and obesity
Pathogenesis	Insulin resistance with relative insulin deficiency	Insulin resistance, central obesity, dyslipidaemia, and hypertension
Prevalence	90–95% of diabetes cases	Affects >20% of adults in developed countries
Key Symptoms	Often asymptomatic early, polyuria, polydipsia, fatigue, gradual onset	Often asymptomatic; features include obesity, hypertension, dyslipidaemia
Risk Factors	Obesity, sedentary lifestyle, family history, age	Obesity, inactivity, genetic factors
Treatment	Lifestyle modification, oral agents, insulin if needed	Weight loss, exercise, and management of individual risk factors
Complications	Microvascular and macrovascular complications	Cardiovascular disease, fatty liver, PCOS
Diagnosis	Hyperglycaemia, insulin resistance, higher C-peptide	Clinical criteria: waist, BP, triglycerides, HDL, glucose
Other Types	Monogenic diabetes, secondary diabetes, LADA, MODY	Inherited metabolic disorders (e.g., PKU, Gaucher's), mitochondrial
Prevention	Preventable with lifestyle changes	Preventable with lifestyle changes

2. MATERIAL AND METHODS

PLANT MATERIAL

The Botanical Survey of India (BSI), Dehradun, Uttarakhand, India, authenticated the plant vide letter no.Tech./Herb (Ident.)/2022-23/518 With 1187 that was obtained from wildly grown area of Kumaon Region (Almora) Uttarakhand, India.

EXTRACT PREPARATION

After being harvested, the plant was let 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 upto 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 ^{o}C 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.

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)

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.

Where,

Ac is the control group's absorbance.

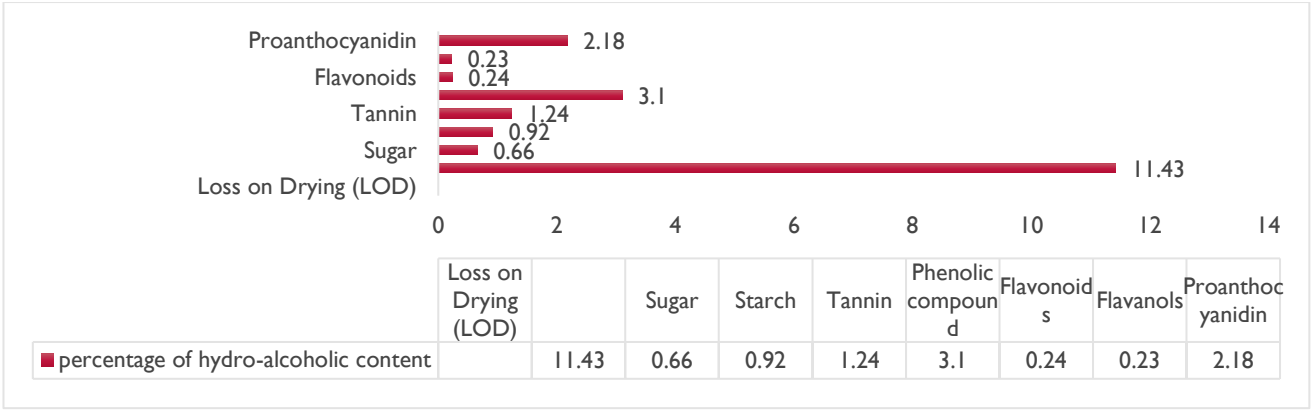
As = Absorbance at various concentrations of the sample.

3. RESULTS AND DISCUSSION

Table 2: 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	+	+	+	-	-

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

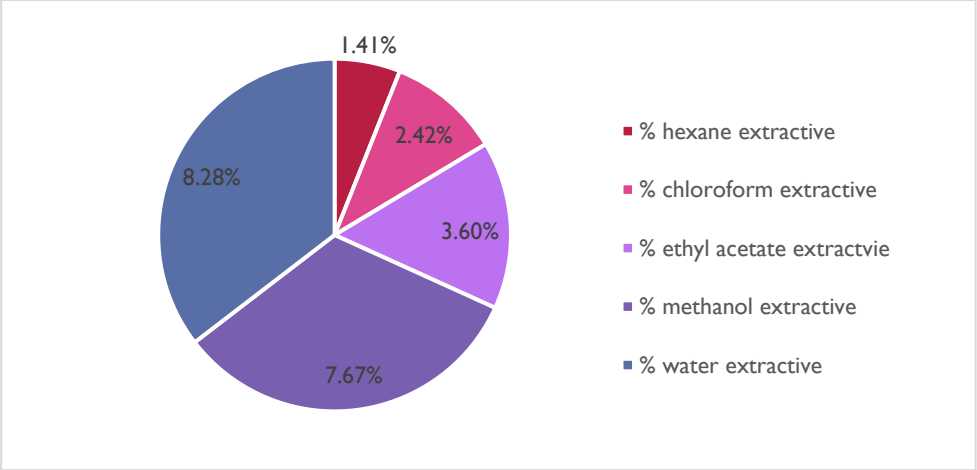


	Loss on Drying (LOD)		Sugar	Starch	Tannin	Phenolic compound	Flavonoids	Flavanols	Proanthocyanidin
percentage of hydro-alcoholic content		11.43	0.66	0.92	1.24	3.1	0.24	0.23	2.18

Table 4. 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 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.

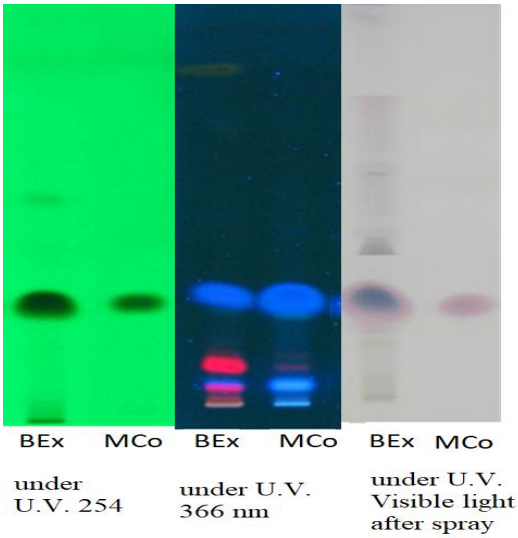


Fig. 3. HPTLC PROFILE of Plant extract (Stem Part) (BEX- Bark Extract ; MCo- Marker Compound Geniposidic acid)

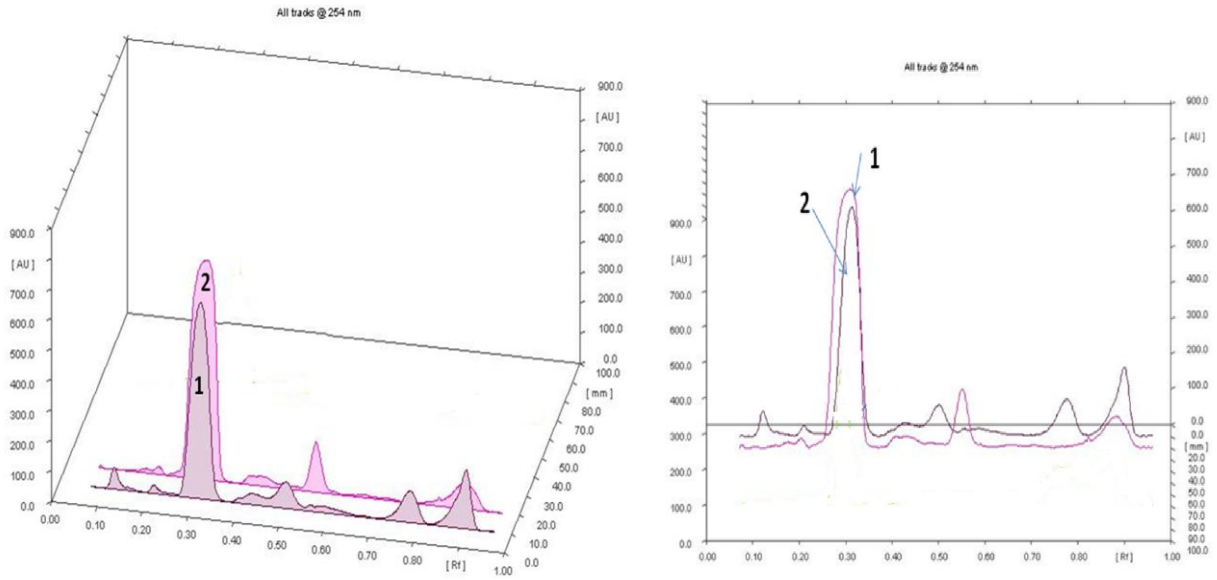
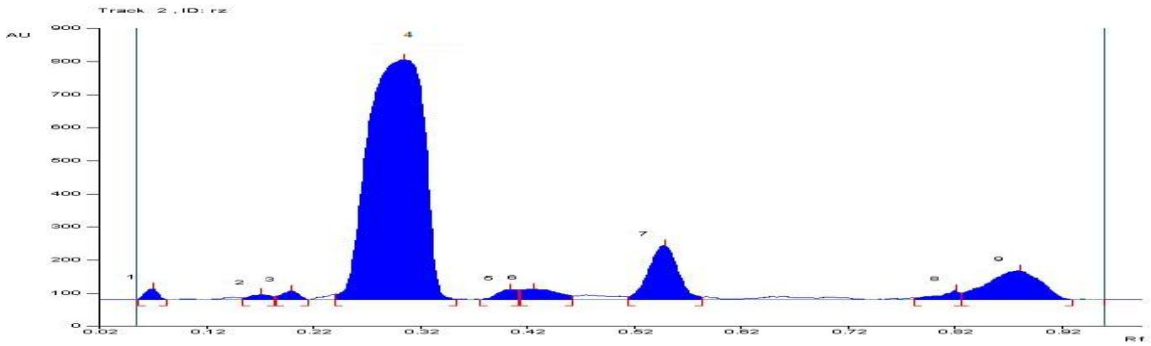


Fig. 4. For plant samples and markers at 254 nm, the UV scan profile is both filled and line.



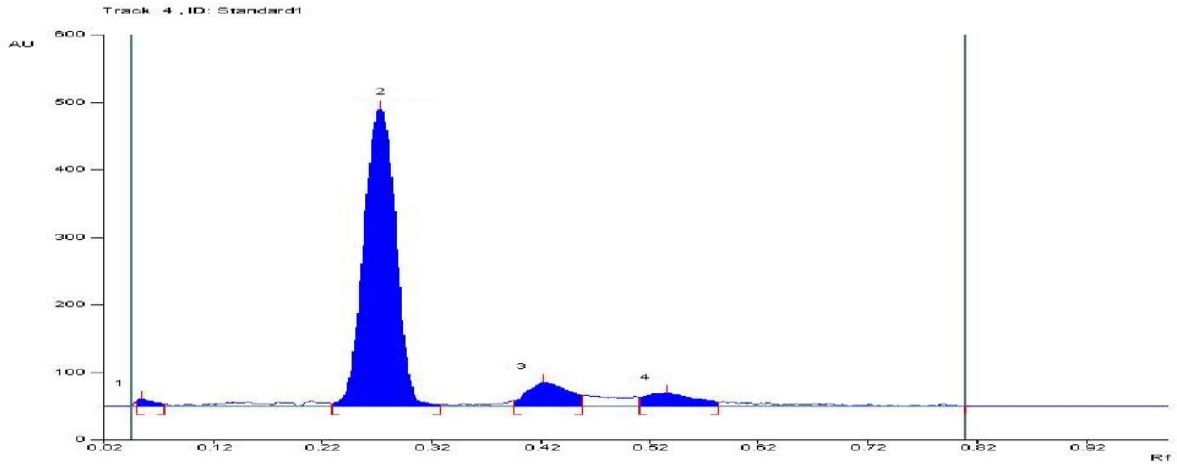


Fig. 5. Densitometric scan profiles at 254 nm for the extract and the marker compound (geniposidic acid).

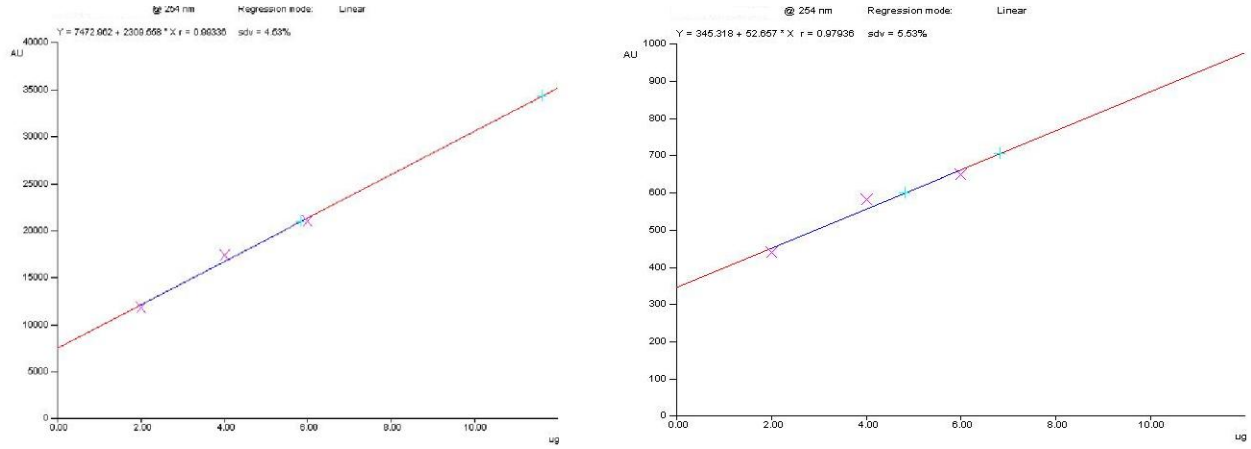


Fig. 6. This graph displays linearity (a) in relation to the peak's area (b) in relation to the peak's height.

Tab. 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**

ANTIDIABETIC BIOACTIVITY

Table 6: shows % inhibition activity

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

4. 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

REFERENCES

1. Joshi, B. C., Juyal, V., Sah, A. N., & Mukhija, M. (2022). Ethnopharmacology, botanical description and phytochemistry of premna barbata: an unexplored medicinal plant species from lamiaceae family. Current Traditional Medicine, 8(6), 60-66.
2. Munir, A. A. (1984). A taxonomic revision of the genus Premna L.(Verbenaceae) in Australia. Journal of the Adelaide Botanic Garden, 1-43.
3. Dianita, R., & Jantan, I. (2017). Ethnomedicinal uses, phytochemistry and pharmacological aspects of the genus Premna: a review. Pharmaceutical biology, 55(1), 1715-1739.
4. Joshi, B. C., Juyal, V., Sah, A. N., & Mukhija, M. (2023). In-Vitro Antiproliferative Efficacy, Antioxidant bioactivity and LC-MS Based Metabolite Profiling of Premna barbata Stem Bark.
5. Singh, C., Mehata, A. K., Muthu, M. S., & Tiwari, K. N. (2024). Premna integrifolia: A Review on the Exploration of its Potential Pharmacological and Therapeutic Properties. Current Traditional Medicine, 10(2), 37-50.
6. Yadav, D., Masood, N., Luqman, S., Brindha, P., & Gupta, M. M. (2013). Antioxidant furofuran lignans from Premna integrifolia. Industrial Crops and Products, 41, 397-402.
7. Singh, C., Mehata, A. K., Muthu, M. S., & Tiwari, K. N. (2024). Premna integrifolia: A Review on the Exploration of its Potential Pharmacological and Therapeutic Properties. Current Traditional Medicine, 10(2), 37-50.
8. Roza Dianita, R. D., & Ibrahim Jantan, I. J. (2017). Ethnomedicinal uses, phytochemistry and pharmacological aspects of the genus Premna: a review.
9. Sudo, H., Takushi, A., Hirata, E., Ide, T., Otsuka, H., & Takeda, Y. (1999). Premnaodorosides D–G: acyclic monoterpenediols iridoid glucoside diesters from leaves of Premna subscandens. Phytochemistry, 52(8), 1495-1499.
10. L. X. Guo, J. H. Liu, and F. Yin,"Regulation of insulin secretion by geniposide: possible involvement of phosphatidylinositol 3- phosphate kinase," European Review for Medical and Pharmacological Sciences, vol. 18, no. 9, pp. 1287–1294, 2014.
11. J. Yan, C. Wang, Y. Jin et al., "Catalpol ameliorates hepatic insulin resistance in type 2 diabetes through acting on AMPK/NOX4/PI3K/AKT pathway," Pharmacological study, vol. 130, pp. 466–480, 2018.
12. P. Jiang, L. Xiang, Z. Chen et al., "Catalpol alleviates renal damage by improving lipid metabolism in diabetic db/db mice," American Journal of Translational study, vol. 10 no. 6, pp. 1750–1761, 2018.
13. J. Liu, H. R. Zhang, Y. B. Hou, X. L. Jing, X. Y. Song, and X. P. Shen, "Global gene expression analysis in liver of db/db mice treated with catalpol," Chinese Journal of Natural Medicines, vol. 16, no. 8, pp. 590–598, 2018.
14. C. Liu, Y. Hao, F. Yin, Y. Zhang, and J. Liu, "Geniposide protects pancreatic β cells from high glucose-mediated injury by activation of AMP-activated protein kinase," Cell Biology International, vol. 41, no. 5, pp. 544–554, 2017.
15. Williamson E., Okpako D.T., Evans F.J., 1996. Selection, Preparation and Pharmacological Evaluation of Plant Material, Wiley, Chichester.
16. Goldfrank L., 1982. The Pernicious Panacea: Herbal Medicine. Hospital Physician, Vol. 10, pp. 64–86.
17. Malik C.P., Singh M.B., 1980. PlantEnzymology and Histoenzymology, Kalyani Publishers, New Delhi, pp. 278.
18. Soldati F., 1997. The registration of medicinal plant products, what quality of documentation should be required. The industrial point of view In: World Congress on Medicinal and Aromatic Plants for Human Welfare, Vol. 2, pp. L-48.
19. Vulto A.G., Smet P.A., 1988. In: Dukes, M.M.G. (Ed.). Meyler's Side Effects of Drugs, Elsevier, Amsterdam, 11th Ed. pp. 999–1005.

20. Gruenwald J., 1997. The market situation and marketing of herbal medicinal products (HMP) in Europe. In: World Congress on Medicinal And Aromatic Plants For Human Welfare, Vol. 2, pp. L-33.
21. Brevoort P., 1997. The current status of the US botanical market in: World Congress on Medicinal and Aromatic Plants For Human Welfare, Vol. 2, p. L-42.
22. Israelsen L.D., 1997. United States regulatory status of botanical preparations in:World Congress on Medicinal And AromaticPlants for Human Welfare, 2, pp. L-44.
23. Brevoort P., 1997. The current status of the US botanical market in World Congress on Medicinal and Aromatic Plants for Human Welfare, Vol. 2, pp. L-42.
24. Elisabetsky E., 1987. Pesquisas em Plantas medicinais. Cieˆncia e Cultura, Vol. 39 (8), pp. 697–702.
25. Rouhi A.M., 1997. Seeking drugs in natural products. Vol. 7, pp. 14–29.
26. Reid W.V., Laird S.A., Meyer C.A., Ga´mez R., Sittenfeld A., Janzen D.H., Gollin M.A., Juma C., 1993. Biodiversity Prospecting: Using Genetic Resources for Sustainable Development. World Resources Institute (USA).
27. Payne G., Bringi V., Prince C., Shuller M., 1991. The quest for commercial production of chemicals from plant cell culture, Plant Cell and Tissue Culture in Liquid Systems, Oxford University Press, Oxford.
28. Hamburger M., Hostettmann K., 1991. Bioactivity in plants: the link between phytochemistry and medicine. Phytochemistry,Vol. 30 (12), pp. 3864–3874.
29. Borris R.P., 1996. Natural products study: perspectives from a major pharmaceutical company. Journal of Ethnopharmacology, Vol. 51 (1/3), pp. 29–38.
30. Williamson E., Okpako D.T., Evans F.J., 1996. Selection, Preparation and Pharmacological Evaluation of Plant Material, Wiley, Chichester..

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