PHARMACOGNOSTIC EVALUATION AND PRELIMINARY PHYTO-CHEMICAL SCREENING OF LEAVES AND BARK OF *PREMNA BARBATA* WALL. EX SCHAUER

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ABSTRACT

Background: *Premna Barbata Wall. Ex Schauer is broadly used as local medicine by various tribes and medicinal practitioners for the treatment of various diseases in Garhwal, Kumaun region in India and western Himalayan Countries. Despite the popular ethanobotanical utilization of the plant especially the bark for the treatment of Herpes simplex, still there has been no conclusive study mentioned regarding pharmacognostic and phytochemical investigation of the plant.*

Aim: *Thus, the current study was focused to study and explore the pharmacognostical and phytochemical parameters of Premna Barbata Wall. Ex Schauer.*

Material and Methods: *Leaf and bark samples of Premna Barbata Wall. Ex Schauer were subjected to morphological and Microscopical studies. Physicochemical parameters were also studied and preliminary phytochemical analysis with various solvents like, acetone, ethyl acetate, petroleum ether, methanol and aqueous extract of plant part were performed according to polarity. Leaf and bark powder of plan material was treated with different reagents used for powder microscopy and samples were observed for fluorescence under UV light and visible light of short and long wavelength.*

Results: *Microscopical studies showed different cell constituents such as collenchymas, xylem vessels, palisade cells, warty trichomes and lower and upper epidermis in leaf; cork cells, phelloderm, multiseriate medullary rays, and fibers in bark part. Physicochemical parameters including loss on drying, extractive value, ash value, swelling and foaming index were evaluated for both leaf and bark part and their values were documented. Fluorescence analysis gave different range of fluorescence color for powdered drug sample. Phytochemical investigation reported the appearance of chemical constituents such as alkaloids, glycosides, terpenoids, resins and flavanoids in both leaf and bark extracts. Methanol was considered as the best solvent as it extracted maximum chemical constituents and percentage yield.*

Keywords: *Premna Barbata Wall. Ex Schauer, Phytochemical, Physicochemical, Microscopical, Fluorescence.*

INTRODUCTION

Herbs are making a comeback and herbal rebirth is spreading all over the world. Around 75% of the population of world depends and believes on plants and plant extracts for health care. On an average about 30% of the entire plant species, at a time or other was used for medicinal purposes. It is believed that out of the 2, 50,000 higher plant species, more than 80,000 have medicinal values. India is in the midst of the world's 12 biodiversity centers with the presence of over 45000 various plant species. Out of these, around 15000-20000 plants possess good medicinal properties. Only around 7000-7500 plants species are used for their medicinal properties by traditional practioners and communities[1]. In ancient times mankind almost depends on nature for both health and illness. Primitive peoples treated illness by using plant, animals and mineral parts[2].

The genus Premna belonging to family Verbenaceae contains more than 200 distinct species which are widely spread in tropical and subtropical areas of world[3]. Phytochemical investigation reveals that the genus Premna is rich source of iridoid glycosides, diterpenoids, flavanoids. Secondary metabolites isolated from this genus possess diverse biological activity such as antibacterial, cytotoxic, antioxidant, hepatoprotective and anti-inflammatory[4]. *Premna barbata* Wall. Ex Schauer plant included in family Verbenaceae is widely spread in the eastern and northern part of India, Nepal, Myanmar and Pakistan, Australia, Africa and Pacefic Island[5]. After detail studies of surveys carried out on ethanobotanical plants of Garhwal and Kumaun regions, Uttarakhand, India and in Nepal it was concluded that *Premna barbata* Wall. Ex Schauer possess diverse medicinal properties. It is use in wound healing, treatment of throat infection, arthritic pain, dropsy, herpes complex disease, urine infections by peoples of different community[6-9].

Detail literature studies states that there is no published work till date on pharmacognostic evaluation of this plant. Keeping in view this fact present study was undertaken to develop quality standard for leaves and bark portion of this plant.

MATERIALS AND METHODS

PLANT COLLECTION AND AUTHENTIFICATION

Plant *Premna barbata* Wall. Ex Schauer was collected from the forest, fields & adjacent areas of Kasardevi, Almora Uttarakhand during the month of July, identified and preserved. Two set of herbarium specimens were submitted for identification of plant sample to Botanical Survey of India, Northern Regional Center, 192, Kaulagarh Road, Dehradun-248195. The stem was shade dried and powdered using mechanical grinder. Powder samples were stored in air tight containers and calculated amount of the powder was used in pharmacognostic studies and phytochemical analysis.

CHEMICALS AND INSTRUMENTS

All chemicals and reagents used for pharmacognostic evaluation were of analytical grade. Compound microscope was used for the study. The photography was done by using camera Sony cyber-shot (24 mega pixels). Eyepiece micrometer, stage micrometer and camera lucida were used for determination of quantitative microscopical characters.

PHARMACOGNOSTIC STUDIES

MORPHOLOGICAL EVALUATION

Organoleptic evaluation of leaf and bark parts of the plant were performed with the help of sense organs for identification of important characters such as size, shape, fracture, color, odour and taste[10].

MICROSCOPICAL EVALUATION

Qualitative microscopical evaluation of leaf and bark part was carried out by taking free hand transverse section of fresh sample. Free hand sectioning of fresh leaves and bark were carried out, for section cutting plant samples were sandwiched in between potato pith sectioned with the help of sharp blade or razor using chloral hydrate as clearing agent and safranin as staining agents. Photomicrography was performed. Leaf constants were determined using camera lucida. Shade drying of leaves and barks was done and samples were powdered and stored in airtight containers and used for powder microscopy. Powder microscopy of leaves and bark part were performed and studied as per the standard methods by clicking the images of various fragments of plant tissues. Different reagents such as phloroglucinol-HCl, Ruthenium Red, Sudan Red-I and Iodine solution were used for powder microscopy[10-13].

PHYSICOCHEMICAL EVALUATION

Dried powder of leaves and bark were subjected to physicochemical analysis. Physiochemical constants such as ash value, extractive value, foreign matter, moisture content was performed and studied[11].

EXTRACTION AND PHYTOCHEMICAL SCREENING EVALUATION

Preliminary phytochemical analysis of leaves and bark specimens was carried on different extracts prepared through successive extraction method in soxhlet apparatus. Dried powdered leaves and bark (50 gm) were extracted in a soxhlet apparatus with various solvents according to their polarity. The initial phytochemical screening of the leaf and bark extract was performed as per WHO guidelines to determine the presence of active plant metabolites such as alkaloids, flavonoids, tannins, phlobatannins, triterpenes, steroids and saponins[14-15].

FLUORESCENCE ANALYSIS

Fluorescence analysis has been performed with various chemical reagents as per the mentioned standard procedures. A small pinch of stem bark and leaf powder is putted on watch glass and 1-2 drops of reagent which is freshly prepared is added, it is mixed by gentle inclining the watch glass and after a certain time, the watch glass is settled inside the UV chamber and color were observed in visible light, short (254 nm) and long (366 nm) ultra violet radiations. Color was observed and recorded by use of different reagents in various radiations[16].

RESULT AND DISCUSSION

PLANT AUTHENTIFICATION

Sample of plant was taxonomically identified by botanist of Botanical Survey of India, Northern Regional Center, 192, Kaulagarh Road, Dehradun-248195. One set of the sample was deposited in the herbarium of Botanical Survey of India.

Figure 1: Identified herbarium specimen of *Premna barbata* Wall. Ex Schauer

MACROSCOPICAL EVALUATION

Premna barbata Wall. Ex Schauer is a small tree of around 4-6 m height usually grows in forest, fields and adjacent areas of Garhwal and Kumaun region of Uttarakhand, India and its neighboring countries. Leaves are simple, opposite with ovate-lanceolate shape, entire margin with acute apex around 3-12 cm long and 2-5 cm in width. Color of the leaves is dark green with characteristic odour having astringent taste. Dried stem bark is curved, recurved and flat in shape, 0.2-0.8 cm in thickness, around 11 cm long and up to 6 cm in width. Outer surface of bark is grayish brown with grooves and furrows while inner surface is wrinkled and yellowish white in color. Bark possesses no odour and taste is astringent.

Figure 2 : Morphological features of *Premna Barbata* Wall. Ex Schauer leaf and bark part

MICROSCOPICAL ANALYSIS

QUALITATIVE LEAF ANALYSIS

Transverse section of leaf shows epidermal cells which are somewhat rectangular in shape and upper epidermal cells is larger in size than the lower one. 6-7 layers of collenchyma cells are present beneath the upper epidermis region. Collenchyma cells are large having thick wall and oval shape. Warty trichomes with irregular shape are present with in upper epidermis. Palisade cells are double layered while mesophyll contains spongy parenchyma. Vadscular

bundles are covered with endodermis and shows presence of lignified xylem and phloem vessels.

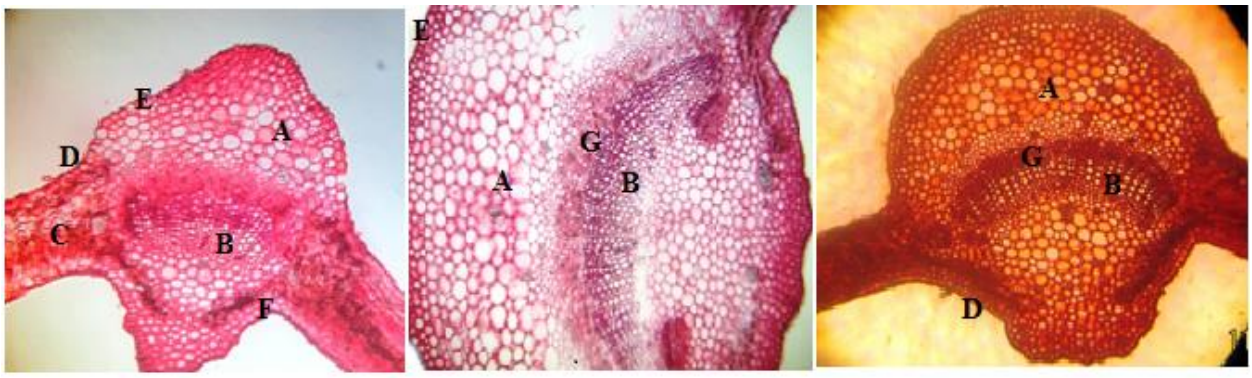

Figure : 3 Transverse section of *Premna barbata* Wall.Ex Schauer leaf (A) Collenchyma (B) Xylem vessels (C) Palisade cells (D) Trichome (E) Upper epidermis (F) Lower epidermis (G) Phloem

QUALITATIVE BARK ANALYSIS

Microscopical study of stem bark shows the presence of cork cells in periderm region, cells are arranged radially in rows having dark brown content and tabular shape. Phellogen having rectangular cells which are thin walled with 4-6 layers. Cortex possesses thin walled elongated cells. Secondary phloem is also visible containing medullary rays, phloem fibers and phloem parenchyma. Secondary phloem acquires large portion of bark and is differentiated into collapsed phloem and non collapsed phloem zone. Medullary rays are elongated marginal cells, thick walled and compact. Prismatic calcium oxalate crystals are also present in the bark.

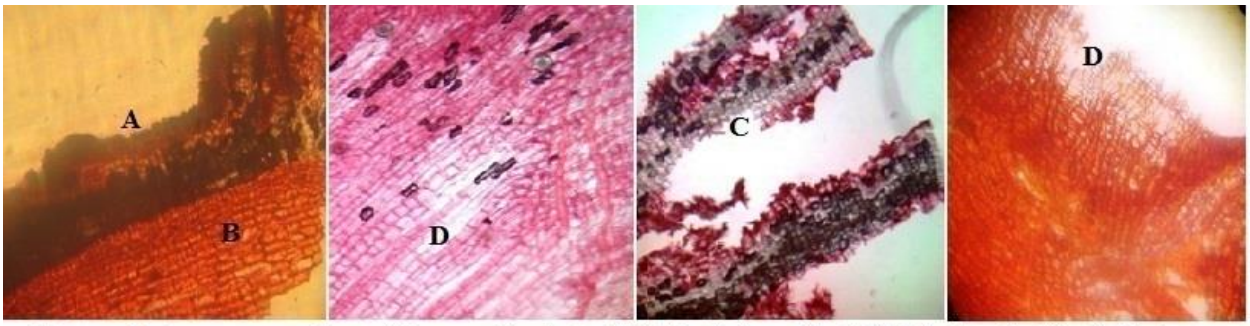

Figure :4 Transverse section of *Premna barbata* Wall. Ex Schauer bark (A) Cork cells (B) Phelloderm (C) Phloem (D) Medullary rays

POWDER MICROSCOPY

Shade dried leaf and bark samples were finely powdered and pinch of powder was placed in different slides which is further treated with various reagents like iodine soloution, phloroglucinol + Conc. Hcl, reuthenium red and sudan red III. Powder microscopy of leaf powder revealed the existence of epidermal cells rectangular or polygonal in shape having thick cell wall, clusters of acicular calcium oxalate crystals, spiral shaped xylem vessels and starch grains that are mostly simple in appearance. Bark powder characters such as presence of multiseriate medullary rays,small fragments of cork cells, sclereid cells and fibers of various shapes and thickness were observed and studied.

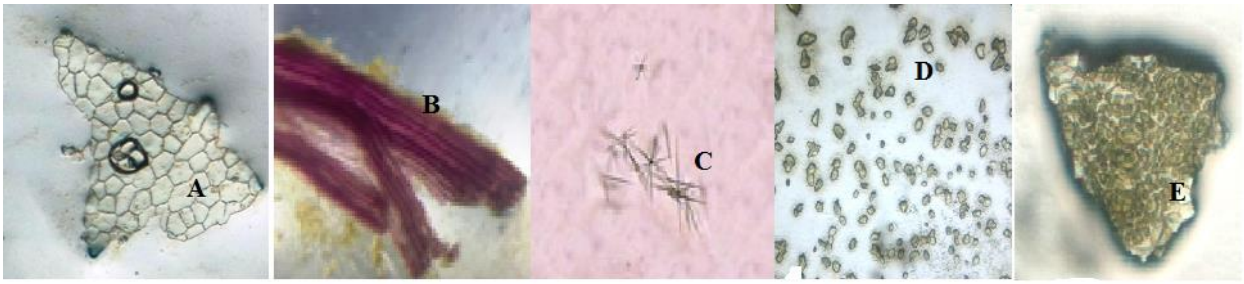

Figure : 5 Powder characteristics of *Premna barbata* Wall. Ex Schauer leaf (A) Epidermal cells (B) Xylem vessels (C) Acicular calcium oxalate crystals (D) Starch grains (E) Stomata

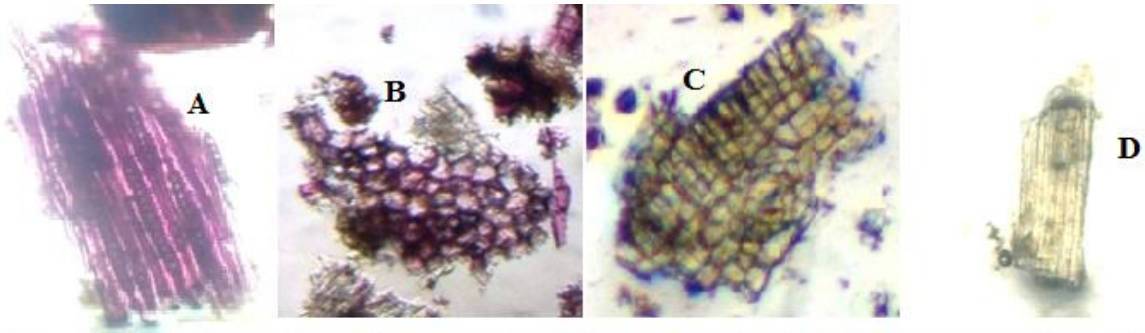

Figure : 6 Powder characteristics of *Premna barbata* Wall. Ex Schauer bark (A) Medullary rays (B) Cork cells (C) Sclereids cells (D) Fiber

QUANTITATIVE ANALYSIS

Quantitative analysis revealed that the fresh leaf sample contains paracytic stomata. Leaf parameters such as stomatal no. of both surface i.e upper and lower surface were found to be 11 and 14 respectively. Stomatal index of upper surface was 10.14 while of lower surface is nil. The vein islet no was calculated as 30.

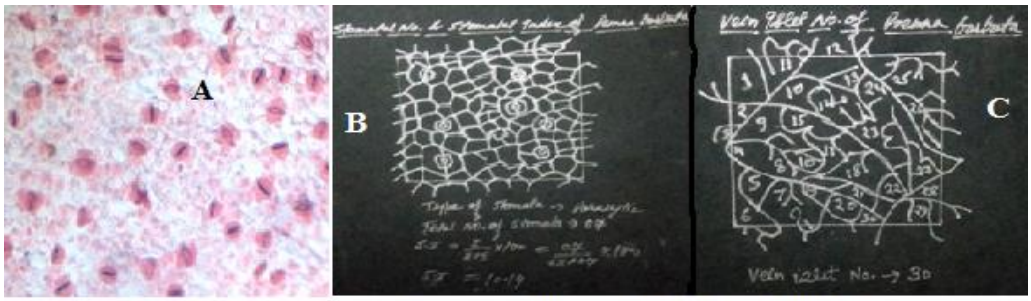

Figure : 7 Quantitative microscopy of leaves of *Premna barbata* Wall. Ex Schauer (A) Paracytic stomata (B) Stomatal No. (C) Vein islet No.

Table 1: Leaf constants data of *Premna barbata* Wall. Ex Schauer

S.No	Leaf Surface	Values(per sq.mm)
1	Stomatal No. of upper surface	11
2	Stomatal No. of lower surface	14
3	Stomatal index of upper surface	10.14
4	Stomatal index of lower surface	Nil
5	Vein islet No.	30

PHYSICOCHEMICAL EVALUATION

Physicochemical constants such as moisture content, extractive value, ash value, swelling index and foaming index were determined for leaf and stem bark of *Premna barbata* Wall. Ex Schauer. Loss on drying determined the amount of moisture content in crude drug samples. Ash value detected adulteration as well as determined purity and quality of crude drug. Extractive value indicated the percentage of secondary metabolites obtained with solvent from a given amount of crude plant material. Swelling index gave an idea about the amount of swelling material such as gums and that material which contain large quantity of pectin and mucilage. Saponin containing compounds which give persistent foam in aqueous solution were determined by foaming index. Results for physicochemical parameters were represented in Table 2 and 3.

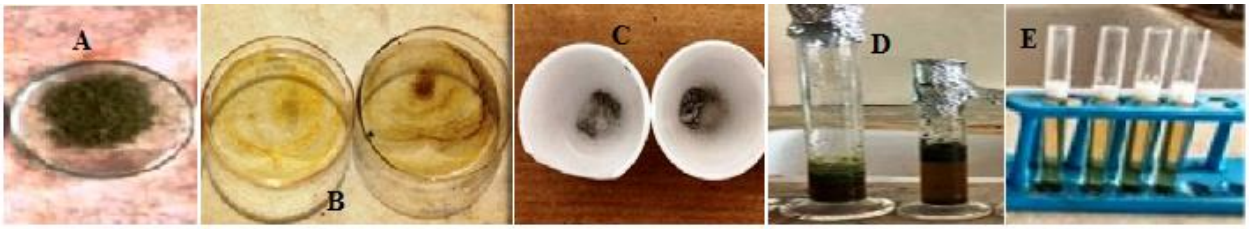

Figure : 8 Physicochemical parameters of *Premna barbata* Wall. Ex Schauer leaf (A) Loss on drying (B) Extractive value (C) Ash value (D)Foaming index (E) Swelling index

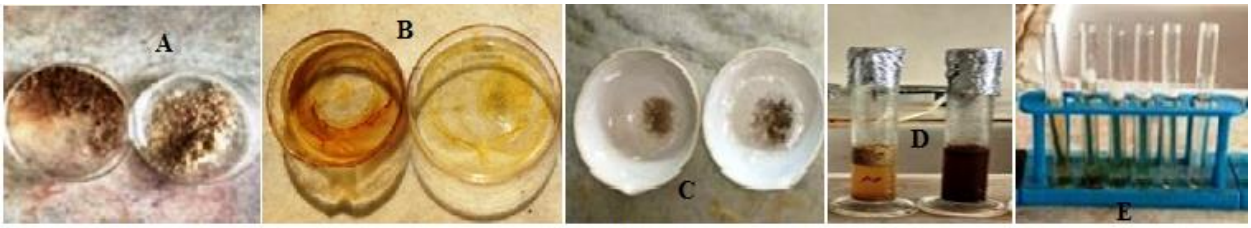

Figure : 9 Physicochemical parameters of *Premna barbata* Wall. Ex Schauer bark (A) Loss on drying (B) Extractive value (C) Ash value (D) Swelling index (E) Foaming index

Table 2: Physicochemical parameters of *Premna barbata* Wall. Ex Schauer leaf.

S.No	Physicochemical parameters	Results (%)
1	Loss on drying	0.108
2	Water soluble extractive	14.7
	Alcohol soluble extractive	11.4
3	Total ash	8.3
	Water soluble ash	4.1
	Acid insoluble ash	1.2
4	Swelling index	119
5	Foaming index	5.12

Table 3: Physicochemical parameters of *Premna barbata* Wall. Ex Schauer bark.

S.No	Physicochemical parameters	Results (%)
1	Loss on drying	0.090
2	Water soluble extractive	19.7
	Alcohol soluble extractive	13.1
3	Total ash	6.7
	Water soluble ash	3.5
	Acid insoluble ash	0.8
4	Swelling index	108.7
5	Foaming index	Nil

Fluorescence Analysis of Powders

Fluorescence characters imparted by various plant samples play a vital role in determination of quality and purity. Powder of leaf and stem bark was treated with different chemical reagents and were observed in day light and ultraviolet light. Fluorescence report of *Premna barbata* Wall. Ex Schauer powdered leaf and stem bark is tabulated in Table 4 and 5.

Table 4: Fluorescence characteristics of *Premna barbata* Wall. Ex Schauer leaf.

S.No	*Premna barbata* (Leaf)	Visible	Short UV-254	Long UV-365
1	Powder + 1N NaOH in water	Yellow	Amber green	Yellowish green
2	Powder + 1N NaOH in alcohol	Light green	Faint green	Faint green
3	Powder + acetic acid	Yellowish green	Light red	Green
4	Powder + methanol	Light green	Light red	Green
5	Powder + H_2SO_4	Brown	Brownish green	Light green
6	Powder + petroleum ether	Light brown	Light brown	Faint green
7	Powder + Hcl	Amber	Amber	Amber green
8	Powder + water	Cream	Cream	Cream
9	Powder + nitric acid	Faint yellow	Cream	Light green
10	Powder +acetone	Amber green	Light red	Green

Table 5: Fluorescence characteristics of *Premna barbata* Wall. Ex Schauer bark.

S.No	*Premna barbata* (Bark)	Visible	Short UV-254	Long UV-365
1	Powder + 1N NaOH in water	Faint yellow	No color	Faint green
2	Powder + 1N NaOH in alcohol	Faint yellow	No color	Faint green
3	Powder + acetic acid	Faint yellow	No color	Faint green
4	Powder + methanol	Faint yellow	No color	Faint green

5	Powder + H_2SO_4	Brown	Brown	Black
6	Powder + petroleum ether	No color	No color	No color
7	Powder + Hcl	Light yellow	Light green	Green
8	Powder + water	No color	Cream	No color
9	Powder + nitric acid	Faint yellow	Faint yellow	Faint yellow
10	Powder +acetone	Faint yellow	Faint yellow	Faint yellow

Extraction and Phytochemical Screening

Extraction process was carried out by using various solvents according to their polarity. Various solvents such as petroleum ether, ethyl acetate, acetone, methanol and water was utilized for extraction of leaf and stem bark portions. Percentage yield of various extract in different solvents was calculated. Furthermore leaf and stem bark extracts were used for phytochemical screening for identification of different secondary metabolites present. Phytochemical investigation shows the presence of flavanoids, glycoside and terpenoids in leaf sample while stem bark showed appearance of mainly tannins and resins. Results of percentage yield and phytochemical analysis of powdered leaf and stem bark is tabulated in Table 6-9.

Table 6: Calculated % yield of various extracts of *Premna barbata* Wall. Ex Schauer leaf.

S.No.	Solvent	Colour of extract	Yield of extract (% in grms)	% yield
1	Petroleum Ether	Dark Green	1.8	3.6
2	Ethyl Acetate	Green	1.5	3.0
3	Acetone	Light Green	1.9	3.8
4	Methanol	Greenish Black	1.2	2.4
5	Water	Brownish Black	2.4	4.8

Table 7: Calculated % yield of various extracts of *Premna barbata* Wall. Ex Schauer bark.

S.No.	Solvent	Colour of extract	Yield of extract (% in grms)	% yield
1	Petroleum Ether	Dark Brown	2.1	4.2
2	Ethyl Acetate	Brown	2.4	4.8
3	Acetone	Brownish Black	3.5	7.0
4	Methanol	Light Brown	2.3	4.6
5	Water	Brownish White	2.7	5.4

Table 8: Results of phytochemical screening of different extracts of *Premna barbata* Wall. Ex Schauer leaves extracts.

S.No.	Secondary Metabolites	Extract of leaves in various solvents			
		Pet. Ether Extract	Et. Acetate Extract	Acetone Extract	Methanol Extract	Water Extract
1	Carbohydrates	-	-	-	-	+
2	Glycosides	-	-	-	+	-
3	Alkaloids	+	-	-	-	-
4	Saponins	-	-	-	-	+
5	Proteins & amino Acid	-	-	-	-	+
6	Terpenoids	+	+	-	+	-
7	Tannins	-	-	-	+	+
8	Phenolic Compounds	-	+	+	+	-
9	Resins	-	-	-	+	-
10	Flavanoids	-	-	+	+	-

Table 9: Results of phytochemical screening of different extracts of *Premna barbata* Wall. Ex Schauer bark extracts.

S.No.	Secondary Metabolites	Extract of leaves in various solvents			
		Pet. Ether Extract	Et. Acetate Extract	Acetone Extract	Methanol Extract	Water Extract
1	Carbohydrates	-	-	-	-	+
2	Glycosides	-	-	-	-	-
3	Alkaloids	+	-	-	-	-
4	Saponins	-	-	-	-	+

5	Proteins & amino Acid	-	-	-	-
6	Terpenoids	+	+	-	+
7	Tannins	-	-	-	+
8	Phenolic Compounds	-	+	+	+
9	Resins	-	-	-	+
10	Flavanoids	-	-	+	+

CONCLUSION

The current study is to scrutinize the flora diversity of Kumaun and Garhwal region of Uttarakhand for medicinal value. Observation and results from this study gave beneficial data and information on pharmacognostic, Phytochemical and physicochemical properties of *Premna barbata* Wall. Ex Schauer. Pharmacognostic study of cells and their arrangement play important role in standardization of this crude drug which will be helpful in prevention of adulteration and substitution .Preliminary phytochemical screening of stem bark and leaf indicates the presence of some secondary metabolites which in future will play essential role in herbal medicine. As not so much work is published and available on this plant, the results reproduced from this current study serve as a standard in further study of this plant.

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