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PHYTOCHEMICAL EVALUATION OF *PREMNA SERRATIFOLIA* L.

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ABSTRACT

Standardization of crude drugs is a code of conduct and an essential need of a time. The broad spectrum of standardization gives surety of correct substance in correct amount for desired therapeutic effects. It also provides all possible careful measures taken from collection, manufacturing till drug dispensing for clinical application. The plant selected for the present investigation is *Premna serratifolia*, belonging to the family Lamiaceae. The aerial parts of the plant were shade dried and ground to fine powder and subjected to organoleptic, fluorescent and physico-chemical analysis. The plant powder was subjected to Soxhlet extraction using methanol as the solvent. Phytochemical screening (Qualitative and Quantitative) of the plant extract was done according to the standard biochemical procedures. Anti-inflammatory activity of the plant extract was tested using Albumin denaturation assay. The organoleptic analysis revealed the characteristic colour, taste, odour and nature of the powder of *Premna serratifolia.* In fluorescence analysis, on treatment with different solvents, colour changes (specific) could be noticed in the plant powder. The results of the physico-chemical analysis provided important parameters in detecting adulteration or improper handling of drugs. Qualitative and quantitative analysis in the plant confirmed the presence of many important bioactive phytochemicals. Bioactivity study indicated the anti-inflammatory potential of the plant comparable to that of the standard Aspirin. Further work should be carried out to isolate, purify and characterize the active constituents responsible for the specific activity of the plant and to elucidate the possible mechanism of action of the extract.

Keywords: *Premna*, Phytochemical, Pharmacognostical, Physio-chemical, Bioactivity, Organoleptic

INTRODUCTION

Medicinal plants have been a vital source of both curative and preventive medical therapy preparations for human beings, which also has been used for the extraction of important bioactive compounds. In recent years, many researchers have focused on medicinal plants derived from natural products due to their wide range of pharmacological significance **[1]** Pharmacognostical standardization is the study of physical, chemical and biological properties of natural drugs, drug substances or potential drugs through prescribed standards, constant parameters, definitive qualitative and quantitative values that carry an assurance of quality, efficacy, safety and reproducibility **[2, 3]**. A major contributor to pharmacognosy is the discipline of phytochemistry. Phytochemistry is the study of phytochemicals, which are chemicals derived from plants. The research on phytochemicals and use of phytochemicals is increasing more because of the harmful side effects of the synthetic compounds. The compounds contributing to the fundamental metabolism of plants are termed as primary metabolites. In contrast, secondary metabolites are derived from primary metabolites and do not have fundamental role in maintenance of plant life processes **[4, 5]**. They are divided into three large categories namely alkaloids, terpenes and phenolics **[6]**.

Inflammation is an evolutionarily conserved process of protection and a critical survival mechanism. It is composed of complex sequential changes in the tissue to eliminate the initial cause of the cell injury, which may have been caused by infectious agents or some other substances. Steroidal and non- steroidal anti-inflammatory drugs are currently the most widely used despite their renal and gastric negatively secondary effects. These drugs block COX-1and COX-2 enzyme activity. COX enzyme assists with prostaglandin production. Now there is a need for the new safe potent, nontoxic or less toxicant-inflammatory drugs.

The plant selected for the present study *Premna serratifolia*, belonging to the family Lamiaceae (Mint family) is a small tree or shrub. *Premna* is derived from the Greek “premnon” (stump of a tree), referring to the dwarf stature and *serratifolia* means margins of foliage are “toothed like a saw”. It is commonly known as “Headache tree” in English and “Kozhichedi”, “Munja” in Malayalam.

Premna serratifolia L. (Lamiaceae) is an important medicinal tree found in various part of the world. In herbal medicines root of the plant was used as a key ingredient. The roots have various medicinal

properties and used for the treatment of diabetes, chyluria, inflammation, swelling, headache, bronchitis, liver complication and fever. Pharmacological studies of *P. serratifolia* confirmed its anti-inflammatory, antimicrobial, hepatoprotective, antioxidant and antidiabetic activities **[7]**. There is a great demand for this plant in the pharmaceutical industry, since it is one of the key ingredients of many Ayurvedic preparations like Arishtam, Avaleham, Kwatham, Ghritham and Thailam. However, it has been observed from the existing literature that this plant has not been much explored in the context of scientific research. Very few phytochemical aspects have been carried out in this plant. Scarce information is available about biological potential of *P. serratifolia*. Exploration of chemical constituents is required for establishing mechanisms of action of possible markers and structure-activity-relationship among the constituents present in the plant which will give the basis for its therapeutic use. So, more research studies focusing on the phytochemical aspects needs to be carried out to explore the true potential of the plant.

The aim of the present work was a systematic study on the quality control parameters, chemical composition and biological activity to ascertain and validate the ethno-medicinal and pharmaceutical potential of *Premna serratifolia*.

MATERIALS AND METHODS

Plant material

The plant selected for the present study, *Premna serratifolia* was collected from Karunagapally, Kollam. The aerial parts of the plant were used for the study.

I. POWDER ANALYSIS

Fresh plants of *Premna serratifolia* were collected in a polythene bag. Dirt was removed from the collected material. The aerial parts were shade dried and then powdered in an electric grinder and sieved with fine mesh sieve. The powder was then used for the organoleptic study and solvent extraction.

A. Organoleptic study

Organoleptic refers to the evaluation by means of the organs of sense and includes the macroscopic appearance of the plant material, its colour, odour, and taste, and the feel of the powder to the touch **[8]**. The plant powder characteristics like the colour, odour, taste and nature were evaluated.

B. Fluorescence analysis

The crude drug powder was treated as such with seven different reagents. Each solution was loaded on an activated thin gel layer slide and the fluorescence under normal light, short UV (256 nm) and long UV (365 nm) was observed **[9]**.

C. Physicochemical characterization

Different physico-chemical parameters were determined according to

the official methods and guidelines on quality control for medicinal plant materials.

1. Loss on drying **[10]**

2. Foreign matter **[11]**

3. pH **[12]**

4. Swelling index **[13]**

5. Foaming index **[13]**

II. PHYTOCHEMICAL SCREENING

A. Preparation and yield of extract [11]

About 15 g of the powdered plant material was subjected to extraction by Soxhlet apparatus using 100 ml methanol. The extract was concentrated under reduced pressure and preserved in refrigerator until further use. The percentage of the crude extract was determined using the following equation.

Percentage yield (%) = (Weight of the crude extract) / (Weight of the sample) ×100

B. Qualitative analysis

Different phytochemical constituents were tested using standard biochemical procedures **[14]**. The different compounds tested are given below:

Tannins	Saponins
Flavonoids	Alkaloids
Terpenoids	
Phlobatannins	Glycosides
Simple phenolics	
Coumarins	Quinones
Acids	Flavanols
Lignin	Steroids
Gums and Mucilage	

C. QUANTITATIVE ANALYSIS

1. Determination of Alkaloids **[14]**

2. Determination of phenols **[15]**

D. BIOACTIVITY STUDY: ANTI-INFLAMMATORY ACTIVITY

Inhibition albumin denaturation [16]

The reaction mixture consisted of test extract and 1% aqua solution of bovine albumin fraction. The pH of the reaction mixture was adjusted using small amounts of 1N HCl. The sample extract was incubated at 37℃ for 20 min and then heated at 51℃ for 20 min. Aspirin was used as a standard. Percent of inhibition of protein denaturation was calculated.

Percent inhibition (%) = [{Abs control-Abs sample}/Abs control] ×100

RESULTS

I. POWDER ANALYSIS

A. Organoleptic study

Colour: Brownish green

Smell: Pungent

Taste: Bitter

Texture: Fine

B. Fluorescence analysis

The dry powder was subjected to fluorescence analysis with different reagents in normal light, short UV and long UV. The colour changes observed are summarized **(Table 1)**.

C. Physicochemical characterization (Table 3)

Table 1: Fluorescence analysis of *Premna serratifolia*

Powder + Reagent	Visible (400-800 nm)	UV short (256 nm)	UV long (365 nm)
Powder as such	Green	Green	Green
Powder + 1N NaOH	Brown	Brown	Green
Powder +1N HCl	Brown	Brown	Black
Powder + 50% KOH	Green	Green	Brown
Powder + 1N NaOH in MeOH	Brown	Brown	Black
Powder + Conc. H_2SO_4	Green	Green	Green
Powder + Conc. HNO_3	Yellow	Green	Black
Powder + Iodine water	Green	Yellow	Yellow

A total of five physicochemical parameters were evaluated in *Premna serratifolia* **(Table 2)**. The plant moisture content was reported in low amounts. Foaming index was found to be more than 100units and no considerable swelling was observed. The pH indicated the acidic nature of the plant. Foreign matter was found to be in low amounts.

Table 2: Physico-chemical characters of *Premna serratifolia*

PARAMETERS	VALUES
Loss on drying	9.36 %
Foreign matter	2.8%
pH	5.06
Swelling index	NIL
Foaming index	>100 ml

II. PHYTOCHEMICAL SCREENING

A. Yield of extract

The methanol extract was prepared by Soxhlet extraction. The yield of the extract was 3.8%.

B. Qualitative analysis

A total of 15 phytochemicals were qualitatively analysed in methanol extract of the plant. Most of the compounds were present in the extract. Phlobatannins, gums and mucilage and quinones were absent in the plant extract. The results of the phytochemical screening tests are provided **(Table 3 and Plate 2)**.

C. Quantitative analysis

The quantitative analysis of two phytochemicals were conducted in the methanol extract of *Premna serratifolia* **(Table 4)** by standard procedures. The amount of phenols was found to be higher when compared with alkaloids.

D. BIOACTIVITY STUDY: ANTI-INFLAMMATORY ACTIVITY

Albumin denaturation assay

Protein degradation is considered to be the cause of inflammation and this assay reflects the ability of the extracts to block protein denaturation. The methanol extract of *Premna serratifolia* was effective in inhibiting heat induced albumin denaturation **(Table 5)**. The IC_{50} value

(calculated by Probit analysis) of the methanol extract of *Premna serratifolia* was 19.58 µg/ml which was comparable to the standard Aspirin (IC_{50} value 18.92 µg/ml).

Table 3: Phytochemicals tested in *Premna serratifolia*

Sl. No.	PHYTOCHEMICALS	PRESENT/ABSENT
1.	Tannins	+
2.	Saponins	+
3.	Flavonoids	+
4.	Alkaloids	+
5.	Terpenoids	+
6.	Phlobatannins	-
7.	Glycosides	+
8.	Simple Phenolics	+
9.	Coumarins	+
10.	Quinones	-
11.	Acids	+
12.	Flavanols	+
13.	Lignin	+
14.	Steroids	+
15.	Gums and mucilage	-

Table 4: Quantitative analysis of *Premna serratifolia*

Sl. No.	PHYTOCHEMICALS	AMOUNT (mg/g)
1.	Alkaloids	24.04
2.	Phenols	68.41

Table 5: Anti-inflammatory assay in *Premna serratifolia*

Sl. No.	Concentration of extract (µg/ml)	Standard Aspirin (% of inhibition)	Test Extract (% of inhibition)
1	20	56	52
2	40	77	69
3	60	91	85
4	80	98	94

DISCUSSION

The pharmacognostic techniques used for standardization of crude plant drugs involve morphological or organoleptic, microscopic, physical, chemical, chromatographic, spectrophotometric and biological evaluations **[17]**. The organoleptic investigations (colour, odour, taste, texture and nature) were performed and tabulated. *Premna serratifolia* plant powder has brownish green colour, pungent smell, bitter in taste and fine texture. Fluorescence analysis can be used for the identification of the drug and for checking adulteration **[18]**. In the present study, the fluorescence analysis of *Premna serratifolia* plant powder was analysed under visible light and UV light **(Table 1)**. The fluorescence analysis is adequately sensitive and enables the precise and accurate determination over a satisfactory concentration range without several time-consuming dilution steps prior to analysis of pharmaceutical samples **[19]**.

The physical or physicochemical parameters are useful in establishing quality profile of a crude drug and constitute an important component of qualitative

evaluation **[17]**. They help in detecting adulteration or improper handling of drugs **[18]**. The physico-chemical parameters in *Premna serratifolia* were analysed **(Table 2)**. Low moisture content (<14%) is preferable for an herbal drug. It prevents the decomposition of crude drugs due to chemical change or microbial contamination. It discourages the growth of microbes such as mites, bacteria, fungi or yeast **[18]**. The moisture content of *Premna serratifolia* plant was 9.36% **(Table 2)** which suggests that the plant could be recommended for drug preparation. Herbal drugs should be free from moulds, insects and other animal contamination. The permissible limit of foreign matter is not more than 2%. 2.8% of foreign matter was observed in the plant powder of *Premna serratifolia* **(Table 2)**. The pH of 5% aqueous solution of the plant was 5.06. The acidic nature of the powder may be due to presence of acidic salts **(Table 2)**. The swelling index is the volume in millilitres occupied by 1 gram of a drug, including any adhering mucilage, after it has swollen in an aqueous liquid for 4 h. The swelling index was absent in the plant suggesting the absence of gums and mucilage. The foaming index of was found to be > 100 ml indicating the presence of saponins in *Premna serratifolia.*

Phytochemical screening is the scientific process of analyzing, examining, extracting, experimenting, and thus identifying different classes of phytoconstituents present in various parts of the base for the discovery of drugs, the active components could be further taken for investigation and research. The purity of the crude drug is ascertained by quantitative estimation of active chemical constituents present in them **[17]**. In the present study, the powdered material of *Premna serratifolia* were subjected to Soxhlet extraction using methanol. The yield of the concentrated extract was found to be 3.8 %.

Plants synthesize a wide range of chemical compounds which are classified based on their chemical class, biosynthetic origin and functional groups into primary and secondary metabolites. Plant-produced chemical compounds or phytochemicals like alkaloids, glycosides, flavonoids, volatile oils, tannins, and resins have been used in a wide range of commercial and industrial applications such as flavors, aromas and fragrances, enzymes, preservatives, cosmetics, bio-based fuels and plastics, natural pigments and bioactive compounds. The research on phytochemicals and use of phytochemicals is increasing more because of the harmful side effects of the synthetic compounds. The preliminary phytochemical screening detects the presence or absence of various compounds in the plant by specific coloured products produced in reaction to the addition of specific chemicals **[17]**.

The methanolic extract of *Premna serratifolia* was subjected to preliminary phytochemical screening. The chemicals present were tannins, saponins, flavonoids, terpenoids, alkaloids, glycosides, simple phenolics, coumarins, acids, lignin, steroid and flavanols **(Table 3)**. Quantitative estimation of alkaloids and phenols were carried out using standard procedures. The compounds were found to be in considerable amounts **(Table 4)**.

Analysis of the bioactivity test results indicated that *Premna serratifolia* have strong anti-inflammatory potential. In this study, whole plant material was used. The methanol extract was screened for anti-inflammatory activity using albumin denaturation assay. Number of anti-inflammatory drugs are known to inhibit the denaturation of proteins. Mizushima and Kobayashi have employed protein denaturation as in vitro screening model for anti-inflammatory compounds. Inhibition albumin denaturation is most widely employed method for estimating anti-inflammatory activity. The main function of anti-inflammatory agent is to inhibit the function of cycloxygenase (COX) enzyme that is responsible for conversion of arachidonic acid to prostaglandin (PG).

Protein denaturation is a process in which proteins lose their secondary and tertiary upon the application of extraneous agents, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured **[20]**. Denaturation of proteins is the cause of inflammation and arthritic diseases. Production of auto antigens in certain arthritic diseases may be due to denaturation of proteins in vivo **[21]**. The results of anti-inflammatory study indicated that the extract of *Premna serratifolia* was potentially active and comparable to Aspirin, so it could be used as a potent source for anti-inflammatory drugs. The study clearly demonstrates that the methanolic extracts of *Premna serratifolia* effectively inhibited the denaturation of BSA in vitro, in a manner comparable to the reference anti-inflammatory drug, aspirin. The results suggest that important anti-inflammatory drugs can be isolated from the plant and administered for inflammation after several pre-clinical and clinical trials.

CONCLUSION

Traditional knowledge regarding the usage of *Premna serratifolia* are many but the scientific research available today to support this knowledge is limited. The data obtained in the present work will be useful in the synthesis of new drugs of pharmaceutical importance. The anti-inflammatory activity of the plant may be due to the synergistic action of the phytochemicals present in the methanol extract. These findings provide scientific

evidence to support traditional medicinal uses of *Premna serratifolia* and indicate a promising potential for the development of lead compounds from the plant.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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