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# PHARMACOGNOSTICAL, PHYTOCHEMICAL, AND BIOACTIVITY STUDIES OF PREMNA INTEGRIFOLIA L: EXPLORING MEDICINAL PROPERTIES

**KAROO LAL MEENA**

**ASSISTANT PROFESSOR ,DEPARTMENT OF BOTANY**

**GOVT COLLEGE KARAULI**

## ABSTRACT

*Premna integrifolia L. is an important medicinal plant with potentially healing properties. The current investigation was centred on the use of the root bark of P. integrifolia L. for the purpose of pharmacognostical and phytochemical analysis. The procedure of establishing the validity of P. integrifolia L. root bark in both its crude form and formulations that incorporate it as a powdered component is simplified by the use of pharmacognostical techniques, which are of great assistance in this investigation. Premna integrifolia Linn., a remarkable woody medicinal plant that belongs to the Verbenaceae family, has been used extensively in three different medical systems: Ayurvedic, Siddha, and Unani. The purpose of this study is to give a complete overview of the facts on the phytochemistry of P. integrifolia, as well as its traditional applications and ecological biodiversity. The phrases "Premna integrifolia," "ecological biodiversity," "traditional applications," and "phytoconstituents of P. integrifolia" were used to search through a number of web sites in order to get information on the plant. The following types of sources were also consulted: journals, pharmacopoeias, Ayurvedic literature, Indian classical texts, and other academic publications. In relation to the botanical origins of Agnimanthā, there exists a significant disparity amongst the three Ayurvedic Formularies of India that have been successfully published.*

*Keyword's: Premna integrifolia L., phytochemical, pharmacognostical*

## INTRODUCTION

Over the course of more than half a century from the first introduction of medical treatment, phytochemicals have developed into an essential component of several medications. To be more specific, one hundred percent of the pharmaceuticals that were authorised between the years 1940 and 2002 were either natural goods or were developed with the use of data collected from natural things. The Siddha school of medicine, homoeopathy, and ayurveda are all examples of ancient medical techniques that are practiced in India. A significant portion of India's culture has a long history of making use of traditional medicine. Within each of these systems, plants are the primary source of the medicinal compounds that are used. Because of this, a sizeable portion of the pharmaceutical medications that are used today are derived from or manufactured from plants that are used for therapeutic purposes. The plant known as Premna integrifolia Linn., which is also commonly known as Araṇī or Agnimantha, plays a significant role in the formulation of "Daśamūla," a ten-herb combination that is used in Indian medicine. On a large scale, this combination is used to treat a considerable number of different illnesses. When it comes to the botanical origins of Agnimantha, each of the

three Ayurvedic Formularies of India (AFIs) that have been published has been found to have distinctive characteristics. In the first edition (Part I), the genuine botanical source is referred to as Clerodendrum phlomidis Linn.f. However, Premna integrifolia Linn. and Premna mucronata Roxb. are taken into consideration as potential alternatives. On the other hand, the second edition of Part I identifies P. integrifolia as the genuine plant source. However, P. mucronata and C. phlomidis are also recognised as potential alternatives for this plant. The true Agnimantha, on the other hand, is recognised as C. phlomidis Linn.f. in Part II of the first edition of AFI. Premna obtusifolia R. Br. and P. mucronata Roxb. are mentioned as alternatives for this species. It is reported that both of these plants are considered to be viable substitutes to the original material. As a result of the fact that the cause for this variation is not provided, it is not evident why there is such a variance in the listing of botanical sources for Agnimantha. There are several Ayurvedic remedies that include its root extract as an active component. These treatments include Ariṣṭam, Avaleham, Kvātham, Ghṛtam, and Tailam. Over the course of the Vedic period, the stem and sticks of the P. integrifolia plant were used for the purpose of generating fire. It was believed that this plant had a great deal of significance. The purpose of this study is to conduct an in-depth investigation on the phytochemistry, traditional use, and ecological biodiversity of P. integrifolia.

**Pharmacognostical**

The study of pharmacognosy, often known as the study of crude medicines, focuses on medicines that are acquired from natural sources. These medicines include medicinal plants, animals, fungus, and other life things that are derived from plants and animals. Pharmacognosy is defined as "the study of the physical, chemical, biochemical, and biological properties of drugs, drug substances, or potential drugs or drug substances of natural origin as well as the search for new drugs from natural sources." This definition comes from the American Society of Pharmacognosy. In line with the definition that was supplied by the American Society of Pharmacognosy, this term is presented here.
When it comes to screening commercial variants, replacements, adulterants, and any other quality control procedures that may be applied to medicines, the pharmacogenetic assessment is a beneficial tool that may be employed. This evaluation evaluates the pharmaceuticals in order to identify any potential issues. Assisting in the process of gathering information on the biochemical and physical characteristics of crude medicine, the equipment is straightforward and reliable, and it is utilised to assist in the process.

**Phytochemical**

Compounds known as phytochemicals are derived from plants as their biological source. Phytochemicals are compounds that are produced by plants via either their primary or secondary metabolism. Phytochemicals are also known as phytochemical enzymes. From the Greek term phyto, which literally translates to "plant," The vast majority of the time, they are responsible for the biological activity that takes place inside the plant host. This activity not only helps the plant grow, but it also helps the plant defend itself against other organisms, such as competitors, diseases, or predators.

Plants are the source of phytochemicals, which are chemical substances that they produce. The primary purpose of these substances is to aid plants in warding off diseases that are brought on by fungi, bacteria, and viruses that affect plants. In addition, phytochemicals are consumed by a variety of species, including insects. We may trace its origins back to the Greek word φυτόν (phyton), which is a term that signifies "plant." Many

phytochemicals have been utilised in traditional medicine, while others have been utilised as poisons. Some of these phytochemicals have been used.

When referring to plant molecules that are now the focus of research but have not yet been shown to have any impact on health, the term "phytochemicals" is often used. These chemicals are not regarded to be elements that are essential for human health. Regulatory agencies that are responsible for food labelling in both Europe and the United States have made suggestions to the industry in order to limit or prohibit health claims about phytochemicals that are shown on food product or nutrition labels. These recommendations have been made in order to protect consumers from potential health risks.

**Premna integrifolia Linn.**

There is an important woody medicinal plant known as Premna integrifolia Linn., which is a member of the family Verbenaceae. This plant has played a vital part in the medical systems of Ayurveda, Siddha, and Unani. It is the goal of this research to present a comprehensive summary of the information that has been gathered on the ecological biodiversity, traditional use, and phytochemistry of P. integrifolia. Several online databases were searched using the terms Premna integrifolia, ecological biodiversity, traditional applications, and phytoconstituents of P. integrifolia in order to acquire information about the plant. This was done in order to gather information about the species. Additionally, pharmacopoeias, magazines, Ayurvedic literature, and Indian classical texts were used in addition to other sources. Naturally occurring substances, especially those that are derived from plants, have been used for the purpose of assisting in the maintenance of human health ever since the beginning of medicine. The practice of traditional medicine has been there since the beginning of time, and over the course of human history, it has gained widespread recognition and been used by the people. Since the beginning of time, people have believed that plants are a wonderful source of various chemicals that have medical properties. Because they have a low number of negative effects and have a positive influence on human health, medicinal products that are derived from plants have been drawing the attention of scientists all over the world for a substantial period of time. This is due to the fact that they have a positive impact on healthcare. Although plants that have a long history of use in ethnomedicine may be a rich source of compounds that can be used to cure a number of ailments and infectious diseases, the pharmaceutical industry may gain from the utilisation of these plants since these plants can be used to treat a wide range of illnesses. It is considered that medicinal plants are a reservoir of these compounds because they contain a large variety of bioactive chemicals that have a wide range of therapeutic properties. These substances may be found in medicinal plants. Anti-inflammatory, antiviral, anticancer, antimalarial, and analgesic properties are only some of the therapeutic advantages that are associated with medicinal plants for their broad range of therapeutic applications.

According to the World Health Organization (WHO), a broad variety of medicines may be derived from a variety of medicinal plants because of their medical properties. To add insult to injury, around eighty percent of the world's population that is still in the process of developing depends on traditional medicines to fulfil their fundamental medical needs. People in India have depended on their own traditional medicines for the treatment of a wide range of illnesses, including fever, malaria, and diarrhoea, for millennia. These cures have been used to maintain their health and treat a number of ailments. There are a great deal of plant resources that may be found in India.

## OBJECTIVE

1. To investigate the phytochemical and pharmacognomic assessment of Premna integrifolia Linn's root bark

2. To investigate the phytochemistry, traditional applications, and biodiversity of Premna integrifolia L

## MATERIALS AND METHODS

The root barks of P. integrifolia were chosen for collection at the Indian Medical Practitioners Co-operative Pharmacy and Stores (IMPCOPS) in Chennai, Tamil Nadu, India. This was the place where the root barks were picked. C. Arunachalam, who works as a Research Officer in Botany at the Captain Srinivasa Murthy Regional Ayurveda Drug Development Institute in Chennai, was the one who verified the authenticity of the root barks. In addition to that, the voucher specimen, which was assigned the number 00641/2014, was sent to the Botany Department. Using a pulvarizer to crush the root bark into powder, washing it under running water from the tap, allowing it to air dry and lastly putting it in a container that was airtight were the steps that were taken to eliminate dust from the root bark.

### Pharmacognostical and Physiochemical Evaluation

A morphological investigation of an anatomical sectioning was performed using powder microscopy, and the procedures that are typically followed were followed. An Olympus BX 51 Fluorescence microscope and a Nickon D 7000 Camera were the tools that were used for the investigations that were carried out. The assessment of the pH, total ash, acid insoluble ash, loss on drying at 105 degrees Celsius, and water-soluble extractives extracted from the powder of P. integrifolia root bark that was carried out.

### Preliminary Phytochemical Screening

In order to extract the root bark powder at a concentration of ten grammes per one hundred millilitres, a procedure known as cold maceration was used. other to the use of petroleum ether as the first solvent, the other solvents that were utilised were chloroform, ethyl acetate, methanol, and water. For a total of twenty-four hours, the extraction procedure was carried out three times without interruption. Following the completion of the extraction procedure, each extract was subjected to a layer of filter paper labelled "Whatman No. 1."
Until the filtrate was fully dry, we evaporated it again and over again. Over the course of the experiment, the polarity of the solvents was gradually increased, and they moved from being non-polar to being polar. Following the isolation of a broad range of compounds with varied degrees of polarity, a screening procedure was carried out in order to identify phytoconstituents contained within the compounds. During the process of successively extracting root bark from P. integrifolia, a number of qualitative analyses were carried out. These analyses included the following: alkaloid, triterpenoid, coumarin, steroid, tannin, saponin, flavonoid, quinone, flavanone, anthocyanin, anthraquinone, phenol, protein, carbohydrate, glycoside, amino acid, acid, and furan. Standard operating procedure was utilised in this instance.

### Making the Root Bark Hydro Alcoholic Extract (HAE)

The HAE was produced by a method known as cold percolation, which was carried out at no higher than room temperature. In order to produce each extract, 1x10 g/100 mL was taken, and the root bark was defatted with petroleum ether, ethanol, and water in the proportions of 50:50, 60:40, and 70:30 (volume/volume) respectively for a period of 72 hours while being shaken regularly. This process was repeated three times. Whatman No. 1 filter paper was used in order to filter these extracts prior to their utilisation throughout the process. Until the filtrate was fully dry, we evaporated it again and over again. In order to conduct an analysis of each extract, both thin-layer chromatography (TLC) and high-performance thin-layer chromatography (HPTLC) were carried out.

**TLC and HPTLC Finger Printing Standardisation**

Using a Linomat applicator, the various ratios of HAE, such as 50:50, 60:40, and 70:30 (volume/volume), were observed on an aluminium plate that had been pre-coated with Silica gel-60TG, F254 and had a thickness of 0.2 millimetres. The plate had been covered with Silica gel. The TLC plate was loaded with three distinct ratios of extracts, each consisting of ten microliters, fifteen microliters, and twenty microliters. Using the solvent system that was composed of toluene, ethyl acetate, and formic acid in the proportions of 40:60:10, a chromatogram was made, and then the chromatogram was dried. At a temperature of 105 degrees Celsius, the plates were heated in an oven that heated hot air until the colour of the dots became visible. In addition, the plates were submerged in a solution that included sulfuric acid and vanillin at a concentration of 5%. Under ultraviolet light at 254 and 366 nanometers, the plates were analysed for their characteristics. On the other hand, the same was placed through a CAMAG HPTLC machine, and the Win cats application was used to record the Rf values as well as the fingerprinting data.

**RESULTS**

P. integrifolia is a shrub that has mature root bark that is totally margined and has a flat, curved, and re-curved appearance respectively. Although it may grow to be up to 12 centimetres in length and up to 5 centimetres in breadth, its thickness can vary from 0.3 to 1 centimetre. Its length can reach up to 12 centimetres. The surface of its exterior is rough and exfoliating, and it has a silvery-gray look from the outside. On the other hand, the surface of its interior is rough, wrinkled, and brownish in pigmentation. It has a surface that is filthy and fragmented, yet it does not smell or taste astringent despite its appearance. Upon examination of the root bark, it was discovered that the outer periderm is made up of thin-walled rectangular cells that are tangentially elongated and include four to six rows of tabular cells. This is then followed by dead phloem, which is made up of collapsed parenchyma cells that are packed with tannin content and embedded with starch grains that range from round to oval in shape, cluster crystals of calcium oxalate, and rosette crystals. Within the inner periderm, there are cells that are lignified and have thin walls. These cells are rectangular in shape and are tangentially elongated. Afterwards, a polygonal parenchymatous cortex with a ring of stone cells that is discontinuous is found in the surrounding tissue. Phloem parenchyma that is non-collapsed, thin-walled, and radially running makes up the inner zone of secondary phloem. Additionally, a group of sclerenchymatous fibres may be seen in specific spots inside this zone. Powder microscopy revealed a dirty grey fractured surface, a fragment of polygonal lignified cork cells in surface view and tangential cells in sectional view, a fragment of tangential longitudinally cut medullary ray associated with fibres, a fragment of parenchyma cells filled with tannin content and a few starch grains, a fragment of fibres having pegged, forked with sharp ends, a few fragments of idioblast cells, stone cells, numerous rosette crystals of calcium oxalate, and a few starch grains that were round to oval, simple and compound, and had 2-4 components. All of these findings were

discovered through powder microscopy. The use of powder microscopy allowed for the observation of each of these observations.

Every single one of the findings that were discovered during the physiochemical examination of the root bark of P. integrifolia may be found in Table 1. At a temperature of 105 degrees Celsius, the ash content was found to be 21.97%, the acid-insoluble ash was found to be 0.29%, the alcohol-soluble extractive was found to be 10.69%, the water-soluble extractive was found to be 6.98%, the pH was found to be 5.1, and the loss on drying was found to be 10.3%. Phytochemical screening was performed for the goal of activity-guided fractionation, and the results of this screening are shown in Tables 2 and 3. To illustrate the high-performance thin-layer chromatography (HPTLC) profile of high-absorbent ethyl acetate (HAE) in toluene, ethyl acetate, and formic acid (40:60:10) at a volume of 10µL, Table 4 and Figure 5 have been shown. Figure 6-11 provides one with the opportunity to see graphical depictions of HPTLC finger printing in a number of different ratios. The HAE with a ratio of 50:50 (v/v) contains eight compounds when measured with a deuterium lamp at 254 nm. In contrast, the ratio of 60:40 (v/v) has nine compounds, while the ratio of 70:30 (v/v) contains seven compounds. With reference to the wavelength of 366 nm, the different ratios of HAE, which are 10µL at 50:50 (v/v), contain ten compounds, sixty: forty (v/v) contains ten compounds, and seventy-thirty (v/v) contains eight compounds according to scanner 3. A blue hue is produced by the Camag HPTLC gadget when it is seen via the lens of a mercury lamp.

## DISCUSSION

In the process of standardizing herbal composition, the first and most significant stage is the authentication technique known as the macro-microscopic approach. This is the stage that includes the most critical steps. Both P. serrati folia L. and C. phlomidis L. are considered to be two separate species in the field of botany; yet, they are often referred to as Agnimantha due to the fact that they share resemblance. It was observed that the macroscopic and microscopic features of the dried root bark of P. integrifolia L. would be valuable in verifying the botanical identity of the plant even when it is in its dried form. This was the case during the process of confirming the identification of the plant. In spite of the fact that taxonomic identification makes it easy to identify plants while they are still in their fresh form, it is much more difficult to do so once they have been dried. This is due to the fact that numerous properties of plant components change as they dry. It is possible that the morphology and microscopic examination of plant medicines will be of assistance in detecting the botanical source of the medication in the event that these pharmaceuticals are bought from the market in their unprocessed condition.

**Table 1: Calculating Physiochemical Parameter Estimates**

| S. No. | Parameters | Results |
|---|---|---|
| 1. | pH | 5.1 |
| 2. | Ash Value (%) | 21.97 |
| 3. | Acid-insoluble Ash (%) | 0.292 |

| | | |
|---|---|---|
| 4. | Alcohol-soluble Extractive (%) | 10.69 |
| 5. | Water-soluble Extractive (%) | 6.98 |
| 6. | Loss on Drying at 105°C (%) | 10.30 |

[Values are mean ± SD (n=3)]

**Table 2: Screening for Phytochemicals in Subsequent Extracts**

| S. No. | Phytochemical | Petroleum Ether | Chloroform | Ethyl Acetate | Methanol | Ethanol | Water |
|---|---|---|---|---|---|---|---|
| 1. | Alkaloids | - | - | - | + | + | + |
| 2. | Triterpenoids | - | - | - | + | + | _ |
| 3. | Coumarins | - | - | + | + | + | _ |
| 4. | Steroids | + | + | + | + | + | + |
| 5. | Tannins | - | - | - | + | + | + |
| 6. | Saponins | - | - | - | + | + | + |
| 7. | Flavonoids | - | - | - | + | + | + |
| 8. | Quinones | - | + | + | + | + | + |
| 9. | Flavanones | - | + | + | + | + | + |
| 10. | Anthocyanins | - | + | + | + | + | + |
| 11. | Anthoquinones | - | - | - | - | - | _ |
| 12. | Phenols | - | - | + | + | + | + |
| 13. | Proteins | - | - | + | - | - | _ |
| 14. | Carbohydrates | - | - | + | + | + | _ |
| 15. | Glycosides | - | - | + | + | + | _ |
| 16. | Amino Acids | - | - | - | - | + | _ |
| 17. | Acid | + | - | - | - | - | + |
| 18. | Furan | - | - | - | + | + | + |

**Table 3: HAE's Phytochemical Analysis and Screening**

| S. No. | Phytochemical | 50:50 v/v | 60:40 v/v | 70:30 v/v |
|---|---|---|---|---|
| 1. | Alkaloids | + | + | + |
| 2. | Triterpenoids | + | + | + |
| 3. | Coumarins | + | + | + |
| 4. | Steroids | + | + | + |
| 5. | Tannins | + | + | + |
| 6. | Saponins | + | + | + |
| 7. | Flavonoids | + | + | + |
| 8. | Quinones | + | + | + |
| 9. | Flavanones | + | + | + |
| 10. | Anthocyanins | + | + | + |
| 11. | Anthoquinones | - | - | - |
| 12. | Phenols | + | + | + |
| 13. | Proteins | - | - | - |
| 14. | Carbohydrates | + | + | + |
| 15. | Glycosides | + | + | + |
| 16. | Amino Acids | + | + | + |
| 17. | Acid | - | - | - |
| 18. | Furan | + | + | + |

**Table 4: Different ratios of HPTLC fingerprinting were performed of HAE - 10µl**

| S. No | Mobile phase: Toluene: ethyl acetate: formic acid(40:60:10) | | |
|---|---|---|---|
| | | 254 nm | 366 nm |
| 1. | HAE-50:50 v/v | 7 | 7 |

| | | | |
|---|---|---|---|
| 2. | HAE-60:40 v/v | 9 | 8 |
| 3. | HAE-70:30 v/v | 7 | 8 |

**Fig 1: Image of Pulmonaria integrifolia**

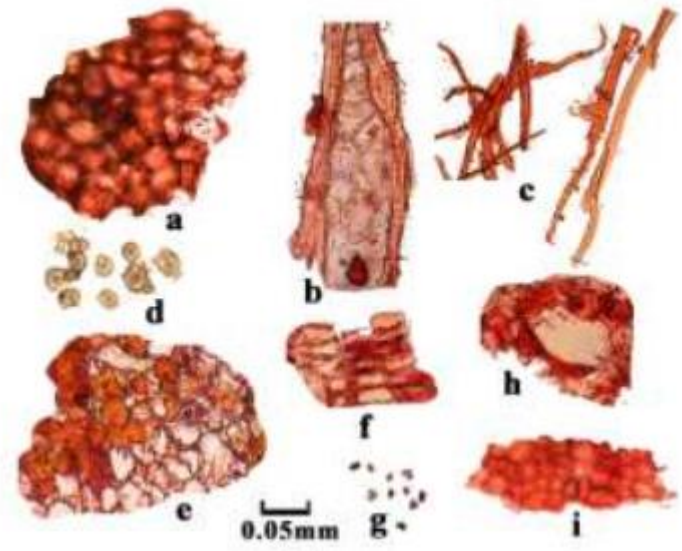


**Fig 4: An examination of P. integrifolia L. powder microscopy a. cork cells in surface view; b. tangentially cut medullary ray associated with fibres c. fibres d. rosette crystals of calcium oxalate e. parenchyma with tannin content f. lignified cork in sectional view g. starch grains h. idioblast cell i. stone cells**

The root bark powder of P. integrifolia has a pH value that is somewhat acidic owing to the natural composition of the powder. Following the completion of the study, it was found that the percentage of total ash, acid insoluble ash, loss via drying at 105 degrees Celsius, water soluble extractive, and alcohol soluble

extractive was found to be negligible. Following the alcoholic extract, it was discovered that steroids were contained in the extracts of petroleum ether. The alcoholic extract came in second place. Quinones, flavanones, and anthocyanins were found to be present in extractives that ranged from chloroform that contained alcohol to alcohol itself. Coumarins, phenols, polysaccharides, and glycosides were discovered to be present in the extracts that were obtained by converting ethyl acetate to alcohol. Alcoholic extracts were the only ones that contained the triterpenoids, alkaloids, tannins, and furan that were discovered in the plant. A number of the components, such as triterpenoids, coumarins, proteins, carbohydrates, glycosides, and amino acids, were not discovered in the aqueous extracts; however, all of the other phytochemicals were present in the extracts. Hydroalcoholic extracts included all phytoconstituents in varied proportions (50:50, 60:40, and 70:30 v/v), with the exception of anthoquinones, proteins, and acids. These ratios were observed during the course of the experiment. The findings of the HPTLC analysis showed that hydroalcoholic extracts with a volume-to-volume ratio of sixty percent to forty percent included a bigger quantity of constituents. According to Ayurvedic medicine, the root barks of P. integrifolia are utilised in the production of Dasamula medication, which is subsequently utilised to treat a wide range of illnesses. By using the components that are present in the root bark, it is likely that researchers will be able to discover the mechanism of action that is associated with that plant.

## CONCLUSION

The present review reveals a wide range of properties, including those that are analgesic and antinociceptive, antibacterial, anticancer, antitumor, cytotoxic, antimicrobial, anti-obesity, hypolipidemic, antioxidant, antiparasitic, antiulcer, gastroprotective, cardioprotective, hepatoprotective, hypoglycemic, immunomodulatory, and neuroprotective. The principal extractive solvents that are used in the investigation of the pharmacological properties of P. integrifolia are methanol and ethanol, according to the findings of the research investigation. The phytoconstituents that are present in this solvent are often in high concentrations. These phytoconstituents include phenols, flavonoids, amino acids, vitamins, carbohydrates, and a variety of other substances. Among the possible explanations is the fact that the extracts include the phytocompounds that are accountable for the effects. The leaves and roots of the plant were the primary areas of concentration for the researchers as they investigated the pharmacological qualities of the plant. The pharmacological effects of the different sections of the P. integrifolia plant are discussed in this website, which is an excellent resource for learning about these effects. An investigation into the biological activity of the active components of P. integrifolia, an investigation into the clinical safety of the plant, and an investigation into the traditional use of the plant are all possible directions for additional study. If the data presented here is able to assist in the establishment of research methods for the creation of contemporary pharmaceuticals and Ayurvedic formulations for the treatment and cure of a broad variety of ailments, then it may show promise as a new resource for the advancement of pharmaceutical innovation.

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