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# PHYTOCHEMICAL INVESTIGATION AND STRUCTURE ELUCIDATION OF BIOACTIVE MOLECULES OBTAINED FROM PREMNA SERRATIFOLIA (ARNI)FOR THEIR ANTI ARTHRITIC ACTIVITY

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## Introduction

Medicinal plants plays very significant part in the primary healthcare system of India . Most of the people living in tribal area ,are aware of the medicinal properties of the plants and they are using the parts of plants for the treatment of their health issues. Plant Arni (Premna Serratifolia) has been used for the treatment of arthritis in many regions of India, Mostly it is found in the forest of southern India and West Bengal also it recoreded its occurring in Satpura Plateau .Phytochemical present in the plant Arni shown to be beneficial for the prevention of arthritis as well as for well long term health.

This is the source of essential chemical compounds such as lignans, flavonoids, terpenoids, and steroids, irridiod glycosides , alkolides, phenolic compounds.

Few studies were reported to validate the ethno-pharmacological claim regarding the antiarthritic and anti-inflammatory properties of Premna serratifolia. Different parts of P. serratifolia including fruit, roots, barks, and leaves have been used in folk medicine for the treatment of a number of illnesses, such as stomach disorders, diabetes, cough, rheumatism, inflammatory, and cardiovascular disorder . Pharmacological studies conducted so far confirm on the ethnomedicinal uses of P. serratifolia including antioxidant, antiarthritis, antiparasitic, and cardio- and gastroprotective activities . Studies conducted on P. serratifolia mainly involve roots, barks, woods, and stems from the plant. Only few papers have been published regarding the pharmacological properties of the leaves of P. serratifolia, such as tumor cell suppression activity cytotoxic activities on neuroblastoma and melanoma cell lines , and anti-inflammatory and anticancer activities using animal model .In present study we are attempting to identify the phytochemical constituent and its structural analysis of the bio-active molecules obtained from Premna Serratifolia. Analysis of chemical compound for their anti-arthritic activity is the aim of this study. Chromatography and Spectroscopy techniques used to separate , identify the structure of chemical compounds obtained from the plant Premna Serratifolia.

In past study phytochemical investigations on the Premna Serratifolia reveals the isolation and structural identification of number of iridoid glycosides.This type of compounds have important role for the treatment of many mankind diseases, antiarthritic activity also observed along with antidiabetic,neuroprotective, nerve growth factor-potentiating,and hepatocurative activities.

## Literature Review

Bioactivity guided fractionation of Premna serratifolia leaves succeeded into isolation of two terpenoids and one steroid compound with significant cytotoxic activity. Here we report the isolation of these cytotoxic terpenoids/steroids from this plant for the first time which could be developed as anticancer agents. Mahesh Biradi and Kiran Kumar Hullatti (2017) . Premna integrifolia is an important constituent of famous herbal formulation “Dashmula” of Indian Ayurvedic system of medicines. The plant is known to possess hypoglycaemic, anti-inflammatory, antiarthritic and broad-spectrum antimicrobial activities due to the presence of several diterpenoids and spermine alkaloids in its decoction. In order to develop chemical markers for quality assurance of this herb in Ayurvedic

formulation, we report here the isolation of three novel diterpenoids from the root bark of *P. integrifolia* namely 1β,3α,8β-trihydroxy-pimara-15-ene (**1**), 6α,11,12,16-tetrahydroxy-7-oxo-abieta-8,11,13-triene (**2**) and 2α,19-dihydroxy-pimara-7,15-diene (**3**). 1,3-Dihydroxy and 2-hydroxy diterpenes belong to a limited number of families and their isolation is also interesting from chemotaxonomic point of view. These diterpenoids were also evaluated for antibacterial activity. Deepti Yadav,Neerja Tiwari,Madan M.Gupta (2010) . Three new lignoids, premnan A (1), premnan B (2), and tauntangyiol C (3), were isolated from Premna serratifolia wood, a traditional cosmetic plant in Myanmar, together with a new lignoid, premnan C (4) assumed to be an artifact, one natural new lignoid (5), and three known lignoids (6–8). The structures of the new compounds 1–4 were elucidated based on 1D and 2D NMR, IR spectroscopy, and HRESIMS. The absolute configurations of 1–4 were also determined by optical rotation, circular dichroism (CD) data analyses, and comparisons with the reported literature. So-Yeun Woo,Shotaro Hoshino (2019)Flavonoids can act as free radical scavengers and terminates radical chain reaction that occurs during oxidation of triglycerides in the food system. The quantitative estimation of phenols, terpenoids and flavonoids in P. serratifolia gives an insight into their phytochemical repository which can provide a rich data in understanding the basic pattern of growth and metabolism. At the same time phenols, terpenoids and flavonoids can be used as chemical markers in taxonomic studies. Agarwal M, Agarwal Y, Ltankar P, Patil A, Vyas J, Ketkar A(Phytochemical & HPTLC Studies of Various Extracts of Annonasquamosa (Annonaceae) Int pharm Tech Res, 2012 . Further investigations are required to transform the experience-based claims on the traditional uses of Premna species into evidence-based information. The present knowledge obtained mainly from experimental studies was critically assessed to provide evidence and justification for their traditional uses to propose future research prospects for this plant. Phytochemical studies on Premna species have led to characterization of diterpenoids, iridoid glycosides, and flavonoids as the charactetistic chemical composition of the genus. The in vitro and in vivo evaluation of biological properties of the extracts and isolates from various species of Premna on antimicrobial, antioxidant, anti-inflammatory, immunomodulatory, cytotoxic, antihyperglycaemic, and other activities should lead to further detailed investigations to identify the bioactive compounds and their mechanisms of action. The antimalarial, hepatoprotective, cardioprotective and gastroprotective effects of the plant extracts should encourage further studies on these plants for use as preventive agents. Toxicological evaluation should be conducted to address

any adverse side effects which may occur. The roles and mechanisms of the bioactive compounds should be addressed appropriately to understand the contribution of individual compound to the activities as well as to become potential lead molecules for development into drug candidates. Attempts should be made to carry out more preclinical studies of the standardized extracts and bioactive compounds of Premna species, which include determination of modes or mechanisms of action in different animal models, bioavailability, pharmacokinetics and toxicological studies before submission of potential candidates to serious randomized human trials is possible. As more scientific evidences on therapeutic effects are discovered, Premna species will be recognized as a valuable source of drug leads and pharmaceuticals. Roza Dianita & Ibrahim Jantan ,2017

## Materials and Methods

Plant collection & sample extraction

Healthy plants of Arni (Premna Serratifolia L.) collected from the BOTANICAL GARDEN BHOPAL MadhyaPradesh. This plant generally found in the forest of Sheopur ,Neemuch , Mandsour & South-West forest of Betul District in Madhyapradesh .Sample extracted for the phytochemical screening prepared by using Soxhlet extraction method.This process is otherwise known as continuous hot extraction. The apparatus is called Soxhlet extractor made up of glass. It consists of a round bottom flask, extraction chamber, siphon tube, and condenser at the top. A dried, grinded, and finely powdered plant material is placed inside porous bag (thimble) made up of a clean cloth or strong filter paper and tightly closed. The extraction solvent is poured into the bottom flask, followed by the thimble into the extraction chamber. The solvent is then heated from the bottom flask, evaporates, and passes through the condenser where it condenses and flow down to the extraction chamber and extracts the drug by coming in contact. Consequently, when the level of solvent in the extraction chamber reaches the top of the siphon, the solvent and the extracted plant material flow back to the flask. The entire process continues repeatedly until the drug is completely extracted, a point when a solvent flowing from extraction chamber does not leave any residue behind. This method is suitable for plant material that is partially soluble in the chosen solvent and for plant materials with insoluble impurities.

## Mechanisms of separation in chromatography

### TLC . (THIN LAYER CHROMATOGRAPHY)

This technique also involves the use of adsorption mechanism to separate a compound from a mixture. Separation is based on the interaction between the compounds in a mixture and stationary phase. It is applicable in the separation of compounds with low molecular weight.The stationary phase usually is 100g of silica gel dissolved in distilled water to make a slurry. Meanwhile, in some instances Sephadex is applicable. The solution of silica gel is then poured into a glass plate with dimension 20cm × 20cm to produce a thickness of 1.5mm. It is then kept for 1h at 105°C to solidify. Afterward, 10mL of extract is injected into the lower part of the plate and allowed to spread. The plate is then carefully inserted into the separation chamber containing mobile phase and allowed to stand for 30min. The compounds contained in the mixture will ascend to various positions on the plate based on their solubility. Each compound separated is identified by calculating its retardation factor which is the ratio of distance traveled by the compound to the distance traveled by the solvent and compare it with that of a known compound).The compounds spotted are scrapped at different position using spatula and finally re-extracted using various solvents.Advantages include less time-consuming, producing clear spots, and stable to acid as solvent.

### CC. (COLOUM CHROMATOGRAPHY)

It involves the use of several mechanisms such as adsorption chromatography, molecular sieve, and ion exchange to achieve the desired outcome. The column is made up of a long glass tube (5–50mm in diameter, 5 cm–1 m long) with a tap and glass wool filter at the bottom. In addition, silica gel, alumina, cellulose, or Sephadex are used as stationary phase, whereas the mobile phase is liquid. The process begins by packing 30g of silica gel (70/35) into a transparent glass column (80cm long, 5cm diameter) without introducing air bubbles. Subsequently, the extract to be partitioned is added from the top. Least polar solvent (n-hexane) was first added as a mobile phase and allowed to stand for 1h in a closed column. The bottom of the column opened and various fractions of n-hexane collected at an interval. In addition to that, other solvents such as chloroform, ethyl acetate, n-butanol, and methanol added. Fractions of these solvents were collected individually at different time intervals and finally characterized.

HPLC. (High performance liquid chromatography)

This technique uses the mechanism of adsorption to achieve effective separation. It is suitable for the partitioning of both organic and inorganic compounds. The mobile phase is a suitable solvent, whereas the stationary phase is solid particles tightly joined together. Separation is initiated via interaction of the compounds in the mixture with the solid particle of the stationary phase.The apparatus consists of a solvent reservoir, sample injector, pressure pump, HPLC tube, and diode detector. The process begins by injecting the mixture to be separated at the bottom of HPLC. In addition, a suitable solvent is poured into the solvent reservoir. The tap is now opened to allow the movement of solvent downward, which is then pushed by a pressure pump to mix up with the injected sample. Finally, the mixture moved into the diode detector, which separated the compounds, removed the waste, and pumped the final content to processing units.

## SPECTRAL ANALYSIS ,STRUCTURE ELUCIDATION AND IDENTIFICATION

Mass Spectroscopy(MS).

This method is useful in the identification of compounds based on chemical structure and molecular weight. The aim is to sequence and identify the unknown compound in a mixture. The substances usually identified include oligonucleotides and peptides. The process begins by bombarding an organic molecule with an electron and converts it into very energetic charged ions. The signal was first detected using electron ionization energy of 70eV; also, the sample spectra are detected and recorded as percentage peak. Compounds are identified based on their relative molecular mass and molecular weight. This can be achieved by plotting mass of the fragmented ions against the charges of individual ion. Notably, MS provides abundant information on organic molecules.

Ultraviolet Spectroscopy(UV) and Visible Spectroscopy

Ultraviolet and Visible Spectroscopy The absorption spectra of plant constituents can be measured in very dilute solution against a solvent blank using an automatic recording spectrophotometer. For colourless compounds, measurements are made in the range 200 to 400 nanometres (nm); for coloured compounds, the range is 200 to 700 nrn. This method is suitable for qualitative and quantitative analysis of compounds present in theplant's extract. Various secondary metabolites such as phenols, anthocyanins, tannins, and polymer dyes could be detected at certain frequencies. Total

phenolic content and other secondary metabolites can be established using this technique. Specific frequencies were used to identify flavonoids (320nm), phenolic compounds (280nm), anthocyanins (520nm), and phenolic acids (360nm).

Nuclear Magnetic Resonance (NMR)

This technique pays more attention to the physical properties of the bioactive molecule such as number and array of the carbon atom, presence of isotopes of carbon, hydrogen atom, and protons. It also described how atoms are arranged in a molecule.

Infrared Spectroscopy (IR)

This method tries to assess functional groups present in a compound. Knowledge of the functionalgroup helps in defining the physical and chemical properties of a given compound. Also, single, double, and multiple bonds are identified through this process. The technique involves passing an organic compound through infrared radiation, which is absorbed in certain frequencies. Liquid samples are identified using sodium chloride plates, whereas solids samples are determined using potassium bromide milled together and compressed into a thin pellet. The result is recorded as a spectrum that is percentage transmittance. Lastly, the spectra are analyzed; the peaks obtained at certain wave number are compared with standard reference.

## Conclusion

Study proves that Premna Serratifolia is an important source of phytochemicals , thus used for the treatment of many diseases in mankind .The results of the preliminary phytochemical screening of the extract of Premna serratifolia Linn., revealed the presence of phytoconstituents such as alkaloids, steroids, flavonoids, phenolic compounds, tannins and glycosides specifically iridoid glycosides. Phytochemical obtained from the plant used as anti-arthritic drug economically.

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