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PHYTOCHEMICAL ANALYSIS AND PHARMACEUTICAL ACTIONS OF METHANOLIC EXTRACT OF PREMNA SERRATIFOLIA (ARNI)

Swati Chandel[1]

Research Scholar

Department of Chemistry , Govt. Dr. Shyama Prasad Mukherjee Science and Commerce College

Kolar ,Bhopal (Madhyapradesh)

Dr.Asha Verma[2]

Professor

Department of Chemistry ,Govt. Dr. Shyama Prasad Mukherjee Science and Commerce College

Kolar, Bhopal (Madhyapradesh)

Abstract:

Preliminary screening of phytochemical compounds in Premnaserratifolia revealed the presence of sterols and triterpenes, resin, alkaloids, Flavonoid-luteolin , glycosides, tannins, phenolic compounds, carbohydrates, amino acids and unsaturated aromatic hydrocarbons .Premna serratifolia methanolic extract and petroleum ether extract were tested phytochemically in this instance. The methanolic extract included a variety of phytoconstituents, according to the results. Phytochemical estimation of Premnaserratifoliaconfirmed the existence of alkaloids and carbohydrates, glysoside, saponin, flavonoids and phenol.

Keywords:Premna Serratifolia,Phytochemical,extract

Introduction:

Medicinal plants are a reservoir of biologically active compounds with therapeutic properties that over time have been reported and used by diverse groups of people for treatment of various diseases.Bioactive compounds obtained from the medicinal plant work more significantly if they are properly separated and apply as per the need. The leaves of P. Serratifolia are commonly consumed as food ingredient inl India. Prematured fruits of the plants is popular food ingredient in tribal area.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 disorder , headache,cough ,inflammation.Study on this plant also reveals its use asgastroprotective , anti oxident ,antiarthritis and cardioprotectiveactivity.The present study mainly focussed on the bioactive compounds obtained from the plant PremnaSerratifolia , responsible for the antiarthritic actiivity

Materials and Methods:

a.Plant Material

The entire plant of PremnaSerratifoliawas collected from village Koudiya(Patalkot)District Chindwara Madhya Pradesh India in the period between the month of April and May . is Generally Found in forest at the foothills of the mountains of Satpura range. Height of this plant goes upto 7-8 meter.Flowering occurred in the period of month April and May.The whole plant sample of Premna Serratifolia was dried , later it crushed and powdered form used for the preparation of the extract.

b.Extraction

Soxhlet extraction

Dried and powder of PremnaSerratifolia successively defatted with petroleum ether and then placed in a thimble of Soxhlet apparatus. The extraction was carried out using Methanol solvent system at 40-60^{0}C temperature of the heating mantle for 8-10 hours. After the extraction process, the extract of sample was filtered and concentrated to dryness. Extracts were collected in air tight container (Alara et al., 2019). Extraction yield of all extracts were calculated using the following equation below:

Formula of Percentage yield = Actual yield X 100

c.PhytochemicalScreeening

Qualitative Phytochemical Estimation of Extracts

Detailed phytochemical testing was performed to identify presence or absence of different phytoconstituents in extracts of PremnaSerratifolia using standard procedures (Kokate et al., 2006). The extracts were subjected to following tests:

Tests for carbohydrates:

- **Molisch test:** To 1ml of extract, 2-3 drops of alcoholic α-naphthol solution was added. Conc. sulphuric acid was added along the side of the test tube. The appearance of purple ring at the junction of two liquids was observed, which verifies the existence of carbohydrates in the test samples.

- **Fehling's test**: To 1 ml of extract, similar quantity of Fehling's solution A and B was included and warmed on a water bath for few minutes. The development of brick red precipitate was observed.

- **Benedict's test:** Equal volume of Benedict's reagent and extract were mixed within a test tube and heated in the water bath for 5-10 minutes. Solution displays as red, yellow, or green based on the amount of sugar reduction present in the test solution which revealed the existence of reducing sugar.

- **Barfoed's test:** 1 ml of extract and Barfoed's reagent were mixed in a test tube and heated on water bath for 2 minutes. Red colour due to formation of cupric oxide indicates the presence of monosaccharide.

Test for alkaloids:

Prior to filtering, each test extract was individually treated with dil. hydrochloric acid. The filtrate from each test extract was exposed to the subsequent examinations:

- **Mayer's test:** A few drops of Mayer's reagent were applied around the tube's sides to 2-3 ml of filtrate. Alkaloids are present when a white or creamy precipitate forms.

- **Hager's test:** A couple of drops of Hager's reagent were added to 1-2 milliliters of filtrate in a test tube. The existence of alkaloids is indicated by the formation of a yellow precipitate.

- **Wagner's test:** In a test tube, a few drops of Wagner's reagent were applied to 1-2 ml of filtrate. Alkaloids are present when a reddish-brown precipitate forms.

Test for flavonoids:

- **Lead acetate test:** Lead acetate solution was added in small amounts to the extract for treatment. The presence of flavonoids may be indicated by the formation of a yellow precipitate.

- **Alkaline reagent test:** In a test tube, the extract underwent treatment individually with a few drops of sodium hydroxide. The development of a indicates the presence of flavonoids by bright yellow color that becomes less colorless when a couple of drops of diluted acid are added.

- **Shinoda test:** 5 milliliters (or 95% ethanol) were added to the extract. A small amount of magnesium turning was added to the mixture, and then powerful hydrochloric acid was added drop by drop. The development of a pink hue signifies the existence of flavonoids.

Test for glycosides:

- **Borntrager's test:** Diluted sulfuric acid was added to 3 ml of extract, allowed to boil for 5 minutes, and then filtered. An equal volume of either the cooled filtrate was mixed with either benzene or chloroform, and it was thoroughly shaken. Ammonia was added to the organic solvent layer after it had been separated. The ammonical layer's formation of a pink to red tint shows the presence of anthraquinone glycosides.

- **Legal's test:** Dissolved in pyridine was one milliliter of extract. Addition of 1 milliliter of sodium nitropruside solution, and the solution was alkalinized with 10% sodium hydroxide solution. There are cardiac glycosides present when a pink to blood red hue appears.

- **Keller-Killiani test:** In a test tube, 2 ml of extract was mixed with 3 ml of glacial acetic acid and 1 drop of 5% ferric chloride. By the side of the test tube, carefully add 0.5 mL of strong sulphuric acid. The cardiac glycosides' existence is shown by the formation of blue color in the acetic acid layer.

Test for protein and amino acids:

- **Biuret's test:** One milliliter of a 10% sodium hydroxide solution was added to the extract and heated in a test tube. A drop of 0.7% copper sulphate solution was added to the above mixture. The appearance of a violet or pink tint signifies the existence of proteins.

- **Ninhydrin test:** 3 ml of the passage was cooked in a water bath for 10 minutes with 3 drops of 5% Ninhydrin solution. The amino acid content is shown by the formation of blue color.

Test for saponins:

- **Foam test:** 1 ml of extract was dissolved in 20 ml of distilled water and shacked for 15min in a graduated cylinder. Formation of persistent foam around 1cm layer was observed.

Test for triterpenoids and steroids:

- **Salkowski's test:** The extract was chloroform-treated and filtered. The filtrate was agitated and allowed to stand after a few drops of strong sulphuric acid were added. Sterol is present if the lower layers turn crimson. The triterpenes' existence is indicated by the presence of a golden yellow layer at the bottom.

- **Libermann-Burchard's test:** Chloroform was used to treat the extract. A couple of droplets of acetic anhydride were added to this solution, which was then heated and cooled. Sulphuric acid concentrate was poured into the test tube through the sidewalls. The steroid presence is shown by the production of a brown ring connecting the two layers, while the presence of triterpenoids is indicated by the formation of a deep red color at the junction of two layers.

Test for tannin and phenolic compounds:

- **Ferric chloride test:** Distilled water was used to dissolve some of the extract. 2 mL of 5% ferric chloride solution was introduced into this solution. When phenolic chemicals are present, they are indicated by the formation of blue, green, or violet hue.

- **Lead acetate test:** Distilled water was used to dissolve some of the extract. Lead acetate solution was added in small amounts to this solution. The presence of phenolic compounds is shown by the formation of white precipitate.

- **Gelatin Test:** A small amount of extract was dissolved in distilled water. Two milliliters of a 1% gelatin solution with 10% sodium chloride was added to this solution. The existence of chemicals called phenolics is shown by the formation of white precipitate.

Quantitative Phytochemical estimation

Spectrophotometric Quantification of Total Phenolic Content

The plant extract's total phenolic content was calculated utilizing the Folin-Ciocalteu Assay. The PremnaserratifoliaExtract (0.2 mL from stock solution) was mixed with 2.5 ml of Folin-Ciocalteu's phenol reagent. After 5 min, 2 ml of a 7.5 % Na_2CO_3 solution was incorporated into the blend.This mixture was diluted up to 7 mL with distilled water. The mixture was maintained in the dark for 90 minutes at 23°C before the absorbance at 760 nm was measured. The TPC was calculated by extrapolating the calibration curve created by creating a gallic acid solution (20 to 100g/ml). The phenolic compound estimation was performed in triplicate. TPC was calculated using milligrams of gallic acid equivalents (GAE) per gram of dried material(Saeed et al., 2012).

Spectrophotometric Quantification of Total Flavonoid Content

The amount of flavonoids was ascertained using Aluminium chloride method (Chang et al., 2002). 0.5 ml of Premnaserratifoliaextracts, 0.15 ml of $NaNO_2$ (5%) and 0.15 ml of $AlCl_3.6H_2O$ (10%) were combined in a 10 ml test tube. 2 ml of NaOH (4%) was added after 5 minutes. The solution was well mixed, and the absorbance at 510 nm was measured. The Total flavonoid standard curve was created using the same process as previously described, employing rutin standard solution (20 to 100g/ml). Total flavonoids were calculated as milligrams of rutin equivalents per gram of dried fraction(Senguttuvan et al., 2014).

Result and discussion:

The present investigation showed that the total weight of PremnaSerratifolia Whole plant used were 660 gm. After performing extraction of PremnaSerratifolia, the percentage yield of leave extract in different solvents like petroleum ether and Methanol extract were discovered to be 0.03% (0.185 gm) and 1.97 % (10.466 gm) respectively.

Plant collection

S. No.	Plant name	Plant part used	Weight
1.	*PremnaSerratifolia*	Whole	660 gm

Pharmacognostical evaluation of *PremnaSerratifolia*

Parameters	Value in percentage (%)
	PremnaSerratifolia
Loss on drying	45
Total ash value	9.52
Water soluble ash	1.20
Acid insoluble ash	0.82
Water extractive value	12.08
Alcoholic extractive value	85.12

5.3 Percentage yield

Table 1:Percentage yield of extracts

S. No.	Plant name	Solvent	Color of extract	Theoretical weight (gm)	Yield (gm)	% Yield
1.	*PremnaSerratifolia*	Petroleum Ether	Dark Yellowish	570 gm	0.185	0.03
2.	*PremnaSerratifolia*	Methanol	Dark Brownish	530 gm	10.466	1.97

Phytochemical analysis of*PremnaSerratifolia*extracts

S. No.	Experiment	Result	
		Petroleum Ether extract	**Methanolic extract**
Test for Carbohydrates			
1.	Molisch's Test	-	+
2.	Fehling's Test	-	+
3.	Benedict's Test	-	-
4.	Barfoed's test	-	+
Test for Alkaloids			
1.	Mayer's Test	-	+
2.	Hager's Test	-	+
3.	Wagner's Test	+	+
Test for Terpenoids			
1.	Salkowski Test	+	+
2.	Libermann-Burchard's Test	-	-
Test for Flavonoids			
1.	Lead Acetate Test	-	+
2.	Alkaline Reagent Test	+	+
Test for Tannins and Phenolic Compounds			
1.	$FeCl_3$ Test	-	+
2.	Lead Acetate Test	-	+
3.	Gelatine Test	+	-
Test for Saponins			
1.	Froth Test	+	+
Test for Protein and Amino acids			
1.	Ninhydrin Test	-	-
2.	Biuret's Test	-	+
Test for Glycosides			

1.	Legal's Test	-	+
2.	Keller Killani Test	-	+
3.	Borntrager's Test	-	-

Quantitative Phytochemical analysis Premnaserratifolia Methanolic extract

1.Total Phenolic Content (TPC) Estimation

Table 9 : Standard table for Gallic acid

S. No.	Concentration (µg/ml)	Absorbance (nm)
1.	20	0.120
2.	40	0.221
3.	60	0.354
4.	80	0.465
5.	100	0.572

The most significant are the phenolics secondary metabolites present in plants **(Harikumar *et al.*, 2008)**. They contribute to the antioxidant activity of plants due to their redox properties, act as hydrogen donators, reducing agents, and oxygen scavengers **(Wang *et al.*, 2006)**. This leads to prevention of various diseases associated with oxidative stress such as cardiovascular, neurodegenerative diseases and cancer **(Dimitrios*et al.*, 2006).**

Total Flavonoid Content (TFC) Estimation:

Table 2 : Standard table for Rutin

S. No.	Concentration (µg/ml)	Absorbance (nm)
1.	**20**	**0.088**
2.	**40**	0.162
3.	**60**	0.234
4.	**80**	0.308
5.	**100**	0.405

Flavonoids contribute to majority of plant secondary metabolites. The 3', 4'-orthodihydroxyconfigurations in ring B and a carbonyl group at C4 in ring C, imparts better electron donating properties leading to free radical scavenging activity. The C2–C3 double bond's existence

conjugated to the C4 carbonyl in flavonols is responsible for electron delocalization from ring B, which would indirectly increase the radical-scavenging activity. The flavonoid's antioxidant capacity also depends about the quantity and placement of the hydroxyl groups (Chua et al., 2011).

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