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Pharmacognostical and Preliminary Phytochemical Studies on the Bark Extracts of *Pterospermum Acerifolium*

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Aim: *Pterospermum acerifolium* Linn is usually a perennial, evergreen plant belonging to family Sterculiaceae. It is found in sub-Himalayan tract and outer Himalayan valleys and hills up to 4,000 ft., Bengal, Chittagong, Khasia Hills, Manipur, Darjeeling and Odisha and extensively planted in the Bombay State. Sometimes it is used for packing-cases, planks, turnery articles and plywood. Hill people use the white tomentum from the under surface of the bark to stop bleeding Flowers: used as a general tonic .Flowers and bark: charred and mixed with kamala applied in suppurating small-pox. The extracts of these barks and barks are used in traditional medicine because of their antibacterial and antifungal activity. The bark extracts of various solvents were subjected to pharmacognostical and phytochemical analysis. Variable fluorescence nature of the plant was also noted against day and UV light. Bark extracts contain alkaloids, flavonoids, saponins, phenolic compounds, tannins, cardiac glycosides etc., which could be a reason for the plants pharmacological activity. These observations would be of great value in the authentication of this plant in its crude form.

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1. INTRODUCTION

The plant *Pterospermum acerifolium* been studied by different researchers taking solvent of different nature investigation of chemical constituents were done. Also isolation and characterization of constituents has been reported through the different publication of the studies. The review literature deals with the research work done with the different extracts by different researchers in different parts of the world.

Boscialin, a natural product structurally related to ionones, was first isolated by Séquin *et al.* from the barks of the African tree *Boscia salicifolia* Oliv..The extracts of these barks are used in traditional medicine because of their antibacterial and antifungal activity. Later, related boscialin glucosides were isolated from barks of *Pterospermum acerifolium*. The authors reported that 4'-O-β-D-glucopyranoside-boscialin showed significant insulin releasing effect on isolated rat pancreatic islets ¹.

Fresh flower, bark and leaf extracts of *Pterospermum acerifolium* contained kaempferol1-3-0-galactoside as the major pigment, and in some cases other glycosides of luteolin, kaempferol and quercetin were also present

2. MATERIALS AND METHODS

2.1 Plant material

The plant material was properly collected from the area of salipur, Orissa in the month of September 2008.

The collected barks of *Pterospermum acerifolium* were properly authenticated by Botanists of Ravenshaw College; Cuttack was washed under running tap water to clean adhering dust material and then dried under shade. By means of mechanical and hand grinders the dried barks coarsely powdered. The coarse powder was then properly weighed and then used for extraction.

2.2 Drying and size reduction:

After identification and authentication barks were subjected to drying in normal environmental condition

under shade. The dried barks were powdered by pulverization and were stored in air tight container.

2.3 Extraction:

Extraction is the common process for separation of active constituents by the use of different solvents. There is increasing scientific interest in the extraction and isolation of secondary metabolites from plants as biosynthetic, biochemical, phytochemical, Pharmacological and plant tissue culture studies. The secondary metabolites are the compounds derived from the plant that have no apparent function in primary metabolism of the organism and have a history of use as a therapeutic agent. The plant used for extraction should be properly identified and authenticated. The choice of the plant material for extraction depends upon its nature and the components to be isolated. The dried powdered plant material is generally used for extraction. The fresh plant parts when used are homogenized or macerated with a solvent such as alcohol or water. Several plant constituents including chlorophyll and resins are generally interfering in the isolation process. The precise mode of extraction naturally depends on the texture and water content of the plant material. A water immiscible solvent such as petroleum ether is used for the separation of alkaloids and quinines. Extraction itself may be performed by repeated maceration with agitation percolation or by continuous extraction by soxhlet extraction. ²

2.3.1 Extraction by fractionation:-

(a)Petroleum Ether (60°- 80°) Extract ~About 1.5 kg of shade dried powder of barks of *Pterospermum acerifolium* was extracted with petroleum ether (60°- 80°) for 24 hrs by using soxhlet apparatus. After completion of extraction the solvent was removed under reduced pressure and the extractive was determined.

(b)Methanolic Extract ~ The marc left after petroleum ether extraction was dried and extracted

Pattanaik et al.

with methanol for 24hrs. After completion of extraction, the solvent was removed under reduced pressure and the extractive value was determined.

The crude methanol extract, after removal of the solvent, was dissolved in 10% sulfuric acid solution and partitioned with chloroform, ethyl acetate and n-butanol successively to give chloroform, Ethyl acetate, n-Butanol and water soluble fractions respectively.³

Fluorescence Analysis

Fluorescence characteristics of the powdered drug with different chemicals were observed in day light and ultraviolet light at 254 nm. The powdered leaf was treated with various solvents like hexane, alcohol, chloroform, benzene, acetone and ethyl acetate and acids like 1N hydrochloric acid, 50% sulphuric acid and alkaline solutions like aqueous and alcoholic NaOH. Observations of fluorescence analysis were recorded at 0, 24 and 48 hours.⁴

Fluorescence characteristic of the drug powder with different chemical reagent

Organic molecules absorb light usually over a specific range of wave length; many of them reemit such radiations. So if the powder is treated with different chemical reagents and seen in the UV cabinet, different colours will be produced. Therefore it can be used for the identification of the drug.

The fluorescence characteristic of the drug powder with different chemical reagent was studied by observing under UV Light at 254nm.

3. PHYTOCHEMICAL INVESTIGATION^{3, 4,5, 6}

Different extracts obtained from the above extraction processes and from the reflux condensation extraction process were analyzed for different phytoconstituents present in these by the method of qualitative phytochemical analysis.

3.1 Tests for alkaloids

- **Wagner's Reagent Test:**

With alkaloid it shows reddish brown precipitate. It is prepared by dissolving 1.27 gm of Iodine and 2 gm of Potassium Iodide in 5ml of water and the final volume is made up to 200 ml.

- **Mayer's Reagent Test:**

It is other method of detecting alkaloids. To prepare the reagent, 1.36 gm of mercuric chloride is dissolved in distilled water. In another part dissolve 5gm of potassium iodide in 60 ml of distilled water. Then both the parts were mixed and the volume was adjusted to 200 ml. With alkaloids it shows white to buff precipitate.

- **Dragendroff's Reagent Test:**

With alkaloids this reagent gives orange-brown coloured precipitate. To prepare this reagent, 14 gm of sodium iodide was boiled with 5.2 gm of bismuth carbonate in 50 ml glacial acetic acid for few minutes. Then it was allowed to stand for overnight and the precipitate of sodium acetate was filtered out. To 40 ml of filtrate 160 ml of acetate and 1 ml of water was added. The stock solution was stored in amber-coloured bottle. During experiment; to 10 ml of stock solution 20 ml of acetic acid was added and the final volume was made up to 100 ml with water.

- **Hager's Reagent Test:**

This reagent shows characteristic crystalline precipitate with many precipitates. In this case a saturated aqueous picric acid was used for detection of alkaloids.⁷

3.2 Tests for carbohydrates

- **Benedict's Test:**

In this method of test for monosaccharide, 5 ml of Benedict's reagent and 3 ml of test solution when boiled on a water bath and brick red precipitate appears at the bottom of the test tube confirms the presence of the compounds.

- **Fehling's Test:**

In this method 2 ml of Fehling 'A', 2 ml of Fehling 'B' and 2 ml of extract were boiled. The presence of

reducing sugar is confirmed if yellow or brick red precipitate appears at the bottom of the test tube confirms the presence of the monosaccharide.

- **Molisch's Test:**

When the aqueous or alcoholic solution of the extract and 10% alcoholic solution of α -naphthol were shaken and concentrated Sulphuric acid was added along the side of the test tube, a violet ring at the junction of two liquids confirms presence of carbohydrates.

3.3 Tests for glycosides

a) Test for cardiac glycosides

- **Keller-Killiani Test:**

To an extract of the drug in glacial acetic acid few drops of Ferric Chloride and concentrated Sulphuric acid is added. A reddish brown colour is formed at the junction of the two layers and upper layer turns bluish green.

- **Legal Test:**

To a solution of glycoside in pyridine, sodium nitropruside solution and sodium hydroxide solution were added. A pink to red colour will confirm the presence of glycosides.

b) Test for anthraquinone glycosides

- **Borntrager's test:**

To perform Borntrager's test, 0.1gm of the powdered drug was boiled with 5 ml of 10% Sulphuric acid for 2 minute. It was filtered while hot, then cooled and the filtrate was shaken with equal volume of benzene. The benzene layer was allowed to separate completely from the lower layer, which was pipetted out and transferred out to a clean test tube. Then half of its volume of aqueous ammonia (10%) was added and shaken gently and the layers were allowed to separate. The lower ammonia layer will show red pink colour due to presence of free Anthraquinone.

- **Modified Borntrager's test:**

The C-Glycoside of Anthraquinone requires more drastic conditions for hydrolysis and thus a

modification of the above test is to use ferric chloride and hydrochloric acid to affect oxidative hydrolysis.

When 0.1gm of the drug, 5ml of dilute HCl and 5 ml of 5% solution of ferric chloride were added and boiled for few minutes and then subsequently cooled and filtered part is shaken with benzene; the separated benzene layer and equal volume of dilute solution of ammonia shows pink colour.

3.4 Tests for gums and mucilages

- **Ruthenium Red Test:**

In this test 0.08 gm of ruthenium red when dissolved in 10 ml of 10% solution of lead acetate, it stains the mucilage to red colour.

- **Molisch's Test:**

When the aqueous or alcoholic solution of the extract and 10% alcoholic solution of α -naphthol were shaken and concentrated Sulphuric acid was added along the side of the test tube, a violet ring at the junction of two liquids confirms presence of carbohydrates, gums and mucilage.

- **Test with 95% Alcohol:**

When 95% alcohol added to the extract, gums get precipitated out. The precipitate is insoluble in alcohol.

3.5 Tests for proteins and amino acids

- **Biuret Test:**

When 2ml of the extract, 2 ml of 10% NaOH solution and 2-3 drops of 1% CuSO₄ solution were mixed, the appearance of violet or purple colour confirms the presence of proteins.

- **Ninhydrin Test:**

When 0.5 ml of ninhydrin solution is added to 2 ml of the extract and boiled for 2 minute and then cooled. The appearance of blue colour confirms the presence of proteins.

- **Xanthoproteic Test:**

When 2ml of the extract and 1 ml of conc. HNO₃ were boiled and cooled, subsequently 40% NaOH solution

Pattanaik et al.

added drop by drop to it. Appearance of coloured solution indicates the presence of proteins.

- **Millon's Test:**

2ml of the extract and 2 ml of millon's reagent were boiled, subsequently cooled, and then few drops of NaNO_2 were added to it. Appearance of red precipitate and red coloured solution indicates the presence of proteins.

3.6 Tests for tannins and phenolic compounds

- **Test with Lead Acetate:**

Tannins get precipitate with lead acetate.

- **Test with Ferric Chloride:**

Generally phenols were precipitated with 5% w/v solution of ferric chloride in 90% alcohol and thus phenols are detected.

- **Test with Gelatin Solution:**

To a solution of tannins (0.5 - 1%) aqueous solution of gelatin (1%) and sodium chloride (10%) were added. A white buff precipitate confirms the compounds.

3.7 Tests for steroids and sterols

- **Salkowski's Test:**

To 5ml of the solution of the extract in chloroform in a dry test tube, equal volume of conc. H_2SO_4 was added along the side of the test tube. The presence of steroids and sterols are confirmed by the upper chloroform layer showing a play of colours first from bluish red to gradually violet and lower acid layer showing yellow colour with green fluorescence.

- **Libermann Burchard Reagent Test:**

In this method of detection, about 2 ml of the solution of extract in chloroform was placed in a dry test tube. Then 2 ml of acetic anhydride and 2-3 drops of conc. H_2SO_4 was added to it and allowed to stand for few minutes. An emerald green colour develops if steroid or sterols are present.

3.8 Tests for triterpenoids

- **Test with Tin and Thionyl Chloride:**

For detection of triterpenoids the extract was dissolved in chloroform. A piece of metallic tin and 1 drop of thionyl chloride was added to it. Pink colour confirms the result.

3.9 Tests for saponins

- **Foam Test:**

About 1 ml of alcoholic and aqueous extract was diluted separately with distilled water to make the volume up to 10 ml, and shaken in a graduated cylinder for 15 minutes and kept aside. 1 cm layer of foam after standing for 30 minutes indicates the presence of saponins.

3.10 Tests for flavonoids

- **Test with NaOH:**

For the detection of flavonoids, the extract was first dissolved with water. It was filtered and the filtrate was treated with sodium hydroxide. A yellow colour confirms the presence of flavonoids.

- **Test with Sulphuric Acid:** A drop of H_2SO_4 when added to the above, the yellow colour disappears.

4. RESULTS AND DISCUSSION

Good and effective raw material is needed to maintain effective therapeutic potentials of medicinal plants. Moderate pharmacognostical features are noted from different geographic locations. Standardization is needed to maintain the quality of raw materials, used as medicines. In the present study, pharmacognostical, phytochemical and fluorescence features of the plant *Pterospermum acerifolium* Linn. barks were analysed and reported. Morphological evaluation revealed the shape of the selected bark.⁸

4.1 Colour, consistency and extractive values of different extracts of barks of *pterospermum acerifolium*

In the present study much higher extractive values were noted in aqueous extract (9.3 %) whereas lower extractive values were noted for chloroform (0.2%) and

Table 1: Colour consistency profile

SI.No	Solvent Extraction	Colour	Consistency	% w/w of Extract
1	Petroleum Ether (60 ^o C – 80 ^o C)	Yellowish green	Greasy	3.2
2	Methanol	Yellowish brown	Greasy	4.2
3	Chloroform	Reddish Brown	Greasy	0.2
4	Ethyl Acetate	Reddish Black	Greasy	0.62
5	n-Butanol	Reddish Brown	Greasy	0.89
6	Water	Reddish Brown	Greasy	9.32

Table 2: Fluorescence characteristic of extracts

Extracts	Fluorescence under UV light (365nm)
Petroleum Ether Extract (60 ^o C – 80 ^o C)	No Fluorescence
Methanol Extract	No Fluorescence
Chloroform Extract	Light Orange
Ethyl Acetate Extract	No Fluorescence
n-Butanol Extract	Light yellowish orange

Table 3: Fluorescence observation at 254 nm

sl. no.	Treatment	Fluorescence under uv-light (254nm)
1	Untreated	Green
2	Powder treated with sodium hydroxide in methanol	Greenish
3	Powder treated with HCl	Green
4	Powder treated with nitric acid dilute with equal volume of water	Green
5	Powder treated with sodium hydroxide acid in water	Greenish
6	Powder treated with picric acid	Yellowish
7	Powder treated with conc. Sulphuric acid	Brown

8	Powder treated with ferric chloride solution	Green
9	Powder treated with iodine	Blue

Table 4: Moisture content

Sr. No.	% of moisture	Average (%)
1	11%	12%
2	13%	

Table 5: Preliminary phytochemical identification

Tests for	Petroleum ether extract	Chloroform extract	Ethyl Acetate Extract	n-Butanol Extract
Alkaloids	+	+	-	-
Carbohydrates	-	-	-	+
Glycosides	-	-	+	+
Tannins - phenolic compounds	-	-	+	+
Protein and amino acids	-	-	-	-
Gum and mucilage	-	-	-	+
Flavones and flavonoids	-	-	+	+
Saponins	-	-	+	-
Steroids and sterols	-	-	-	-

Petroleum Ether (3.2%). Higher extractive matters were collected using water (9.7 %) followed by ethanol (8.6 %). (Table – 1).

4.2 Fluorescence characteristic of the different extracts of the barks of *pterosperrum acerifolium*

The fluorescence characteristic of different extracts was studied by observing them under UV Light at 365nm. The tests and observations are recorded in the table no – 2.

4.3 Fluorescence characteristic of the drug powder with different chemical reagent

Organic molecules absorb light usually over a specific range of wave length; many of them reemit such

radiations. So if the powder is treated with different chemical reagents and seen in the UV cabinet, different colours will be produced. Therefore it can be used for the identification of the drug.^{9, 10}.The tests and observations are recorded in the table no – 03.

5. CONCLUSION

Medicinal plants containing phytochemical with various biological activities that can be of significant therapeutic index. Different phytochemicals have been found to possess a wide range of beneficial properties, which may help in protection against infectious diseases and disorders. Phytochemicals such as the steroids and saponins are responsible for the activities of the Central Nervous System¹¹.Steroids and triterpenoids shown to have analgesic properties. The terpenoids have shown to decrease blood sugar level in animal studies¹².The saponins possess hypocholesterolemic and antidiabetic properties. It has been found that more highly oxidized phenols are more inhibitory to microorganisms. Flavonoid compounds inhibit mult ipleviruses. Numerous studies have documented the effectiveness of flavonoids such as swertifranchside glycyrrhizin from licorice¹³ and chrysin against HIV.Tannins received a great deal of attention in recent years, since it was suggested that the consumption of tannin-containing beverages, like green teas and red wines can cure or prevent a variety of ills¹⁴.Many human physiological activities, such as stimulation of phagocytic cells, host - mediated tumour activity and a wide range of anti-infective actions have assigned to tannins.Saponins, terpenoids¹⁵, flavonoids, tannins, steroids and alkaloids have anti-inflammatory effects.

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