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EXTRACTION AND CHEMICAL INVESTIGATION OF LEAVES OF PTEROSPERMUM ACERIFOLIUM



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Abstract

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Herbal Medicine can be broadly classified into various basic systems: Traditional Chinese Herbalism, which is part of Traditional Oriental Medicine, Ayurvedic Herbalism, which is derived from Ayurveda, and Western Herbalism, which originally came from Greece and Rome to Europe and then spread to North and South America. Indian system of medicine comprises of Ayurveda, Unani, Siddha, Homeopathy, Naturopathy and Yoga. Each of which uses the herbal constituents in some or the other form. Ayurveda is India's traditional, natural system of medicine that has been practiced for more than 5,000 years. Ayurveda is a Sanskrit word that literally translated means "science of life" or "practices of longevity." At present extracting the *Pterospermum acerifolium*, the extract contains carbohydrates, tannins-phenolic compounds, gums and mucilage's, flavonoids, saponins. From preliminary identification chemical tests it was confirmed that ethyl acetate extract had large amount of flavonoids.

INTRODUCTION

Herbal medicine is the oldest form of healthcare known to mankind. Herbs had been used by all cultures throughout history. It was an integral part of the development of modern civilization. Primitive man observed and appreciated the great diversity of plants available to him. Much of the medicinal use of plants seems to have been developed through observations of wild animals, and by trial and error (Trease and Evans, 1984). As time went on, each tribe added the medicinal power of herbs in their area to its knowledgebase. They methodically collected information on herbs and developed well-defined herbal pharmacopoeias. Indeed, well into the 20th century much of the pharmacopoeia of scientific medicine was derived from the herbal lore of native peoples. Many drugs commonly used today contain at least one active ingredient derived from plant material. Some are made from plant extracts; others are synthesized to mimic a natural plant compound are of herbal origin. Indeed, about 25 percent of the prescription drugs dispensed contain at least one active ingredient derived from

plant material. Some are made from plant extracts; others are synthesized to mimic a natural plant compound (Han van de Waterbeemd, 1996).

Medicinal plants often contain additional active principles other than major active principles and physiologically inert substances such as cellulose and starch unlike the chemical entities, which contain one active ingredient and number of inert substances, which make up the dosage form.

Herbal Medicine can be broadly classified into various basic systems: Traditional Chinese Herbalism, which is part of Traditional Oriental Medicine, Ayurvedic Herbalism, which is derived from Ayurveda, and Western Herbalism, which originally came from Greece and Rome to Europe and then spread to North and South America. Indian system of medicine comprises of Ayurveda, Unani, Siddha, Homeopathy, Naturopathy and Yoga. Each of which uses the herbal constituents in some or the other form. Ayurveda is India's traditional, natural system of medicine that has been practiced for more than 5,000 years. Ayurveda is a Sanskrit word that literally

translated means "science of life" or "practices of longevity." Ayurveda was the system of health care conceived and developed by the seers (rishis) and natural scientists through centuries of observations, experiments, discussions, and meditations (X. Laing *et al*, 2003).

MATERIALS AND METHODS

PROCUREMENT OF PLANT MATERIAL

The leaves of *Pterospermum acerifolium* was collected from Jatara dist. tikamgarh, Madhya Pradesh (India) during the month of October, 2007. The plants were authenticated and the voucher specimen was deposited in the department of Sanjay nikunj Gyaraspur dist. Vidisa.

DRYING AND SIZE REDUCTION

After identification and authentication leaves were subjected to drying in normal environmental condition under shade. The dried leaves were powdered by pulverization and were stored in air tight container.

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 extraction¹.

EXTRACTION BY FRACTIONATION

(a) Petroleum Ether (60°- 80°) Extract:

About 1.5 kg of shade dried powder of leaves 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 determined².

(b) Methanolic Extract:

The marc left after petroleum ether extraction was dried and extracted 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. The above extracts were used for further studies such as colour, consistency and percentage of extract obtained. The observations were recorded. The liquid

extracts were subjected to fluorescence analysis and used for preliminary phytochemical studies to detect various chemical constituents present in different extracts³.

PHYTOCHEMICAL INVESTIGATION (Kokate et al, 2002)

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. The following chemical tests were carried out and the results were tabulated.

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 alkaloids⁵.

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 carbohydrates⁷.

TESTS FOR GLYCOSIDES

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 glycosides⁸.

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 colour⁹.

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.

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 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 NaNO₂ were added to it. Appearance of red precipitate and red coloured solution indicates the presence of proteins¹⁰.

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.

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. H₂SO₄ 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 present¹¹.

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.

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 saponins¹².

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.

SHINODA TEST

A small quantity of extract was taken in a test tube and dissolved in methanol (1 mL). A pinch of magnesium powder was added followed by conc.HCl. Appearance of pink colour indicates the presence of flavonoids, bioflavonoid (Paech and Tracey, 1979a)¹³.

PHARMACOGNOSTIC EVALUATION **(Physical evaluation)**

COLOUR, CONSISTENCY AND EXTRACTIVE VALUES OF DIFFERENT EXTRACTS OF LEAVES OF PTEROSPERMUM ACERIFOLIUM

FLUORESCENCE CHARACTERISTIC OF THE DIFFERENT EXTRACTS OF THE LEAVES OF PTEROSPERMUM 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 below.

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. The tests and observations are recorded in Table no. 04.

DETERMINATION OF ASH VALUES

Principle: After incineration the crude drugs leave an inorganic ash which in the case of many drugs varies within fairly wide limits and is therefore of little value for purpose of evaluation. In other cases the total ash figure is of importance and indicates to some extent of amount of care taken in the preparation of the drug. In the determination of the total ash values the carbon must be removed at a slow temperature (450°C) as possible. If carbon still present after heating at a moderate temperature, the water soluble ash may be separated and the residue again ignited.

The total ash usually consists mainly of carbonates, phosphates, silicates and silica. To produce more consistent ash as a sulphated ash, which involves treatment of the drug with dilute Sulphuric acid before ignition, is used. In this all oxides and carbonate are converted to sulphonates and the ignition is carried out at higher temperature (600°C).

If the total ash were treated with dilute hydrochloric acid, the percentage of acid insoluble ash may be determined. This usually consists mainly of silica, and a high acid insoluble ash in drugs indicates contamination with earthy material¹³.

TOTAL ASH

3 gm of powdered crude drug were accurately weighed in a silica crucible. The powdered drug was gradually increased the heat until free from carbon and after cooling it was kept in a desiccator. Then with reference to the air dried sample ash is weighed and percentage was calculated. The ash value of the powdered drug was recorded in table no-5.

ACID INSOLUBLE ASH

The total ash was boiled for 5 minutes with 25 ml of dilute hydrochloric acid. The

insoluble matter was filtered and collected on ash less filter paper and washed the filter papers with hot water. Then cool the crucible and kept in desiccator. The acid insoluble ash was weighed and calculated with reference to the air dried drug¹⁴.

WATER SOLUBLE ASH: The ashes was boiled for 5 minutes with 25 ml of water, then the insoluble matter was filtered and collected on ash less filter paper and washed the filter papers with hot water and ignite for 15 minutes at a temperature not exceed 450 degree centigrade. The weight of the insoluble matter was subtracted from the pre weighed ash and the difference represented as water soluble ash.

SULPHATE ASH: The platinum crucible was heated for 10 minutes, then allowed to cool in a desiccator and weighed. 1 gm of drug powder was taken in the crucible and the content was accurately ignited gently at first gently, until the substance is thoroughly charred. The residue was cooled and moistened with 1 ml of Sulphuric acid with gentle heat until the white fumes are no longer evolved. Then ignite the sample residue at $800^{\circ} \pm 25^{\circ}$ until all the black particles were disappeared. The crucible was allowed to cool. Then after addition of

few drops of Sulphuric acid, the crucible was allowed to cool and the weight was taken. The Sulphate ash value was calculated and recorded in table no-5¹⁵.

Data shows ash values of leaves of *Pterospermum acerifolium*Linn

MOISTURE CONTENT

The presence of moisture in a crude drug can lead to its deterioration due to either activation of certain enzymes or growth of microbes. Karl- fischer method is a standard procedure for determining moisture content.

Now completely automated equipment is available for moisture determination eliminating the manual aspect of sample handling and weighing. The introduction of crude drug to karl fischer cell, titration and data completion. The moisture content is also determined by automated I R moisture balance¹⁶.

RESULTS AND DISCUSSION

The coarse powder of the shed dried leaves of the plant was subjected to successive extraction by using soxhlet apparatus. The plant materials were treated with solvents for 24 hours. The plant material was extracted with a series of solvents with

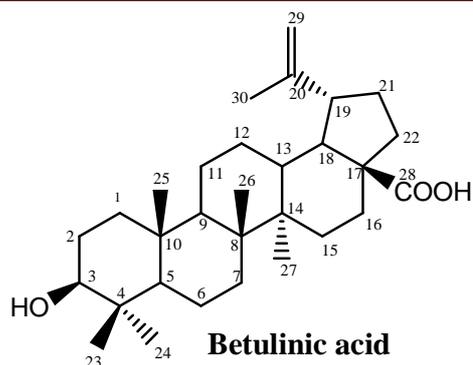
increasing order of polarity, commencing from petroleum ether, Methanol, chloroform, ethyl acetate and n-Butanol to isolate all possible kinds of phytoconstituents present in the leaf part of *Pterospermum acerifolium*. In the different fraction highest yield of n- Butanol fraction is obtained that is 13.3 gm (0.89%) followed by ethyl acetate fraction 9.3 gm (0.62 %) and chloroform fraction 3 gm (0.2%).

The different fractions so obtained were also examined under daylight and UV-Light to find out presence of any fluorescent compounds within them. The colour, consistency and percentage of extractives obtained from each extraction were also studied and pharmacognostical evaluation of powder by treating the crude powder of the drug with other chemical reagents and observing them under UV Light also carried out. After the fluorescence study other phrmacognostical evaluation was done including determination of Ash value in which percentage of Sulphated ash, Water soluble ash, Acid insoluble ash, Total ash and moisture content were also determined. All the extracts were subjected to various chemical tests for preliminary

identification of various phytoconstituents. The extract contains carbohydrates, tannins-phenolic compounds, gums and mucilages, flavonoids, saponins.

From preliminary identification chemical tests it was confirmed that ethyl acetate extract had large amount of flavonoids and saponins therefore ethyl acetate extract was chosen for further study of separation and isolation.

Ethyl acetate extract furnished two compounds, PAL-1 and PAL-2. The structures of these compounds were elucidated by using IR, 1D and 2D NMR, Mass spectral data. PAL-1 was obtained as white crystals with molecular formula $C_{30}H_{48}O_3$ and melting point $306-308^{\circ}C$. IR and NMR data indicated that a pentacyclic triterpinoid of betulinic acid and also by direct comparison with published data confirmed the indentity of PAL-1 as Betulinic acid (M. Rama *et al*, 2006).



CONCLUSION

The plant material was extracted with a series of solvents with increasing order of polarity, commencing from petroleum ether, Methanol, chloroform, ethyl acetate and n-Butanol to isolate all possible kinds of phytoconstituents present in the leaf part of the *Pterospermum acerifolium*. The extract contains carbohydrates, tannins-phenolic compounds, gums and mucilages,

flavonoids, saponins. From preliminary identification chemical tests it was confirmed that ethyl acetate extract had large amount of flavonoids and saponins therefore ethyl acetate extract was chosen for further study of separation and isolation.

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Table 1

Sr. No.	Solvent Extraction	Colour	Consistency	% w/w of Extract
1.	Petroleum Ether (60 ⁰ C – 80 ⁰ 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

Table 2

Extracts	Fluorescence under UV light (365nm)
Petroleum Ether Extract (60 ⁰ C – 80 ⁰ C)	No Fluorescence
Methanol Extract	No Fluorescence
Chloroform Extract	Light Orange
Ethyl Acetate Extract	No Fluorescence
n-Butanol Extract	Light yellowish orange

Table 3

S.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

S.no	TYPES OF ASH	PERCENTAGE(W/W)
1.	Total ash	9.5%
2.	Acid insoluble ash	0.25%
3.	Water soluble ash	3%
4.	Sulphate ash	0.4%

Table 5

S.no	% of moisture	Average (%)
1	11%	
2	13%	12%

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