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A Pharmacognostic and Phyto-Physicochemical Evaluation of *Pterospermum acerifolium* (L) Wild. Flower

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ABSTRACT

Pterospermum acerifolium (L) Wild**.** is usually a perennial, evergreen tree belonging to family Sterculiaceae distributed throughout the world. It is found in the sub-Himalayan tract, outer Himalayan valleys, and hills up to 4,000 ft., Assam, West Bengal, Khasi Hills, Manipur, Darjeeling, Odisha and extensively planted in Maharashtra state. It is commonly known as Bayur Tree, Dinner-plate tree, Kanak Champa or Muchukunda. It is one among widely used ethnomedicinal plants for various diseases in India. Various parts of this tree have been traditionally used for a number of disorders including cancer. Mainly it is used for karna-shula (an earache), chechaka (smallpox), sweta-pradara (leucorrhoea), sotha (inflammation), dust-vrana (ulcers), kustha (leprosy), prameha (diabetes syndrome). In view of growing popularity and global interest in Ayurveda and its drug lore. There is an imminent need for well-coordinated research touching phyto-physicochemical, pharmacological as well as clinical studies of plant drugs. It is especially necessary to satisfy the international bodies and drug regulatory authorities relating to standards and quality control of the drug used. For this, chemical research studies have a very important role to play. These studies reveal the hidden secrets of plant kingdom such as their various compounds or active ingredients responsible for their effectiveness. The studies are also important in view to standardize the drug and to find out the adulteration. The article will help the researchers of Ayurveda as well as in another field of Bio-medical sciences to explore more about the said tree for the larger benefit of society.

INTRODUCTION

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. The research into plants with alleged folkloric use as anti-inflammatory agents should, therefore, be viewed as a fruitful and logical research strategy in the search for new anti-inflammatory drugs. *Pterospermum acerifolium* (L) Wild (Family: Sterculiaceae) commonly known as “Dinner plate tree” (English) and “Muchukunda" (Hindi), is a large deciduous tree of about 50-70 ft. and 5-7 ft. the girth on maturity. Flowers are large 12-15 cm in diameter, axillary, solitary or in pairs. It is widely distributed in the sub-Himalayan tract and outer Himalayan valleys and hills up to 4,000 ft., Assam, West Bengal, Khasi Hills, Manipur, Darjeeling and Odisha and extensively planted in Maharastra State[1]. The tree is commonly known as Bayur Tree, Dinner-plate tree, Kanak Champa, Muchkund (Hindi), Muskanda (Bengali), Matsakanda (Telugu), Moragos (Assamese), Vennangu (Tamil), Mushkundo (Oriya), Karnikar (Marathi). *Pterospermum acerifolium* is known to have antibacterial and antifungal activities, antioxidant activity, anti-inflammatory and anti-nociceptive activity, antipyretic activity, anti-ulcer activity, immunomodulatory activity, anti-diabetic activity, hyperlipidemic activity, anti-helminthic activity, and anticancer activity. In some areas, flowers and bark are charred and mixed with Kamala and applied in suppurating small pox[2]. The 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, an attempt has been made to highlight pharmacognostical, phytochemical, fluorescence features reports of *Pterospermum acerifolium* flower.

Taxonomy of *Pterospermum acerifolium*[3]

Kingdom: Plantae

Division: Magnoliophyta

Class: Eukaryota

Kingdom: Plant

Subdivision: Angiosperm

Class: Eudicots

Sub-Class: Eurosids II

Order: Malvales

Family: Sterculiaceae

Sub-Family: Dombeyoideae

Genus: Pterospermum

Species: acerifolium

Fig 1: Floral Parts

Fig 2: Whole tree

Fig 3: Flower

Fig 4: Leaf

MATERIALS AND METHODS

PROCUREMENT OF PLANT MATERIAL

Botanically identification of Pterospermum acerifolium was done at Shree Shree Marishantaveer Herbal Garden of SJG Ayurvedic Medical College, PG Studies & Research Centre, Koppal-583231, Karnataka and marked as source plant. The source plant was taxonomically studied for the whole year. Specimens of flower, fruit, leaf, and barks were submitted to the Central Research Facility, KLEU's Shri BMK Ayurved Mahavidyalaya, Belgaum-3 Karnataka. The source plant was authenticated to be *Pterospermum acerifolium* Wild by a taxonomist and a reference specimen was deposited in the herbarium vide Specimen no- Sl No-01, CRF/12/147.

COLLECTION AND PRESERVATION

Botanically identified fresh flowers of the Trial drug Pterospermum acerifolium were collected from the source plant at Shree Shree Marishantaveer Herbal Garden of SJG Ayurvedic Medical College, PG Studies & Research Centre, Koppal-583231 Karnataka after authentication in the months of September and October 2011. The flowers were shade dried

and preserved in airtight containers at Rasashala attached to SJG Ayurvedic Medical College, PG Studies & Research Centre, Koppal-583231, Karnataka.

PHARMACOGNOSTICAL STUDY

A taxonomical study of the source plant of Pterospermum acerifolium. The macroscopic features of the fresh wood of *Pterospermum acerifolium* were determined using the method of Evans[4]. Macroscopic and Organoleptic studies of Fresh flower and Flower powder was carried out. Anatomical sections and powdered samples for the microscopy and hem microscopy were carried out according to methods outlined by Brain and Turne[5]. T.S of Sepal, T.S of Petal, T.S of Upper and Lower Epidermis of Petal, T.S of the ovary of Fresh flower and microscopy of Flower powder alone, Powder + Iodine Solution 2%, Powder + phloroglucinol 2% + Conc. HCl was done. Organoleptic Study of fresh flower and flower powder in the same line Colour, Odour, Taste, Touch and other organoleptic characters were studied. Examination of Starch, epidermal trichomes, calcium oxalate crystals, lignin was carried out.

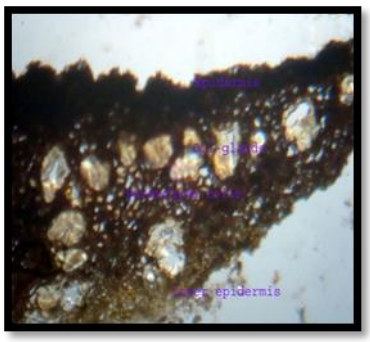


Fig 5: Collenchyma

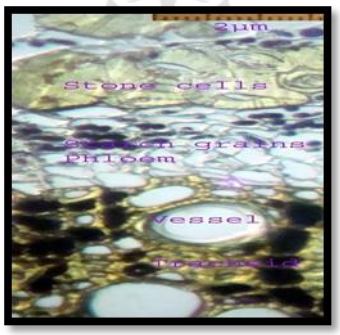


Fig 6: Vascular bundles

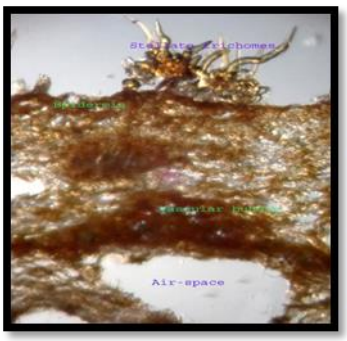


Fig 7: Stone cells

PHYTOCHEMICAL STUDY[6,7]

The physicochemical analysis of the drug which was screened in details was carried out in the Post Graduate Department of Zoology, Bajali College, Barpeta Assam. The study was carried out according to the following plan of work.

1. MOISTURE ESTIMATION: a) Take the sample of coarse powder of about 10 grams in the evaporating dish (evaporating dish should be cleaned with tap water and then with the distilled water, dried at 90°c in the oven for half-an-hour, cool it in the desiccator) b) Take the weight of empty evaporating dish on chemical balance to say weight as 'A' grams. c)

Take 10 grams of coarse powder, a sample of Pterospermum acerifolium Flower powder, weigh it and the weight is 'B' grams. d) Keep this evaporating dish in the oven at 110°c for 2½ hours. Remove the sample from the oven with the help of a pair of the tongue. Keep it in the desiccator for cooling for half-an-hour. After cooling take the weights of this dish to say weight as 'C' grams. e) Again keep this dish in the oven for half-an-hour at 110°c, again after cooling take the weight 'C2' grams. Repeat the procedure till the two consecutive are equal.

2. DETERMINATION OF FOREIGN MATTER: a) Weigh 100 grams of the sample of Pterospermum acerifolium Flower powder. b) Spread the sample on a whitetail or glass plate uniformly without overlapping. c) Inspect the sample with the naked eye or by means of lens 5x or above d) Separate the foreign organic matter (mentioned above) manually. **e)** After complete separation, weigh the matter and determine the percentage w/w present in the sample.

3.ASH VALUE ESTIMATION: Weigh accurately 2 grams of the air-dried drug in a tared platinum or silica dish and incinerate at a temperature not exceeding 600°c for 3 hours until free from carbon, cool and weigh. Calculate the percentage of ash with reference to the air-dried drug.

4.ACID INSOLUBLE ASH: Boil the ash obtained by the method mentioned above. Add 25 ml of dilute hydrochloric acid for 5 mins. Collect the acid insoluble ash in a pre-weighed crucible along with the ashless filter paper kept in a muffle furnace for an hour at around 450°c ± 5°c.

5.WATER SOLUBLE ASH: Boil the ash for 5 minutes with 25 ml distill water, collect the insoluble matter in a crucible or on an ashless filter paper, wash with hot water and ignite for 15minutes not exceeding 450ºC. Subtract the weight of the insoluble matter from the weight of the ash. The difference in weight represents the water-soluble ash. Calculate the percentage of water-soluble ash with reference to the air-dried drug.

6.Sulfated ASH: Heat silica or platinum crucible to redness for 10 minutes, allow to cool in a desiccator and weigh. Put 2 grams of Pterospermum acerifolium Flower powder accurately weighed, into the crucible. Ignite gently at first, until the substance is thoroughly charred. Cool, moisten the residue with 1 ml H2SO4, heat gently until white fumes are no longer evolved and ignite at 800±25°C until all black particles have disappeared. Conduct the ignition in a place protected from air currents preferably in Fume Hood. Allow the crucible to

cool and weigh. Ignite as before, allow cooling and weigh. Repeat the procedure until two consecutive are equal.

7. SPECIFIC GRAVITY ESTIMATION[8] was done with the help of Pycnometer.

8. DETERMINATION OF pH VALUE[8] was done with the help of PH meter.

9.DIFFERENT SOLVENT EXTRACTIVE STUDY[6,7]

Fig 8: Soxhlet Extraction

Fig 9: Extracts of flower

Fig 10: Benzene extract

The flowers were shade-dried in the open air, pulverized using an electric grinder to make powder and stored in airtight containers. The extraction procedures were carried out at Institute for advanced studies in science and technology, Boragaon, Assam following the already established extraction procedure of plant materials[9]. The powder was subjected to successive Soxhlet extraction using solvents of varying polarity; petroleum ether (60oC-80oC), benzene, chloroform, acetone, methanol, and water. The mixture was filtered on the 3rd day using a gauze cloth and the fine filtrate was obtained using Whatman No: 1 filter paper in a Buchner funnel. The filtrate was concentrated using a Büchi Rotavapor R-200 (Büchi Labortechnik, Flawil, Switzerland) into a slurry which was further heated on a water bath at 45 ± 5°C and stored in a vacuum desiccator. The dry extract was stored at 4 °C until the immunomodulatory experimental bioassays were carried out. The percentage yield of the extract was determined.

PRELIMINARY PHYTOCHEMICAL SCEREENING[10]

The Pet. Ether, Methanol and Aqueous extracts were subjected for qualitative preliminary phytochemical screening.

1. A TEST OF CARBOHYDRATES: Molisch's Test (General Test): 2 – 3 ml aq. Extract + few drops of alpha-naphthol solution in alcohol shake and add concentrated H2SO4 from sides of the test tube. Violet ring is formed at the junction of 2 liquids. **(a) Test for reducing Sugars**: (i) **Fehling's Test**: Mix 1 ml Fehling's A and 1 ml Fehling's B solution boil for 1 minute. Add an equal volume of test solution. Heat in boiling water for 5 – 10 minutes. 1st a yellow, then brick red precipitate is observed. (ii) **Benedict's test:** Mix equal volume of Benedict's reagent and test solution in the test tube. Heat in boiling water bath for 5 minutes. The solution appears green, yellow or red depending on the amount of reducing sugar present in the test solution. **(b) Test for Monosaccharides: Barfoed's Test:** Mix equal volume of Barfoed's reagent and test solution.

Heat for 1 –2 minutes in a boiling water bath and cool. Red precipitate is observed. **(c) Tests for Hexose Sugars:** (i) **Selwinoff's Test (for Ketohexose like fructose);** Heat 3 ml Selwinoff'sreagent and 1 ml test solution in boiling water bath for 1 to 2 minutes. The red color is found. (ii) **Tollen phloroglucinol test for galactose:** Mix 2.5 ml concentrated HCl and 1ml 0.5% phloroglucinol. Add 1 – 2 ml test solution. Heat Yellow to red color appears. (iii)**Cobalt Chloride Test:** Mix 3 ml test solution with 2 ml Cobalt chloride. Boil and cool. Add a few drops of NaOH solution. The solution appears greenish blue (glucose) or purplish (fructose) or upper layer greenish blue and lower layer purplish (a mixture of glucose and fructose). **(d) Test for Non-reducing sugars:** (i)Test solution does not give a response to Fehling's & Benedict's Tests. (ii)Hydrolyze Test solution; Fehling's & Benedict's test is positive. **(e) Test for Non-reducing Polysaccharides (Starch): (i)Iodine Test:** Mix 3 ml test solution and few drops of dilute Iodine Solution. Blue color appears it disappears on boiling and reappears on cooling. **(ii)The tannic acid test for starch:** With 20% tannic acid test solution gives a precipitate.

2. A **TEST FOR PROTEINS: (a) Biuret Test (General Test):** To 3 ml T. S., add 4% NaOH and few drops of 1% CuSO4 solution. Violet or pink color appears. (b) **Million's Test for Proteins**: Mix 3 ml. T. S. with 5 ml. Millions Reagent gives a white precipitate. Warm precipitate turns brick red or the precipitate dissolves giving red colored solution. (c) **Xanthoprotein Test (for a protein containing tyrosine or tryptophan):** Mix 3 ml T.S. with 1 ml concentrated H2SO4 i. The white precipitate is formed. Boil ii. Precipitate turns yellow, Add NH4OH iii. Precipitate turns orange.

(d) **Test for Proteins containing Sulphur:** Mix 5 ml T. S. with 2 ml 40% NaOH and 2 drops of 10% lead acetate solution. Boil, the solution turns black or brownish due to PBS formation. **Precipitation Test:** The Test solution gives a white colloidal precipitate with the following reagents:

(i) Absolute alcohol. (ii) 5% HgCl2 solution. (iii)5% Lead Acetate (iv) 5% Ammonium sulphate

3.TESTS FOR AMINO ACIDS: (a) **Ninhydrin Test (General Test):** Heat 3 ml T.S. and 3 drops 5% Ninhydrin solution in boil water bath 10 minutes. Purple or bluish color appears.

(b) **Test for Tyrosine:** Heat 3 ml T. S. and 3 drops Million's reagent solution. The solution shows a dark red color. (c) **Test for Tryptophan:** To 3 ml T.S. and few drops of glyoxalic acid and concentrated H2SO4. Reddish Violet ring appears at the junction of the two layers. (d) **Test for Cysteine:** To 5 ml T.S. and add few drops of 40% NaOH and 10% lead acetate solution then Boil. A black precipitate of lead sulfate is formed.

4.TESTS FOR TANNINS AND PHENOLIC COMPOUNDS: To 2–3 ml of aqueous, methanolic and pet. ether extracts, add few drops of following reagents: (a) 5% FeCl3 solution: Deep blue-black colour. (b) Lead acetate solution: White precipitate (c) Gelatin solution: White precipitate (d) Bromine water: Discoloration of bromine water (e) Acetic acid solution: Red colour solution (f) Potassium dichromate: Red precipitate (g) Dilute iodine solution: Transient red color (h) Dilute HNO3: Reddish to yellow color (i) Dilute KMNO4 solution: Discoloration.

5.TESTS FOR STEROIDS: (a) **Salkowski reaction:** To 2 ml of extract, add 2 ml of chloroform and 2 ml concentrated H2SO4. Shake well. Chloroform layer appears. Red and acid layer shows greenish yellow fluorescence. (b) **Liebermann – Burchard reaction:** Mix 2 ml extract with chloroform. Add 1 – 2 ml acetic anhydride and 2 drops concentrated H2SO4 from the side of the test tube. First red, then blue and finally green color appears. (c) **Liebermann's Reaction**: Mix 3 ml extract with 3 ml acetic anhydride. Heat and cool. Add a few drops of concentrated H2SO4. Blue color appears.

6. TESTS FOR GLYCOSIDES: (a) Test for Deoxysugars (Keller – Killani Test): To 2 ml extract, add glacial acetic acid, one drop 5% FeCl3 and concentrated H2SO4. Reddish

brown color appears at the junction of the two liquid layers and upper layer appears bluish green.

(b) Test for Saponin Glycosides: (i) Foam Test: Shake the drug extract or dry powder vigorously with water persistent. **(ii) Hemolytic Test:** Add drug extract or dry powder to one drop of blood placed on glass slide. Hemolytic zone appears.

7. A TEST FOR FLAVONOIDS: (a) **With NaOH:** The extract was dissolved in water, and then filtered. The filtrate was treated with sodium hydroxide. The yellow color is observed if flavonoids are present. (b) **With H2SO4:** A drop of Conc. H2SO4 acid was added to the above. The yellow color disappears. (c) **With Mg/HCl:** The extracts were dissolved in water, and then filtered. The filtrate was treated with Magnesium and a drop of conc. HCl when added. Pink color development is the indication of the presence of flavonoids.

8. A TEST FOR GUMS AND MUCILAGE: By adding with 95% alcohol if precipitation results show the presence of gums and mucilage.

9. A TEST FOR INORGANIC ELEMENTS: The ash of drug was taken in a test tube and 50% HCl v/v or 50% H No3 v/v was added to. Keep for 1 hr. filter and with filtrate performed following tests. a) **Test for Iron –** To 5 ml test solution add few drops of 5% ammonium thiocyanate.the solution turns blood red. b) **Test for Sulphate –** To 5ml filtrate, add few drops of 5% Bacl2 solution white crystalline Baso4 ppt. Appears that is insoluble in HCl. c) **Test for Chloride –** To 3 ml test solution prepared in NHO3 add few drops of 10% Ag No3 soln. White precipitate. Of AgCl2 observes which is soluble in dilute ammonia solution. d) **Test for Sodium -** To 10 ml filtrate add 2 ml of potassium pyroanthllollate gives a white precipitate. e) **Test for Calcium -** Filtrate with a solution of ammonium carbonate gives a white precipitate which is insoluble in ammonium chloride solution. f) **Test for Potassium -** To 2-3 ml test solution, add a few drops of sodium cobalt nitrate solution. A yellow precipitate of potassium cobalt nitrite observed. g) **Test for Magnesium -** Filtrate with ammonium carbonate solution gives white precipitate but not with ammonium chloride solution.

QUALITATIVE ANALYSIS BY CHROMATOGRAPHY

The T.L.C and H.P.T.L.C are the important and simple analytical tools were used for the qualitative analysis of the raw materials.

FLUORESCENCE ANALYSIS[11]

The Fluorescence properties emitted by *Pterospermum acerifolium* flower powder samples will be examined under Daylight, Long UV of 366 nm and Short UV of 254 nm with the additions of different laboratory chemicals.

Followed by UV VISIBLE SPECTRAL ANALYSIS[12], FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)[13], SCANNING ELECTRON MICROSCOPY (SEM)[14], ATOMIC ABSORPTION SPECTROSCOPY (AAS)[15], NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY[16] (NMR)99 were done.

RESULTS

OBSERVATION DURING THE DRYING THE SAMPLE OF PTEROSPERMUM ACERIFOLIUM FLOWER: Day-1:560 gms, Day-2:556 gms, Day-3:550 gms, Day-4:430 gms, Day-5:404 gms, Day-6:401 gms, Day-7:401 gms, Day-8:401 gms.

PHARMACOGNOSTIC STUDY OF PTEROSPERMUM ACERIFOLIUM FLOWER

MACROSCOPY:

Morphology of drug Pterospermum acerifolium: Polypetalae: As petals are free, 5 in number. Thalamiflorae: Torus small, not expanded. Malvales: Placentation axile, stamens monadelphous. Sterculiaceae: Anthers 2 celled. Helictereae: Flowers hermaphrodite, petals deciduous, androecium columnar below, anthers usually alternating with staminodes. Pterospermum: Anther cells parallel, seeds winged. Bracteoles: Bracteoles laciniate or palmately divided, large tree, bark smooth, leaves 10-14 to 6-12 inches long, roundish or oblong with the stipitate capsule, and hence *Pterospermum acerifolium.*

Macroscopic characters of collected Pterospermum acerifolium Flower:

Size: Sepals: 6 – 7 cm in length, ½ – 1 cm in diameter Petals: 4 – 5 cm in length, ¼ - ½ cm in diameter **Shape**: Bent, curved **Surface:** Externally silky pubescences, Internally ridges

Organoleptic characters of Pterospermum acerifolium Flower: Colour: Sepals: Greenish Brown Petals: Creamish Brown **Odour**: Fragrant **Taste:** Bitter, Astringent **Fracture:** Short, mealy, slightly fibrous

Organoleptic characters of Pterospermum acerifolium Flower Powder: Colour: Brown **Odour**: Fragrant **Taste:** Bitter, Astringent **Touch:** Soft **Shape:** Amorphous

MICROSCOPY OF PTEROSPERMUM ACERIFOLIUM FLOWER

T.S of Sepal: The outline is irregular. The outermost layer is composed of a layer of epidermis consisting of tangentially elongated rectangular cells with the undulating cell wall. The epidermis is enveloped at places with stellate trichomes. Underneath the epidermis 1-2 layered hypodermis is present. Then the wide zone of mesophyll cells is traversed with systematically arranged vascular bundles and air spaces at regular intervals. The cells are made up of parenchyma cells and are filled with Chlorophyll content.

T.S of Petal: The petal is boat-shaped in outline. Below the epidermis mesophyll cells are present. The complete transverse section reveals the numerous oil glands at places in mesophyll area. The cell wall of oil glands is composed of translucent cells. The adhering cells of oil glands are filled with chlorophyll and other cells inclusions. At places, vessels are present in the mesophyll area.

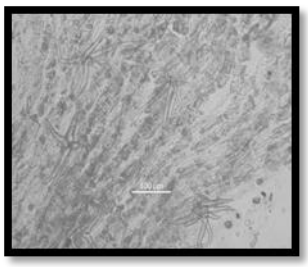


Fig11:Epidermal cells with Trichomes

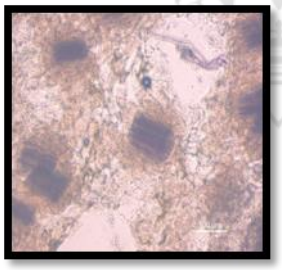


Fig12:Xylem elements

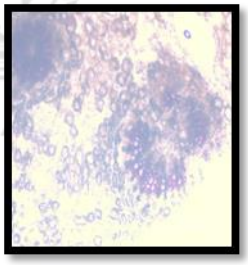


Fig13:Stellate Trichome

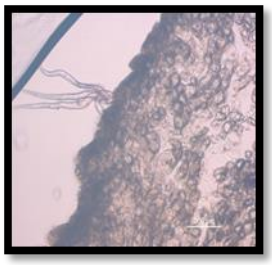


Fig14:Vascular bundle

POWDER MICROSCOPY OF PTEROSPERMUM ACERIFOLIUM FLOWER:

Table 1: Powder microscopy

Contents	Flower
Stone cells	Present
Vessels	Pitted vessels present
Calcium oxalate	Present
Stones	Present
Starch	Traces

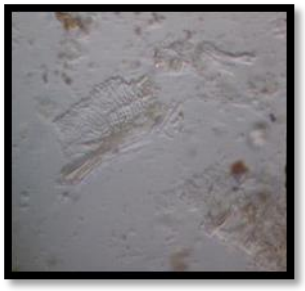


Fig 15: Powder microscopy using Phloroglucinol and conc. HCl

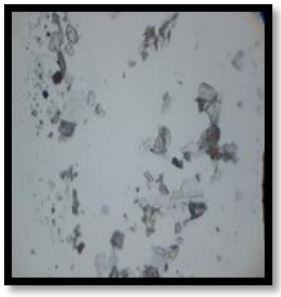


Fig 16: Powder microscopy using chloral hydrate

PHYSICOCHEMICAL ANALYSIS

Determination of Moisture content:

Table 2: Moisture content

Wt. of raw drug (mg)	Wt. after drying (mg)	% of moisture
2000	1830	8.5

Determination of Total Ash:

Table 3: Total Ash

Wt. of raw drug (mg)	Wt. of total Ash (mg)	% of total ash
2000	150	7.5

Determination of Acid Insoluble Ash:

Table 4: Acid insoluble ash

Wt. of raw drug (mg)	Wt. of total Ash (mg)	Wt. of acid insoluble ash (mg)	% of acid insoluble ash
2000	150	1.875	1.25

Determination of Water Soluble Ash:

Table 5: Water soluble ash

Wt. of raw drug (mg)	Wt. of total Ash (mg)	Wt. of water-insoluble ash(mg)	Wt. of water-soluble ash(mg)	% of water-soluble ash
2000	150	141.75	8.25	5.5

Determination of Sulphated Ash:

Table 6: Sulphated ash

Wt. of raw drug (mg)	Wt. of total Ash (mg)	Wt. of sulfated ash (mg)	% of sulfated ash
2000	**150**	**166**	**8.3**

Specific Gravity Estimation: 1.01963

pH Estimation: 5.40 ± 0.10

PHYSICAL CHARACTERS AND SOLVENT EXTRACTIVE VALUES:

Table 7: Extractive values

Sl. No	Solvent	Colour	Consistency	Odor	% of Extractive Value
01	Pet. Ether	Yellowish	Waxy	Characteristic	1.29
02	Benzene	Yellowish	Waxy	Characteristic	1.35
03	Chloroform	Yellowish	Waxy	Characteristic	2.06
04	Acetone	Yellowish Brown	Liquid to Waxy	Characteristic	4.94
05	n Butanol	Reddish Brown	Liquid to Waxy	Characteristic	7.82
06	Water	Yellowish Brown	Liquid to Waxy	Characteristic	12.9

FLUORESCENCE PROPERTIES EMITTED UNDER DAYLIGHT AND UNDER ULTRA-VIOLET RADIATION:

Table 8: Fluorescence Analysis

Sl no	Tests	Day Light	Long UV 366 nm	Short UV 235 nm
1.	Drug powder	Very pale Brown	Dark Brown	Dark Brown
2.	with distilled water	Very light Brown	Brown	Brown
3.	with hexane	Yellowish Brown	Blackish Brown	Blackish Brown
4.	With chloroform	Chocolate Brown	Dark Brown	Dark Brown
5.	with methanol	Yellowish Pale Brown	Yellowish Dark Brown	Yellowish Dark Brown
6.	with acetone	Reddish Brown	Yellowish Brown	Brown
7.	with1N NaOH in water	Chocolate Brown	Bluish Brown	Bluish Brown
8.	with 1 N NaOH in methanol	Brown	Deep Brown	Blackish Brown
9.	with 1N HCL	Yellowish Brown	Deep Brown	Blackish Brown
10.	with H_2SO_4 diluted with an equal volume of water	Faint Brown	Brown	Deep Brown
11.	With HNO_3 diluted with an equal volume of water	Brown	Blackish Brown	Deep Brown

Fig17: Flower under Daylight

Fig18: Flower with NaOH in daylight

Fig19: Flower with NaOH in UV

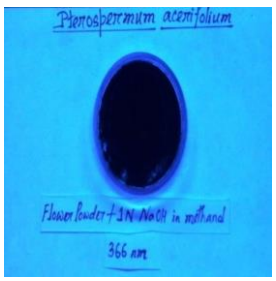


Fig20: Flower with NaOH in UV

PRELIMINARY PHYTOCHEMICAL SCREENING:

Table 9: Phytochemical Screening

Sl.no				
1.	Test for Carbohydrates: a) Benedict's Test b) Fehling's Test c) Polish Test	 - - -	 + + +	 + + +
2.	Test for pentose sugars Solun. + HCl+ Crystals of Phloroglucinol	 -	 -	 -
3.	Test for non-reducing Sugars 3ml soln. + few drops of Iodine	 -	 +	 +
4.	Test for Tannins & Phenolic compounds: a) FeCl3 (5%) b) Potassium c) Dichromate d) Lead acetate e) Dil.HNO_3	 + + + + +	 + + + + +	 + + + + +
5.	Test for Alkaloids: a) Mayer's reagent b)Wagner's reagent c) Dragendroff's reagent d) Hager's reagent	 + - + +	 + - + +	 + - + +
6.	Test for Steroids: a) Salkowski reagent b)Liberman Burchard	 - -	 - +	 - +
7.	Test for Proteins & amino acids: a) Biuret test b) Xanthoprotein test c) ppt test. d) Ninhydrin test	 + + + +	 + + + +	 + + + +
8.	Test for starch: Tannic acid test	+	+	+
9.	Test for Glycosides: Keller-Killiani test	-	+	+
10.	Test for Gum & mucilages: With 95% alcohol	-	+	+
11.	Test for Flavonoids With NaOH With H_2SO_4 With Mg/HCl	 - - -	 - - -	 - - -
12.	Test for Carbohydrates: d) Benedict's Test e) Fehling's Test f) Molish Test	 + - -	 + + +	 + + +

Fig 21: Steroidal test

Fig 21: Saponin test

A TEST FOR INORGANIC COMPONENTS:

Table 10: Inorganic Tests

Sl. no	Test	Result
1.	Test for Iron: Test soln. + Ammonium thiocyanate	+
2.	Test for Calcium a)Solution + Ammonia+ Potassium ferrocynade b)Ammonium carbonate solution + filtrate = insoluble in Ammonium chloride solution	 + +
3.	Test for Copper:Test soln. + Potassium ferocynaide	+
4.	Test for Chlorides:Test soln. + $AgNO_3$	+

TLC PROFILE: 2 spots of orangish-yellow with Rf values 0.27 and 0.85 were seen under long UV before derivatization. They were not seen under visible light. Violet blue 5 spots were seen under visible light (after derivatization) with Rf values of 0.04, 0.14, 0.27, 0.70 and 0.85. Under long UV only 2 spots of blue and red respectively at Rf 0.04 and 0.27 were seen.

Table 11: Rf VALUES

Sl No	Before Derivatization	After Derivatization
01	0.27 and 0.85	0.04, 0.14, 0.27, 0.70 and 0.85

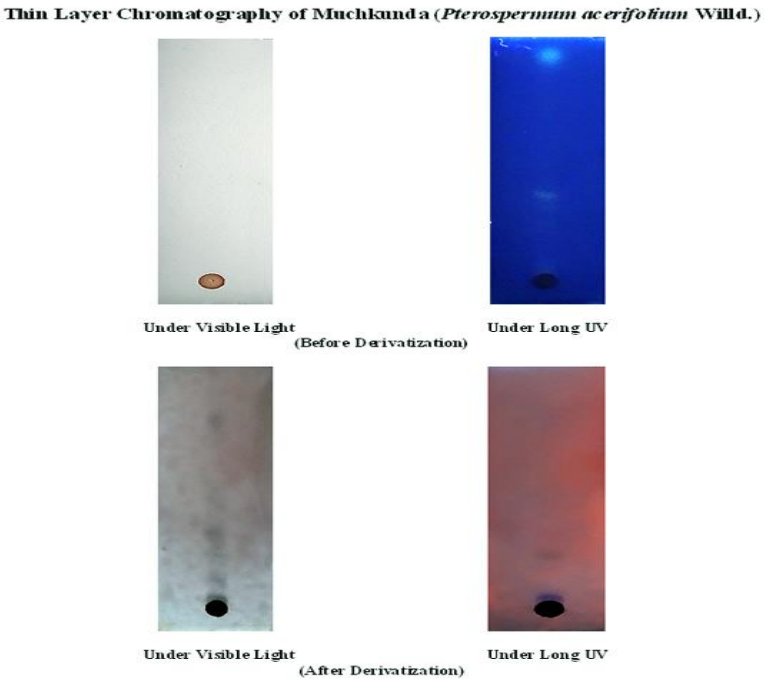
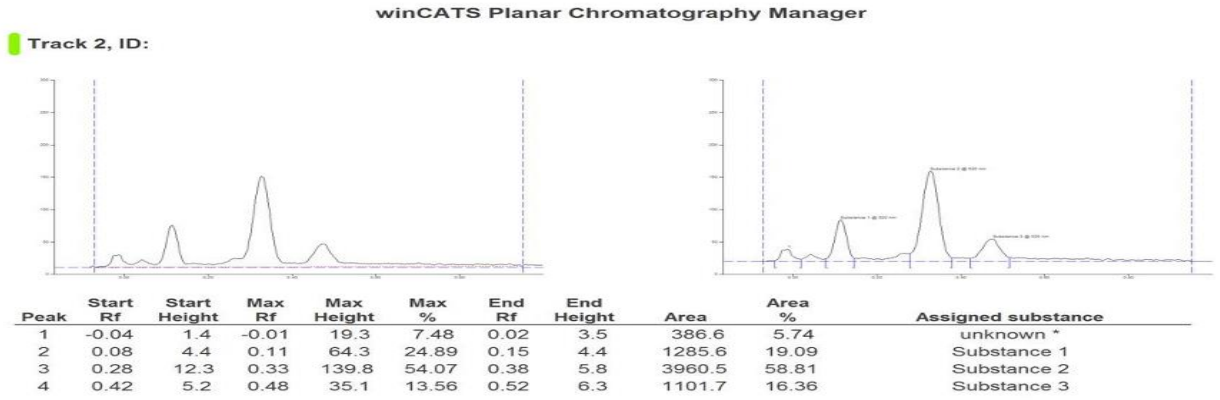


Fig 22: TLC

HPTLC ANALYSIS PROFILE:

Table 12: Rf VALUES

200nm	250nm	300nm	350nm	400nm	450nm
-0.07,-.04,0.04, 0.08,.13,0.42,0 .45,0.48,0.54,0 .69,0.83,0.88,0 .93	-0.09,-0.07,-0.03,0.04, 0.14,0.28,0.42,0.48,0.53 ,0.65,0.69,0.81,0.87,0.93	0.03,0.04,0.08, 0.12,0.14,0.17,0.26,0.32,0.33,0.37,041,0.44,0.48, 0.64,0.77,0.81,0.870.90,0.93	-0.07,-.03,0.04, 0.08,.11,0.14,0.17,0.32,0.33,0.37, 0.42,0.44,0.48,0.64,0.81,83,0.87, 0.90,0.93	-0.07,-.03,0.04, 0.08,.14,0.17,0 .37,0.42,0.44,0 .48,0.69,0.81,0 .87,0.90,0.93	-0.07,-.03,0.04, 0.14,.42,0.65, 0.69,0.81,0.87,0.90,0.93



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.04	1.4	-0.01	19.3	7.48	0.02	3.5	386.6	5.74	unknown *
2	0.08	4.4	0.11	64.3	24.89	0.15	4.4	1285.6	19.09	Substance 1
3	0.28	12.3	0.33	139.8	54.07	0.38	5.8	3960.5	58.81	Substance 2
4	0.42	5.2	0.48	35.1	13.56	0.52	6.3	1101.7	16.36	Substance 3

Fig 23: HPTLC

UV-VIS-SPECTROSCOPY: The UV spectra of the Methanolic, Ethanolic and Chloroform fractions of the sample were recorded and the same has been presented. It shows an absorption peak at 277 nm. The sample shows strong absorption in the range 250-300 nm. This indicates the presence of flavonoid quercetin in the sample as evident from previous studies. This comprehensive analysis of the flavonoid components of herb *Pterospermum acerifolium* will be helpful for the quality control of this herb and its products, and to understand the usage and function.

Table 13: Showing the analysis results of UV-Vis-Spectroscopy

Sl. No	No. of Peaks obtained	Wavelength(nm)
1.	One	277 nm

FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR) ANALYSIS:

Table 14: FTIR Analysis

Sl. no	Wavenumber (cm^{-1})	Probable Chemical Compound
1.	3414.00	Hydroxyl (OH) or Amine (NH_2) group
2.	2924.09	Asymmetric C-H stretching vibration
3.	2866.22	Symmetric C-H stretching vibration
4.	2360.87	Alkyne group
5.	2129.41	Isonitrile group
6.	1739.79	Bending CH Vibration
7.	1620.21	Carbonyl group
8.	1384.89	Bending CH Vibration

ATOMIC ABSORPTION SPECTROSCOPY (AAS) ANALYSIS:

Table 15: AAS Analysis

Sl No.	Element	Concentration (ppm)
1.	Copper (Cu)	0.3221
2.	Chromium (Cr)	0.4032
3.	Zink (Zn)	0.8223
4.	Manganese (Mn)	1.2704
5.	Lead (Pb)	0.0796
6.	Iron (Fe)	9.5551
7.	Nickel (Ni)	0.0347
8.	Calcium (Ca)	227.2983
9.	Magnesium (Mg)	3.5519

NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY (NMR) ANALYSIS

The reports of ^{13}C NMR (300MHz, $CDCl_3$) are as follows,

Table 16: NMR Analysis

Sl. no	δ (Peak)	Probable Chemical group
1.	206.857	Aldehyde or Ketone group
2.	155.910	Amide or Carboxylic group
3.	145.477	Isonitrile group
4.	115.699	Alkene or Aryl group
5.	30.690, 30.433, 30.177, 29.921, 29.151	Alkyl group or Alkyl halide group

DISCUSSION AND CONCLUSION

The flower samples were shade dried in accordance with Ayurvedic pharmacopeia. From Day 1 to Day 8 it was observed that a total reduction in weight from initial 560 grams to a constant of 401 grams was 159 grams. The overall variation of temperature during the whole period of drying the sample was 2°C. The end weight result of 28.32% reduction in weight from initial implies 28-29% of Water and Volatile oil content in the sample which got evaporated during drying of the sample.

Extracts were prepared in Soxhlet apparatus in Non-polar (Pet. Ether, Chloroform), Polar Protic and Polar Aprotic (Methanol, Water) Solvents. The highly nonpolar chemical constituents got extracted in highly nonpolar solvent Pet. Ether with Dipole moment 0.00 D whereas less non polar chemical constituents got extracted in less non polar solvent Chloroform with Dipole moment of 1.04 D. The highly polar chemical constituents got extracted in highly polar solvent Methanol with Dipole moment 1.70 D whereas polar chemical constituents with Amine (NH_2), Hydroxyl (OH) etc which can join water molecules with hydrogen bonding got extracted in less polar solvent Water with Dipole moment of 1.85 D.

The end product after complete preparation were 25ml, 32.25 mg, 51.5 mg, 228 mg, and 322.5 mg from the initial amount of the drug of 25 gram inferring 1.29%, 2.06%, 9.12% and 12.9% extracted drug amount in Pet. Ether, Chloroform, Methanol and distilled water respectively. They all had a characteristic odor, color and were of Semi solid to Waxy inconsistency.

The phytochemical study has a very important role in the standardization of any single or compound drug. As far as the herbal drugs are concerned various phytochemical parameters may be applied to evaluate, standardize as well as to compare any particular drug with the standard drug. Though '*Pterospermum acerifolium* ' is very much devoid from adulteration, it was essential to compare the various dosage form and extracts of *Pterospermum acerifolium* with standard parameters and to give a basis for the experimental and clinical trials so the physicochemical analysis was carried out. The moisture content of the sample is 5.5 %. This tells that it is less prone to any type of infection or fungal growth.

Ash value is usually done to find the presence of inorganic matter in the given sample. Ash value of the flower is 4.5 %. This suggests that the flowers of *Pterospermum acerifolium* have 4.5 % of inorganic matter as its structural parts. This inorganic part can be metals like-iron, copper etc and silica matter.

Acid-insoluble ash is usually done to find out the amount of silica content in the given sample[53]. Acid-insoluble ash of *Pterospermum acerifolium* flower is 0.498%. This tells that there very less amount of silica present in the sample.

Different solvent extractive values of *Pterospermum acerifolium* flower in Pet. Ether, Benzene, Chloroform, Acetone, n Butanol, Methanol, Ethanol, and Water are 1.29, 1.35, 2.06, 4.94, 7.82, 9.12, 9.44, and 12.9 % respectively. Preliminary Phyto-Chemical Analysis of *Pterospermum acerifolium* Flower shows Steroids that was found to be present in Aqueous extract. qualitative analysis by TLC and H.P.T.L.C reveals 2 spots of orangish-yellow with Rf values 0.27 and 0.85 were seen under long UV before derivatization. They were not seen under visible light. Violet blue 5 spots were seen under visible light (after derivatization) with Rf values of 0.04, 0.14, 0.27, 0.70 and 0.85. Under long UV only 2 spots of blue and red respectively at Rf 0.04 and 0.27 were seen. It is observed that the Rf values of the phytoconstituents correspond to Flavonoids which may be responsible for Anti Inflammatory activity of *Pterospermum acerifolium.*

The UV spectra of the Methanolic, Ethanolic and Chloroform fractions of the sample were recorded and the same has been presented. It shows an absorption peak at 277 nm. The sample shows strong absorption in the range 250-300 nm. The UV spectra of the Methanolic, Ethanolic and Chloroform fractions of the sample were recorded and the same has been presented. It shows an absorption peak at 277 nm. The sample shows strong absorption in the

range 250-300 nm. Identification of the phenolic components of chrysanthemum flower (Chrysanthemum morifolium Ramat) Long-Ze Lin *, James M. Harnly Food Composition and Methods Development Laboratory, Beltsville Human Nutrition Research Center, Agricultural Research Service, US Department of Agriculture,10300 Baltimore Avenue, Building 161, BARC-East, Beltsville, MD 20705, the USA reported, Fifteen caffeoylquinic acids and 15 flavonoids were positively identified and the remaining compounds were provisionally identified were UV . max (nm) (MeOH): 277. So the absorption at 277 nm in the sample was expected and can be seen in the UV spectra of the sample. Thus probably indications of the presence of flavonoid quercetin in the sample[53,56]. This comprehensive analysis of the flavonoid components of herb *Pterospermum acerifolium* will be helpful for the quality control of this herb and its products, and to understand the usage and function.

Fourier transform infrared (FTIR) spectroscopy is a measurement technique that allows one to record infrared spectra, indicates towards the presence of Hydroxyl (OH) or Amine (NH_2) group, Alkyne group, Isonitrile group, Carbonyl groups in the drug. Atomic absorption spectroscopy (AAS) is a spectroanalytical procedure for the quantitative determination of chemical elements employing the absorption of optical radiation (light) by free atoms in the gaseous state. The concentrations of different elements obtained in the *Pterospermum acerifolium* flower sample suggestive of highest elemental constituent of Calcium (Ca), Iron (Fe), Magnesium (Mg), Manganese (Mn), Zinc (Zn), Chromium (Cr), Copper (Cu), Lead (Pb), and Nickel (Ni) in decreasing order. The results obtained are described are in parts per million, indicative of safety in oral dosage forms as all the values are below highest permissible limits for Lead, Copper etc. The peaks obtained in NMR Spectroscopy is indicative of the presence of aldehyde or ketone group of compounds, amides or carboxylic group, Isonitriles, alkene or aryl group, alkyl or alkyl halide group of compounds in the drug *Pterospermum acerifolium.*

To conclude, the studies were conducted in view to standardize the drug and to find out the adulteration. The article will help the researchers of Ayurveda as well as in another field of Bio-medical sciences to explore more about the said tree for the larger benefit of society.

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