



Physicochemical and Phytochemical Investigation of the Roots of *Hydnocarpus pentandrus* (Buch.-Ham.) Oken

*Joshi Arun B, Harijan Kiran C

Department of Pharmacognosy, Goa College of Pharmacy, Panaji, Goa, India.

*Corresponding author's E-mail: visitkk@rediffmail.com

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ABSTRACT

The present study was aimed to evaluate the physicochemical and phytochemical parameters of the roots of *Hydnocarpus pentandrus* (Buch.-Ham.) Oken belonging to the family Achariaceae, formally known as *Hydnocarpus pentandra* (Buch.-Ham.) Oken (Flacourtiaceae). The oil extracted from seeds of *Hydnocarpus* species is called chaulmoogra oil, used in leprosy. *H. pentandrus* an endangered species found in Western Ghats region of India has been exploited traditionally against leprosy, rheumatic pain, inflammation and skin diseases. The physicochemical and phytochemical profile confirms the purity and authenticity of *H. pentandrus*. The physicochemical methods revealed the presence of moisture content as 7.1% w/w, total ash as 5.3%w/w, Acid insoluble ash 1.75%w/w, water soluble ash 1.5%w/w, foaming index less than 100, swelling index 0.6 cm, alcohol soluble extractive 3.3% w/w, water soluble extractive 7.2 % w/w, and ether soluble extractive 1.9 % w/w as well as fluorescence analysis which was carried out in visible, short and long wavelength. Phytochemical screening of alcoholic extract of *H. pentandrus* roots revealed the presence of glycosides, flavonoids, carbohydrates, proteins, tannins, saponins, steroids and triterpenoids.

Keywords: Achariaceae, Chaulmoogra oil, *Hydnocarpus pentandrus*, Physicochemical, Phytochemical.

INTRODUCTION

Hydnocarpus pentandrus (Buch.-Ham.) Oken belonging to the family Achariaceae also known as *Hydnocarpus pentandra* (Buch.-Ham.) Oken, family flacourtiaceae. Synonyms are *H. laurifolia* Sleumer and *H. wightiana* Blume. The genus *Hydnocarpus* is the major group of angiosperms. The seed oil from *Hydnocarpus* species is known as chaulmoogra oil and used in leprosy.^{1,2} *H. pentandrus* is found in tropical forests along the Western Ghats, the Konkan southwards and below the Ghats in Kanara and Malabar in damp conditions especially near water. It is also very common in Travancore up to 609 m. Flowering and fruiting is almost throughout the year chiefly during January-may.³ Traditionally the seeds and oil are acrid, astringent, bitter, emollient, vermifuge, anodyne purgative, emetic, carminative, stomachic, haematinic and tonic. They are useful in leprosy, skin diseases, pruritis, leucoderma, dermatitis, bronchopathy, eczema, sprains, bruises, chronic ulcers, dyspepsia, colic, flatulence, diabetes, wounds, ulcers and scald-head.⁴

Flavonolignan Hydnocarpin, Isohydnocarpin, methoxyhydnocarpin along with apigenin, luteolin and chrysoeriol isolated from the seed and seed hull of *H. wightiana*.⁶ Gas-liquid chromatography analysis has shown the oil to contain the following fatty acids - hydnocarpic acid, chaulmoogric acid, gorlic acid, lower cyclic homologues, myristic acid, palmitic acid, stearic acid, palmitoleic acid, oleic acid, linoleic acid and linolenic acid. The oil was used intravenously or intramuscularly in the early part of the twentieth century against leprosy.⁷ The stem bark and leaves contain triterpenes, acetylbutulinic, betulinic ursolic and

acetylursolic acids. Hydnocarpin showed good anti-inflammatory and antineoplastic activity in mice.⁸

The whole plant extract of *H. pentandra* had free radical scavenging activity.⁹ Ethanolic extract of seed hull of *H. Wightiana* showed antidiabetic activity.¹⁰ The methanol extract and their petroleum ether, carbon tetrachloride and chloroform soluble fractions of leaf extract showed significant antibacterial activity.¹¹ Extensive literature survey revealed that no substantial work has been carried out from the roots of *H. pentandrus* hence the effort has been made to establish physicochemical, fluorescence analysis and preliminary phytochemical screening on the roots of *H. pentandrus*.

MATERIALS AND METHODS

Plant Collection

Fresh roots were collected from fully grown plants of *H. pentandrus* from Savardem Sattari Goa in the month of October 2013. It was authenticated by Dr. K. Gopalkrishna Bhatt, dept. of botany, Poornaprajna College, Udupi, Karnataka and by Mr. Dinesh Nayak (Sasyashymala) advisor green belt Mangalore -India.

Preparation of Ethanolic Extract

The roots were then washed thoroughly to remove the soil and adhering materials and dried in shade for 3 days. The dried roots were then powdered and used for physicochemical evaluation, fluorescence analysis and preparation of ethanolic extract. The dried root powder (500g) was exhaustively extracted by maceration with ethanol (95%) for three days. After three days ethanolic layer was decanted off. The process was repeated thrice.

The solvent from the total extract was distilled off using Rotary vacuum evaporator (superfit-supervac) and the concentrate was evaporated to dryness and then used for the preliminary phytochemical investigation.

Physicochemical Investigation^{12,13}

Determination of Swelling Index

Many medicinal plant materials are of specific therapeutic or pharmaceutical utility because of their swelling properties, especially gums and those containing an appreciable amount of mucilage.

Procedure: Carry out simultaneously not less than 3 determinations of any material. Introduce the quantity of the individual plant material, previously reduced to the required fineness and accurately weighed, into a 25ml glass stoppered measuring cylinder. The length of the graduated portion of the cylinder should be about 125 mm, the internal diameter about 16 mm subdivided in 0.2 ml and marked from 0 to 25 ml in an upward direction. Add 25 ml of water unless otherwise indicated in the test procedure, and shake the mixture thoroughly at intervals of every 10 minutes for 1 hour. Allow to stand for 3 hours at room temperature, or as otherwise given. Measure the volume in ml occupied by the plant material, including any sticky mucilage. Calculate the mean value of the individual determinations, related to 1 gm of plant material.

Determination of Foaming Index

Procedure: Reduce about 1 gm of the plant material to a coarse powder, weigh accurately and transfer to a 500 ml conical flask containing 100 ml of boiling water. Maintain at moderate boiling for 30 minutes. Cool and filter into a 100 ml volumetric flask and add sufficient water through the filter to dilute the volume to 100 ml. Place the above decoction into 10 stoppered test tubes (height 16 cm, diameter 16 mm) in a series of successive portions of 1, 2, 3 up to 10 ml and adjust the volume of the liquid in each tube with water to 10 ml. stopper the tubes and shake them in a lengthwise motion for 15 seconds, 2 frequencies per second. Allow to stand for 15 min and measure the height of the foam.

If the height of the foam in every tube is less than 1 cm, the foaming index is less than 100. If in any tube a height of foam of 1 cm is measured, the dilution of the plant material in this tube [a] is the index sought. If this tube is the first or second tube in a series, it is necessary to have an intermediate dilution prepared in a similar manner to obtain a more precise result. If the height of the foam is more than 1 cm in every tube, the foaming index is over 1000. In this case the determination needs to be made on a new series of dilutions of the decoction in order to obtain a result.

Calculation

Foaming index = $1000/a$

Where 'a' is the volume in ml of the decoction used for preparing the dilution in the tube where foaming is observed.

Determination of Loss on Drying

This test determines both water and volatile matter. Drying can be carried out either by heating to 100- 105°C or by drying in a desiccator over phosphorus pentoxide R under atmospheric or reduced pressure at room temperature for a specified period of time. The desiccation method is especially useful for materials that melt to a sticky mass at elevated temperatures.

Gravimetric determination: Weigh accurately about 2-5 gm of the prepared material, or the quantity given in the test procedures, in a previously dried and tared flat weighing bottle. Dry the sample in an oven at 100 - 105°C for 5 hours. Dry until two consecutive weighings do not differ by more than 5 mg, unless otherwise required in the test procedure. Calculate the loss of weight in mg per gm of air dried material.

Determination of Extractible Matter

This method determines the amount of active constituents in a given amount of medicinal plant material extracted with solvents. It is employed for those materials for which as yet no suitable chemical or biological assay method exists.

Procedure:

Cold maceration method

Weigh accurately about 4 gm of coarsely powdered air dried material to a glass stoppered conical flask. Macerate with 100 ml of the required solvent for the given plant material for 6 hours, shaking frequently, then allowing to stand for 18 hours. Filter rapidly taking care not to lose any solvent, transfer 25 ml of the filtrate to a tared flat bottomed dish and evaporate to dryness on a water bath. Dry at 105°C for 6 hours, cool in desiccator for 30 minutes and weigh without delay. Calculate the content of extractable matter in mg per gm of air dried material. For ethanol soluble extractable matter use ethanol as solvent and for water soluble extractable matter use water as the solvent.

Determination of Ash

The presence of ash in the medicinal plants materials is determined by three different methods.

Total ash

This test is designed to measure the amount of material remaining after ignition. "Physiological ash" is derived from the plant tissue itself and "Non Physiological Ash" is the residue after ignition of the extraneous matter adhering to the surface. The present procedure determines both kinds of ashes and is referred to as the "total ash" test.



Procedure

Weigh accurately into a previously ignited and tared crucible, usually platinum or silica, 2 gm of ground material. Spread the material in an even layer in the crucible, ignite the material by gradually increasing the heat to 500-600°C until free from carbon, cool in a desiccator and weigh.

Acid insoluble ash

Acid insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid and igniting the washed insoluble matter left on the filter. This determination measures the presence of silica, especially sand and siliceous earth.

Procedure

To the crucible containing total ash, add 25 ml of hydrochloric acid. Cover with watch glass and boil gently for 5 min. Rinse the watch glass with 5 ml of hot water and add this liquid to the crucible. Collect the insoluble matter on an ash-less filter paper and wash with hot water until the filtrate is neutral. Transfer the filter paper containing the insoluble matter to the original crucible, dry on hot plate and ignite to constant weight. Allow the residue to cool in a desiccator for 30 min and weigh without delay. Calculate the content of acid insoluble ash in mg per g of air dried material.

Water-soluble ash

Water soluble ash is the calculated difference in weight between the total ash and the residue remaining after treatment of the total ash with water.

Procedure

To the crucible containing the total ash add 25 ml of water and boil for 5 minutes. Collect the insoluble matter in ash-less filter paper. Wash with hot water and ignite for 15 minutes at a temperature not exceeding 450°C. Subtract the weight of this residue in mg obtained from the weight of total ash. Calculate the content of water soluble ash in mg per gm of air dried material.

Determination of Fluorescence Analysis¹⁴

Powdered roots of the plant *H. pentandrus* was subjected to analysis under ultraviolet light (254 and 366 nm) and visible light after treatment with various chemical and organic reagents as shown in the table 2.

Preliminary Phytochemical Screening¹⁵⁻¹⁷**Qualitative Analysis**

The preliminary phytochemical studies were performed for testing different phytoconstituents present in the ethanolic extract. The chemical group tests were performed and the results are tabulated in Table-3.

Alkaloids

- Dragendroff's Test: To 2 mg of the ethanolic extract, 5 ml of distilled water was added, 2M hydrochloric

acid was added until an acid reaction occurs. To this 1 ml of Dragendroff's reagent was added. Formation of orange red precipitate indicates the presence of alkaloids.

- Hager's Test: To 2 mg of the ethanolic extract taken in a test tube, a few drops of Hager's reagent were added. Formation of yellow precipitate confirms the presence of alkaloids.
- Wagner's Test: To 2 mg of the ethanolic extract, 1 ml of dilute Hydrochloric acid was added along with few drops of Wagner's reagent. A yellow or brown precipitate indicates the presence of alkaloids.
- Mayer's Test: To 2 mg of ethanolic extract, a few drops of Mayer's Reagent were added. Formation of white or pale yellow precipitate indicates the presence of alkaloids.

Glycosides**Test for Cardiac Glycosides**

- Balget's test: To 2 mg of the ethanolic extract, a solution of sodium picrate was added. Formation of yellow colour indicates the presence of cardiac glycosides.
- Legal's test: To 2 mg of the ethanolic extract, 1 ml of pyridine and 1 ml of sodium nitroprusside was added. Formation of pink colour indicates the presence of cardiac glycosides.
- Keller-Kiliani Test: To 2 mg of ethanolic extract, 1 ml of Glacial Acetic acid, one drop of ferric chloride and 1 ml of concentrated sulphuric acid was added. Appearance of reddish brown colour at the junction of the two liquid layers and development of bluish green colour in the upper layer indicates the presence of cardiac glycosides.
- Liebermann Test: To 2 ml of the ethanolic extract, 3 ml of acetic anhydride was added. Heat and cool. To this add few drops of concentrated sulphuric acid. Formation of blue colour indicates the presence of cardiac glycosides.

Test for Anthraquinone Glycosides

- Borntrager's Test: To 2 mg of the ethanolic extract, 5 ml of dilute hydrochloric acid was added. Boiled and filtered. To the filtrate equal volume of chloroform was added. Shake. To this organic layer ammonia was added. Appearance of pink colour indicates the presence of anthraquinone glycosides.
- Modified Borntrager's Test: To 2 mg of ethanolic extract, 5 ml of dilute hydrochloric acid and 5 ml of 5% ferric chloride was added, boiled and filtered. To the filtrate equal volume of chloroform was added. Shake. To this organic layer ammonia was added. Appearance of pink colour indicates the presence of anthraquinone glycosides.



Test for Coumarin Glycosides

- **FeCl₃ Test:** To the concentrated alcoholic extract of drug, few drops of alcoholic FeCl₃ solution was added. Formation of deep green colour, which turns yellow on addition of conc. HNO₃ indicates the presence of coumarin.
- **Fluorescence Test:** Take moistened powder in a test tube, cover the test tube with filter paper soaked in dilute NaOH. Keep in water bath, after sometime expose the filter paper to UV light. It shows yellowish green fluorescence.

Table 1: Results of physicochemical test on the powdered roots of *H. pentandrus*.

Physicochemical Test	Mean % values
Determination of Swelling Index	0.6 cm
Determination of Foaming Index	<100
Determination of extractive value	
i. Alcohol soluble extractive of sample	3.3 %w/w
ii. Water soluble extractive of sample	7.2 %w/w
iii. Ether soluble extractive of sample	1.9 %w/w
Determination of moisture content	7.1 %w/w
Determination of ash values	
i. Total ash	5.3 %w/w
ii. Acid insoluble ash	1.75 %w/w
iii. Water soluble ash	1.5 %w/w

Carbohydrates

- **Benedict's test:** To 1 mg of the ethanolic extract, 5 ml of Benedict's solution was added and boiled for 5 minutes. Formation of brick red precipitate indicates the presence of carbohydrates.
- **Fehling's Test:** To 2 mg of ethanolic extract, 1 ml mixture of equal parts of Fehling's solution A and B were added and boiled for few minutes. Formation of red or brick red precipitate indicates the presence of reducing sugars.
- **Molisch's Test:** In the test tube containing 2 mg of the ethanolic extract, 2 drops of freshly prepared 20% alcoholic solution of α -naphthol was added. To this 2 ml of concentrated sulphuric acid was added so as to form a layer below the mixture. Red-violet ring appears indicating the presence of carbohydrates which disappears on the addition of excess of alkali.

Flavonoids

- **Shinoda Test:** To 2 mg of the ethanolic extract, 10 drops of dilute Hydrochloric acid followed by a piece of magnesium were added. Formation of pink, reddish or brown colour indicates the presence of flavonoids.
- **Lead Acetate Test:** To 2 mg of the plant extract, lead acetate solution was added. Formation of yellow precipitate indicates the presence of flavonoids.

- **Vanillin-HCL Test:** To the alcoholic solution of the drug vanillin HCl was added, formation of pink colour indicates the presence of flavonoids.

Proteins

- **Biuret's Test:** To 2 mg of ethanolic extract, 5-8 drops of 10% w/v sodium hydroxide solution, followed by 1-2 drops of 3% w/v copper sulphate were added. Formation of violet red colour indicates the presence of proteins.
- **Millon's Test:** To 2 mg of ethanolic extract, 1 ml of distilled water and 5-6 drops of Millon's reagent were added. Formation of white precipitate which turns red on heating indicates the presence of proteins.

Tannins

To 2 mg of the ethanolic extract, a few drops of 5 % ferric chloride solution were added. Formation of green colour indicates the presence of gallotannins, while the formation of brown colour indicates the presence of psuedotannins.

Resins

To 2 mg of the ethanolic extract dissolved in acetone and the solution was poured in distilled water. Formation of turbidity indicates the presence of psuedotannins.

Saponins

- **Foam Test:** Shake the ethanolic extract vigorously with water. Formation of persistent foam indicates the presence of saponins.

Triterpenoids

- **Liebermann-Burchard's Test:** To 2 mg of the ethanolic extract, 1 ml of acetic anhydride was added, boiled and cooled and then 1 ml of concentrated sulphuric acid was added along the sides of the test tube. Formation of brown ring at the junction of the two layers and upper layer turns deep red colour indicates the presence of triterpenoids.
- **Salkowski Test:** To 2 mg of the ethanolic extract, 2 ml of chloroform and 2 ml of concentrated sulphuric acid was added. Shake. Appearance of yellow colour in the lower layer indicates the presence of steroids.

Steroids

- **Liebermann-Burchard's Test:** To 2 mg of the ethanolic extract, 1 ml of acetic anhydride was added, boiled and cooled and then 1 ml of concentrated sulphuric acid was added along the sides of the test tube. Formation of brown ring at the junction of the two layers and upper layer turns green colour indicates the presence of steroids.
- **Salkowski Test:** To 2 mg of the ethanolic extract, 2 ml of chloroform and 2 ml of concentrated sulphuric acid added. Shake. Appearance of red colour in the lower layer indicates the presence of steroids.

Table 2: Results of fluorescence analysis of powdered roots of *H. pentandrus*.

Drug + Reagent	Day light	Short wavelength 254 NM	Long wavelength 366 NM
Powder	Cream	Light brown	Cream
Powder + Water	Cream	Light pink	Light blue
Powder + Ethanol	Cream	Light brown	Light green
Powder + Methanol	Cream	Cream	Aqua blue
Powder + Ammonia	Cream	Light green	Green
Powder + Toluene	Cream	Light brown	Blue
Powder + Acetone	Light cream	Light pink	Sky blue
Powder + Picric Acid	Light yellow	Yellow	Yellow
Powder + Solvent Ether	Cream	Violet	Bright blue
Powder + Pet. Ether	Cream	Light pink	Light blue
Powder + Ethyl Acetate	Cream	Light pink	Light blue
Powder + Chloroform	Cream	Light brown	Light blue
Powder + 10%HCL	Cream	Pinkish tinge	Very light blue
Powder + 10% NaOH	Brown	Brown	Green
Powder + 10% H ₂ SO ₄	Cream	Brown	Bluish green
Powder + 50% Alc. NaOH	Cream	Light brown	Light green
Powder + 50% Aq. NaOH	Orange	Brown	Light Green
Powder + Conc. H ₂ SO ₄	Brownish black	Brownish black	Black
Powder + Conc. HNO ₃	Light brown	Brown	Dark brown
Powder + Conc. HCL	Brown	Brown	Brown

Starch

0.01 gm of iodine and 0.075 gm of KI were dissolved in 5 ml of distilled water and 2.3 ml of extract was added. Formation of blue colour indicates the presence of starch.

RESULTS AND DISCUSSION

The root of *H. pentandrus* was subjected to systemic physicochemical, fluorescence and preliminary phytochemical analysis. The data generated is helpful in determining the quality and the purity of the crude drug, especially in the powdered form. In this study the parameters included for the evaluation of *H. pentandrus* root were moisture content, ash values (total ash, water soluble ash and acid insoluble ash), extractive values using alcohol, water and ether as solvents, swelling index, foaming index. The alcohol soluble extractive indicated the presence of polar constituents like phenols, flavonoids etc. The total ash is particularly important in the evaluation of purity of drugs i.e. the presence or absence of foreign matter such as metallic salts or silica (Table 1). The fluorescence analysis performed showed a wide range of fluorescent colours for the crude drug with different reagents (Table 2). Fluorescence study of root bark powder helps in qualitative evaluation which can be used for its identification. The preliminary phytochemical screening of the ethanolic extract was performed and it was found to contain glycosides, flavonoids, carbohydrates, proteins, tannins, saponins, steroids and triterpenoids (Table 3).

Table 3: Results of preliminary phytochemical screening of the ethanolic extract of *H. Pentandrus* roots.

Preliminary Phytochemical Test	Result
Alkaloids	-
Glycosides	++
Carbohydrates	+
Flavanoids	+
Proteins	+
Tannins	+
Resins	-
Saponins	+
Triterpenoids	+
Steroids	+
Starch	-

CONCLUSION

As no substantial work has been carried out on the roots of *H. pentandrus*, the physicochemical parameters, fluorescence analysis and phytochemical screening performed in this study will guide in pharmacological and therapeutic evaluation of the species and will assist in standardization for quality, purity, and identification of drugs. In conclusion the parameters reported in the study will be useful in the development of further studies on the plant.

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