



RESEARCH ARTICLES

Pharmacognostic investigation of the leaves of *Hydnocarpus pentandrus* (Buch. -Ham)Oken

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Received: 12 March 2025 / Revised: 10 August 2025 / Accepted: 12 August 2025
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Abstract

Pharmacognosy is the study of medicinal drugs derived from plants or other natural sources. According to the American Society of Pharmacognosy, Pharmacognosy is "the study of the physical, chemical, biochemical, and biological properties of drugs, drug substances, or potential drugs or drug substances of natural origin as well as the search for new drugs from natural sources." The present study is of *Hydnocarpus pentandrus* belonging to the family Achariaceae. It includes physicochemical parameters such as total ash, water soluble ash, acid insoluble ash values, phytochemical analysis, quantitative phytochemical screening, extractive values, fluorescence analysis & thin layer chromatography. These are also necessary for the accurate identification of the crude drug *Hydnocarpus pentandrus* (Buch. -Ham)Oken. The leaves yield dulcitol, kaempferol, quercetin, and hydnocarpin, which are used as medicines to treat a variety of illnesses.

Keywords *Hydnocarpus pentandrus* · Phytochemical screening · Ash value · Extractive values

Introduction

A natural product is a chemical substance produced by living organisms such as plants, mushrooms, animals and microorganisms. Relevantly, Pharmacognosy, as is well-known, the science of drugs of natural origins, is one of the main disciplines in pharmaceutical sciences. In other words, pharmacognosy is described as the systematic science of morphological, chemical and biological properties along with history, cultivation, collection, extraction, isolation, bio-assaying, quality control and preparation of crude drugs from natural origin. Seydler, the German botanist, was the first one to use the term "Pharmacognosy" (Brady 1988).

Plants have always been used as medicine by mankind to treat health-threatening diseases, and are still popular for obtaining new drug candidates, as it is the oldest medical

practice for humans. The usage of botanical natural health products is increasing all over the world. It is known that almost 80% of the population in developing countries relies on traditional medicine, mainly composed of herbal prescriptions. Some compounds are used as active ingredients in the form directly isolated from plant extracts; others are synthesized to mimic a natural plant compound. Therefore, natural compounds could be good models for developing novel drug molecules. Modelling or modifying is an important action for the drug industry (Mahady 2001).

There is a rising demand for plant-based medicines, pharmaceuticals, food supplements, health products, cosmetics etc., which are derived from plants. From time immemorial, various societies have resorted to nature, mainly to plants, as medicine and health sources. Today, a large number of people, particularly in underdeveloped and developing countries, use these traditional plants for primary needs of medicinal assistance (Tene 2007). As an estimate of the World Health Organization (WHO), about 80% of the world population still uses these herbs and other traditional medicines for their medicinal use. Polysaccharides from medicinal herbs have received a lot of significance in recent decades because of their notable bioactivities, such as anti-proliferative, antioxidant, anticoagulant, radioprotection effect, antiviral activity, hypolipidemic, antidiabetic, and immunomodulatory activities, which make them worthy for

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medicinal uses. Traditional medicines and medicinal herbs are widely used as curative agents in most underdeveloped countries for maintaining sound health. The antibacterial, antioxidant, and antidiabetic actions of phytochemicals present in these plants could explain their medicinal properties (Cowan 1999). So, these plants should be investigated to have good compression of their qualities, protection, and efficacy (Nascimento 2000).

When the term “natural product” is mentioned, it can be the organism itself (plant, animal and microorganism), a leaf or flower of a plant, an isolated gland or other organ of an animal, an extract or pure substances. Doubtlessly, plants are prolific sources of new bioactive chemicals such as atropine, ephedrine, morphine, caffeine, salicylic acid, digoxin, taxol, galantamine, vincristine, colchicine, etc. Approximately 25% of the prescription drugs dispensed in the United States contain at least one active ingredient of plant origin (Ahmad 2006).

From the previous pharmacological screening, it has confirmed the claimed effect of *Hydnocarpus* species on leprosy has been confirmed. Cyclopentenyl fatty acids isolated from the seed oil have been shown to underlie this effect. Another interesting class of compounds, the flavonolignans, has shown several pharmacological activities. Extracts from different species have been screened and show various pharmacological activities, *in-vivo* and *in-vitro*. Hence, herein, we review the Pharmacognostic investigation of the leaves of *Hydnocarpus pentandrus* and to instigate researchers globally to explore their full potential.

Globally, the genus *Hydnocarpus* includes approximately forty species of shrubs and trees. It is commonly used in the Indian System of Medicine, including Ayurveda and Siddha, four species are found in India (Mudaliar 1936; Sharma and Hall 1991). *Hydnocarpus* species are used widely for both traditional and modern means, primarily in Southeast Asia. *Hydnocarpus pentandrus* (Buch. -Ham)Oken belongs to the family Achariaceae is one of the most medicinally diverse families in the plant kingdom and is a rich source of drugs that are used both in traditional and conventional medicine practices. It is commonly known as *Hydnocarpus* oil tree, chaulmoogra, kuduresotte, Garudaphala, Tuvarak etc. (Joshi and Harijan 2014). It has about 31 genera and 197 species of trees and shrubs that are distributed in tropical and subtropical regions of Africa, Asia and the Americas. It is predominantly found in moist deciduous and semi-evergreen forests up to 850 m (about 2788.71 ft) in the Western Ghats, often near water bodies. In India, it is most commonly found in Maharashtra, Daman & Diu, Goa, Karnataka, Tamil Nadu and Kerala, but it is also cultivated elsewhere (Hooker 1883).

More detailed pharmacognostic standards for the correct identification and detection of adulteration and/or

substitution of these plants have not been reported. Owing to their simplicity and cheaper costs, pharmacognostic studies are an appropriate means by which manufacturers of herbal medicines can establish correct identification of the source materials, intended to produce herbal products. Standardizing these plants through pharmacognostic investigations is a sensible research approach. Pharmacognostic studies are a suitable method for herbal medicine manufacturers to accurately identify the source materials used to make herbal products because of their ease of use and lower costs.

Traditional and medicinal uses of the genus *Hydnocarpus*

***Hydnocarpus pentandrus* (Buch. -Ham)Oken**—The Ayurvedic Pharmacopoeia of India lists the leaves of Tuvataka as *Hydnocarpus pentandrus* (Buch. -Ham)Oken. In Ayurvedic practice, it is commonly known as Tuvataka (Syn. Katukapittha) but has different regional names, including Jangli Badami or Garudaphala in Kannada, Maravattai in Tamil, and Adavibadamu in Telugu (Lucas 2008a).

It is recommended for use in cases with abdominal distension with constipation (Anaha), hemorrhoids (Arsa), sciatica (Grdhrasi), cervical lymphadenitis (Gandamala), abdominal lump (Gulma), fever (Jvara), itching (Kandu), disorders due to Kapha and Vata dosha (Kaphavataja roga), worm infestation (Krumi), diseases of skin (Kustha), inflammation (Sotha), cases with metabolic disorder (Prameha), disorders of the blood (Raktavikara), skin disease (Tvakra-roga), various minor skin diseases (Ksudra Kustha), various major skin diseases (Maha Kustha), diseases of the abdomen (Udara), partial intestinal obstruction (Udavarta), and ulcers (Vrana). Tuvataka is also recommended for treating diabetes (pramehaghna) in the Indian System of Medicine. To cure leprosy, it is traditionally administered orally with honey, ghee or as a tea. The dose administered is 3–5 g of leaves, as a powder (bija churna), or 5–10 drops of leaves oil (Lucas 2008a, b). Ayurveda recognizes three biological systems (Tri dhatus): ‘Vata’ (air, the energy that moves), ‘Pitta’ (fire, the energy that digests) and ‘Kapha’ (mucus, the energy that lubricates the tissues and joints) (Jadhav and Bhutani 2005).

Some of the classical formulations of *Hydnocarpus* species include a formulation called Tuvataka taila, which is used externally (Anonymous 2008, 2000). A preparation called “Tuvataka kalpa”, mentioned in Sushruta Samhita (1000 B.C.) is used in the management of skin diseases where the patient is made to drink the oil of the Chaulmoogra seed. The crushed leaf extract of *Hydnocarpus pentandrus* (Buch. -Ham)Oken. is applied to the scalp to induce hair growth and for its cooling effects (Rajith and

Ramachandran 2010). It is also suggested to have Rasayana properties (Vaidya 1968). Rasayana drugs are said to prevent ageing and increase the strength of the body (Lucas 2008b).

***Hydnocarpus kurzii* (King) Warb—*Hydnocarpus kurzii* (King) Warb.** is known as Niradi-muttu and is used in the treatment of thyroid goiters, leprosy, arthritis, dermatitis, urticaria, eczema, herpes, erythema, and scabies (Mudaliar 1936). On the western coast of India, the seeds have been used for some resistant skin diseases, ophthalmia and in wound dressings. In the treatment of scabious eruptions, seed oil is used with equal amounts of oil of *Jatropha curcas* L., with camphor and lime juice. Another formulation made from an equal proportion of oil and lime water is used as a liniment for a scalded head, burnt skin, leprosy and joint pain. The paste of the seeds is useful in treating eczema, white patches, itching, and infection. Seed oil from *Hydnocarpus wightiana* Blume is reputed to be a remedy in the Konkan region of India for the treatment of ulcers occurring during rains (local-Barsati) in horses (Kirtikar and Basu 1984; Zahid et al. 2013).

In Southeast Asia, cold-expressed seed oil of *Hydnocarpus kurzii* (King) Warb. It was a standard remedy for Hansen's disease (leprosy) even before the advent of chemotherapeutics, such as dapsone and rifampicin (Norton 1994). The stem and leaf of *Hydnocarpus kurzii* (King) Warb., known as Dia Chan Mao in Thailand, has been used traditionally to improve blood circulation (Panyaphu et al. 2011).

***Hydnocarpus anthelminthicus* Pierre ex Laness—Seeds of *Hydnocarpus anthelminthicus* Pierre ex Laness.** are known as 'lukrabo' in Thailand and as 'ta-fung-tsze' in China (Power and Barrowcliff, 1905a). Of the other species of *Hydnocarpus*, *Hydnocarpus annamensis* (Gagnep.) Lescot & Sleumer, which is primarily distributed in the Guangxi province of China, is used in folk medicine for the treatment of rheumatoid arthritis and syphilis (Shi et al. 2006). In Japan, chaulmoogra seeds were used as early as 1716 to treat leprosy (Cole 1933).

In the late nineteenth century, western physicians began using *Hydnocarpus* oil in oral, topical, and parenteral forms to treat lepromatous diseases (Cottle 1879). A typical regimen for a patient included subcutaneous injections of 15 ml of oil beneath the lesions twice weekly for 10 weeks. This protocol resulted in pain and caused fevers, abscesses and phlebitis. In addition, an oral administration was also unfavourable due to the severe emesis induced. Hence, formulations with reduced side effects, such as Alepol (sodium hydnocarpate) and Moogrol (creosoted ester of *Hydnocarpus* oil) manufactured by Burroughs-Wellcome and Antile prol (iodized ethyl esters of chaulmoogra oil-Dean's derivative)

manufactured by Bayer were developed (Bhandari 1932; Semon 1935; Norton 1994; Rae 2005). There is little available toxicological data on these plants, except for one study reporting on ocular side effects of Chaulmoogra (*Hydnocarpus* species) which caused visual disturbances.

Materials and methods

Collection and authentication of plant material

The plant material was collected from Dapoli, Chandra Nagar, Maharashtra, India (17°44'36.3''N 73°09'39.1''E) & authenticated from the Blatter Herbarium, St. Xavier's College, Mumbai (Accession no.- R3060).

The dried leaf material was powdered and passed through a series of sieves to obtain different particle sizes material. The smallest particle-sized material (0.2 mm) was used for carrying out Preliminary phytochemical screening, determination of ash content, extractive values, Fluorescence analysis & for thin layer chromatography, standard methods were used (Chase and Pratt 1949).

Determination of physicochemical parameters of leaves:

The Ash value & Extractive value of leaves were determined separately. The assessment of Ash value involved the examination of Total ash value, Acid-insoluble ash value, and Water-soluble ash value.

Histochemical tests of powdered leaves: The powdered leaves of *Hydnocarpus pentandrus* (Buch. -Ham) Oken were treated by using certain chemicals and reagents. A light microscope was used for additional analysis of the processed plant powder. The powder treated with diluted ammonia and H_2SO_4 , which gives a yellow colour, signifying the presence of flavonoids; the powder treated with $FeCl_3$ reagent gives a dark blue to black colour, signifying the presence of tannins; a few drops of Con. H_2SO_4 reagent gave a yellow colour, indicating the presence of saponins, a few drops of acetic anhydride + Conc. H_2SO_4 (1:1 ratio) reagent gave violet to blue (or) green color, which indicates the presence of steroids.

Powder study of leaf powder using SEM: Scanning Electron Microscopy (SEM)-Energy Dispersive X-ray Spectroscopy (EDS) analysis was carried out to study the surface morphology of leaf powder. The FEI Quanta 200 scanning electron microscope with a tungsten electron source and three modes of operation model was used. The platinum coating is used since the sample is non-conducting. A typical setting at a magnification of 1X to 10000X was adjusted for the study sample. The elemental analysis was additionally done to identify the weight % of major and minor elements

present in the sample using an energy-dispersive X-ray spectrometer (EDS).

Crude fibre Dutch method: Crude fibre is the residue of resistant tissue, which can be obtained after giving treatment to the powder with dilute acid and alkali. (Khandelwal 1991).

Phytochemical screening: Leaf powder was screened for its secondary metabolites using standard methods & different phytochemical tests were performed by using various chemicals. (Khandelwal 1991).

Quantitative phytochemical screening

Estimation of Alkaloids by Harborne's (1973) method

2.5 g of the sample was put into a 250 ml beaker and 100 ml of 10% acetic acid in ethanol was added to it. It was covered and allowed to stand for 4 h. This was filtered, and the extract was concentrated in a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The whole solution was allowed to settle, and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Estimations of total flavonoid content (TF) (Zhishen et al. 1999)

Using this method, 1.0 ml of each sample was mixed with 4 ml of distilled water and then 0.30 ml of a 10% NaNO_2 solution. After 5 min, the mixture was mixed with 0.30 ml of 10% AlCl_3 solution and 2 ml of 1% NaOH solution. As soon as the mixture was well mixed, absorbance at 510 nm was measured in comparison to a blank. The findings were presented as rutin equivalents (mg rutin/g dry extract) after a standard curve (25–150 mg/ml) was plotted.

Estimation of total phenolic content by Folin–Ciocalteu reagent method (Shanmugam et al. 2013)

The Folin-Ciocalteu test was used to determine the dry extracts' total phenolic content. 1 ml of Folin-Ciocalteu's phenol reagent was mixed with 1 ml of the sample (1 mg/ml). After five minutes, the mixture was well mixed with 10 ml of a 7% sodium carbonate solution and 13 ml of deionized distilled water. After 90 min at 23 °C in the dark, the absorbance at 760 nm was measured. After making a solution of gallic acid, the calibration curve was extrapolated to measure the total phenolic content. The phenolic compounds

were estimated in triplicate. The TPC was given in milligrams of gallic acid equivalents (GAE) per gram of dried material.

Estimation of total tannins content

0.1, 0.2, 0.3, 0.4, and 0.5 ml (concentration varying from 10 to 50 μg) working standard solution was pipetted into a series of test tubes. 0.1 ml of the sample was pipetted out into a test tube. Distilled water was added to all the tubes to make up the volume to 7 ml. 1 ml of potassium ferricyanide and 1 ml of FeCl_3 were added to all the tubes and mixed properly. Absorbance was measured spectrophotometrically at 700 nm. The graph was drawn by plotting the concentration of tannin along the X-axis and the optical density reading along the Y-axis. The unknown sample concentration was calculated from the standard curve of the graph. The result was expressed as mg of tannins present in the sample.

Thin layer chromatography

The TLC were obtained using precoated aluminium-backed silica gel GF254 TLC plates (0.25 mm thickness). The TLC plates were developed in a single trough CAMAG chromatograph saturated with the different mobile phases. After development, the plates were dried and observed under 254 nm and 365 nm for fluorescence (Wagner and Bladt 1996).

Fluorescence analysis: The leaf powdered drugs underwent fluorescence analysis by exposing them to ultraviolet radiation. The fluorescence was then observed by following the procedure outlined by Chase & Pratt, 1949.

Results and discussion

Physicochemical evaluation: Ash values represent the inorganic residue left after the combustion of plant materials. These values are crucial for: Quality control, Detection of adulteration or contamination, to check Assessment of purity & for Standardization of crude drugs. When plant materials are burned, organic components are destroyed, and inorganic salts (like silicates, carbonates, phosphates) remain as ash. Depending on their solubility and resistance to acids, these are further classified into different types.

Total ash value (~ 13%): The total ash includes all the inorganic matter present in the plant, both:

Physiological ash: Comes from the natural mineral content of the plant tissue (e.g., potassium, calcium, magnesium).

Non-physiological ash: Originates from external sources like soil, sand, or contaminants introduced during handling or storage. A high total ash value may indicate either high natural mineral content or adulteration/contamination & it helps to identify poor harvesting, drying, or storage practices. In the above results, A total ash value of ~ 13% suggests a significant amount of mineral content, indicating that the minerals are mostly natural.

Acid insoluble ash (~ 1.5%): This is the portion of the total ash that does not dissolve in dilute hydrochloric acid. It typically includes: Silica (sand, siliceous earth), Soil particles & Inorganic adulterants. It measures the amount of extraneous, indigestible material in the plant sample. A low value reflects good cleaning and handling during preparation, and higher levels are unacceptable and may indicate contamination or adulteration.

The value of ~ 1.5% is considered low, suggesting that the sample is relatively clean, with minimal contamination from soil or sand.

Water soluble ash (~ 8.5%): The part of the ash soluble in water, indicating the presence of water-soluble salts like potassium, sodium, chlorides, and sulfates, and Other bio-available minerals. It reflects the quantity of active mineral constituents that can dissolve in bodily fluids. It shows the importance of understanding the nutritional and therapeutic value of the plant, and high water-soluble ash can be a positive indicator of beneficial mineral content.

A value of ~ 8.5% indicates a substantial presence of soluble inorganic salts, suggesting nutritional and potential pharmacological value.

- The high total ash is not concerning because the acid-insoluble fraction is low, suggesting the minerals are mostly from the plant.
- Water-soluble ash being high is favourable for the bio-availability of beneficial salts.

Overall, these values suggest a clean, mineral-rich plant sample suitable for further pharmacological evaluation or formulation.

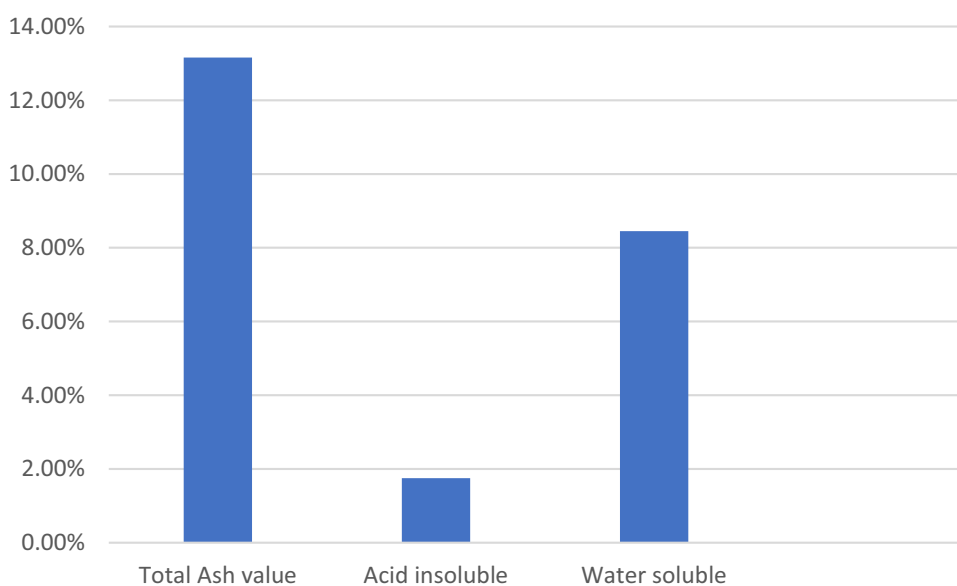
The physicochemical analysis (ash values) reveals that the plant sample contains a high amount of naturally occurring minerals with minimal contamination. These findings support the authenticity, purity, and pharmacological potential of the herbal material. Such ash profiles are critical for ensuring the quality control and standardization of crude drugs in traditional and modern medicine systems (Table 1).

Extractive value: The bar graph presents the extractive values (%) of a plant material using different solvents. Extractive value is an important parameter in pharmacognostic studies to evaluate the amount of active constituents that can be extracted using various solvents. Higher extractive values indicate greater solubility of phytochemicals in that particular solvent. Extractive value refers to the quantity of active constituents in a given amount of plant material that can be extracted using a particular solvent. It is a crucial parameter in the standardization and quality control of herbal drugs.

Significance of extractive value in pharmacognosy

Determines solvent efficiency: Extractive values help in identifying which solvent extracts the maximum number of

Table 1 Ash value of powder



bioactive compounds from the plant material. For instance, polar solvents like water, ethanol, and hydroalcohol often extract polar constituents such as alkaloids, glycosides, flavonoids, etc.

Indicates phytochemical profile: High extractive value with a specific solvent suggests the predominance of certain classes of compounds (e.g., polar or non-polar) in the plant, and it is useful in selecting suitable solvents for targeted phytochemical screening.

Helps in standardization of herbal drugs: It is used as a quality control parameter to ensure batch-to-batch consistency, and it helps in establishing identity, purity, and strength of crude drugs.

Detects adulteration or substitution: Deviations in extractive values from standard/reference values may indicate inferior quality, adulteration, or improper drying/storage of the plant material.

Aids in formulation development: Extractive values guide the selection of extracts for formulation, ensuring maximal therapeutic efficacy by using extracts rich in active constituents. Hydroalcoholic extract showed the highest extractive value (~61%), indicating it is the most efficient solvent system for extracting a wide range of phytochemicals, possibly both polar and moderately non-polar

compounds. Ethanol and distilled water also showed good extraction capacity, signifying that the major bioactive constituents in the plant may be polar (e.g., alkaloids, glycosides, flavonoids). Methanol and acetone, though polar, had moderate extractive values, suggesting selective solubility of certain compounds. Non-polar solvents like petroleum ether and hexane showed the lowest extractive values, indicating fewer non-polar phytochemicals (such as fats, waxes, and oils) present in the plant material. Extractive value is a simple, economical, and effective method to assess the quality and chemical strength of herbal materials. It plays a vital role in drug development, pharmacognostic evaluation, and ensuring the therapeutic potential of herbal products (Table 2).

Hydro alcohol > Ethanol > Distilled water > Methanol > Acetone > Chloroform > Petroleum ether > Hexane.

Histochemical tests of powder

See Table 3.

Elemental analysis of powder

See Table 4 and Fig. 1 and 2.

Table 2 Extractive values of powder

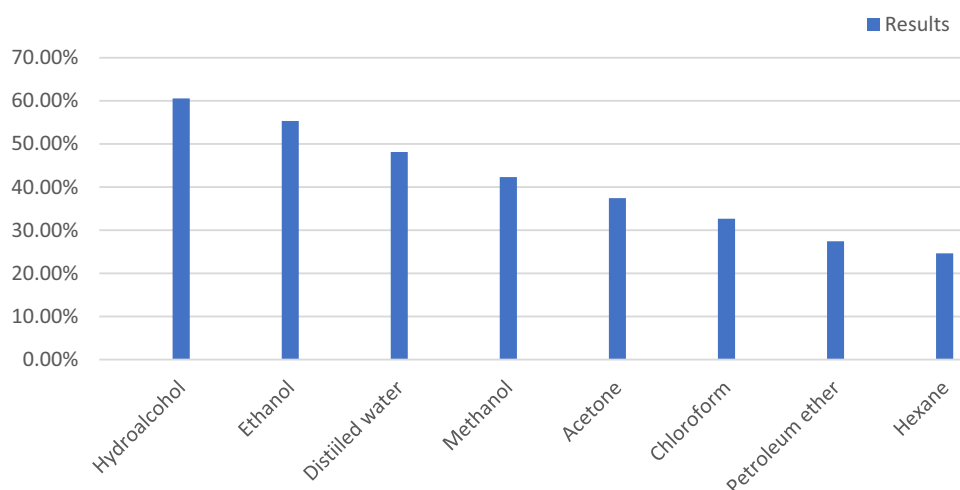


Table 3 Histochemical tests of leaf powder

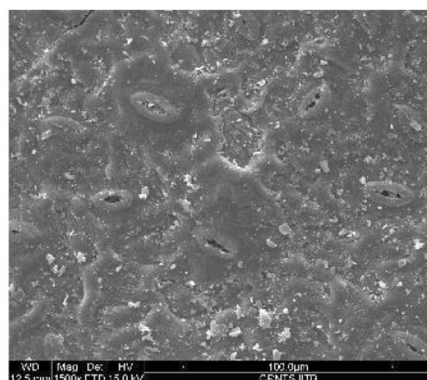
Powder treated with reagents	Test	Observation	Result
Powder + Dil. Ammonia + Conc. H ₂ SO ₄	Flavonoids	Yellow colour	Present
Powder + Dragendorff Reagent	Alkaloids	Brown colour	Present
Powder + Iodine	Starch	Blue colour	Present
Powder + Ruthenium red	Mucilage	–	Absent
Powder + Conc. H ₂ SO ₄	Cellulose	Green colour	Present
Powder + Conc. HCl	CaCO ₃ Crystals	–	Absent
Powder + FeCl ₃	Tannins	Black colour	Present

Table 4 Elemental analysis of *Hydnocarpus pentandrus* leaf powder

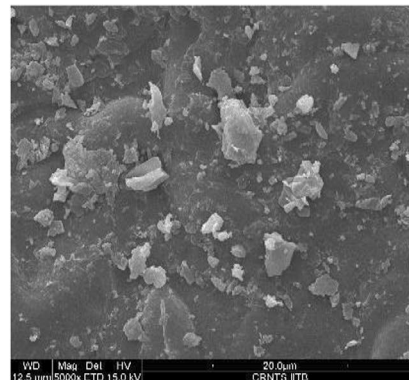
Element	Weight %	Atomic %
C	64.4	74.7
N	4.9	4.8
O	16.4	14.3
Mg	0.2	0.1
Al	0.9	0.5
Si	1.6	0.8
P	8.7	3.9
S	0.6	0.3
K	0.4	0.1
Ca	0.4	0.1
Ni	0.6	0.1
Cu	0.5	0.1
Zn	0.5	0.1

The elemental composition is based on weight % and atomic % reveals the presence of both organic elements (like C, N, O) and trace minerals or inorganic elements (like Mg, Zn, Cu, etc.). The elemental composition reveals the presence of several biologically and pharmaceutically

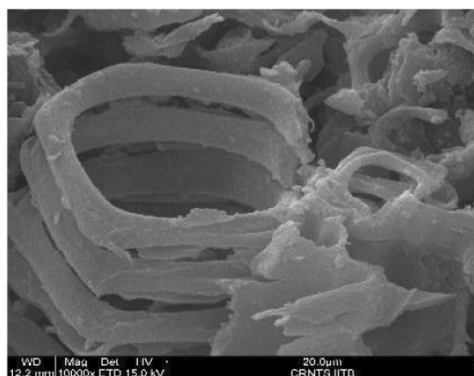
significant elements, each contributing to various medicinal functions. Carbon (C), being the primary constituent of all organic compounds, forms the structural backbone of biomolecules, including drugs, vitamins, and hormones. Nitrogen (N) is equally essential, as it is found in amino acids, nucleic acids, and numerous pharmaceutical compounds, including antibiotics and alkaloids. Oxygen (O) is vital not only for sustaining cellular respiration but also plays a key role in oxidative metabolism and is used therapeutically in oxygen therapy for respiratory ailments. Magnesium (Mg) is widely used in medicine, particularly in antacids, laxatives, and in the management of neuromuscular conditions. Aluminium (Al) compounds are commonly included in antacids and also serve as adjuvants in vaccine formulations. Silicon (Si) finds its use in drug delivery systems, medical implants, and as an inert binder in tablet manufacturing. Phosphorus (P) is a fundamental component of ATP and nucleic acids and plays a significant role in energy metabolism and bone health. Sulfur (S) is used in various medicinal preparations, especially in dermatological treatments and antimicrobial agents, and is a component of several essential amino acids and vitamins. Potassium (K), as an important electrolyte, is

Fig. 1 Elemental analysis of powder. **A** Stomata of *Hydnocarpus pentandrus* (5X), **B** Calcium Oxalate crystals (5X), **C** Tracheids (10X), **D** Scleriform fibers (5X)

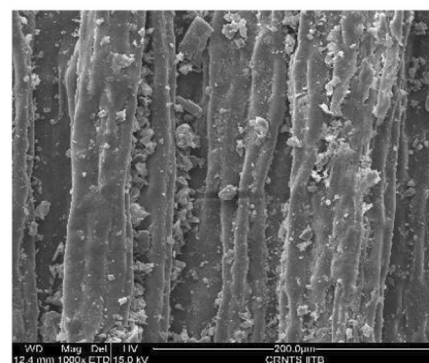
(a)



(b)



(c)



(d)

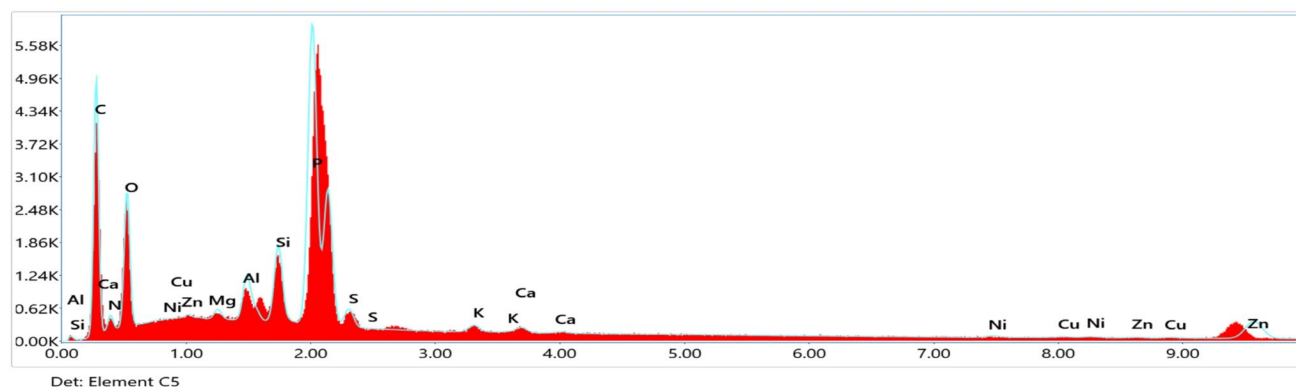


Fig. 2 Energy Dispersive X-ray spectroscopic spectrum of the sample

used in treating electrolyte imbalances, cardiac arrhythmias, and muscle-related conditions. Calcium (Ca) is well-known for its role in bone health, and its compounds are used in supplements and antacids. Nickel (Ni), though not extensively used, may play a role in certain enzymatic processes. Copper (Cu) is involved in enzymatic redox reactions and is utilized in antimicrobial applications and wound healing. Lastly, Zinc (Zn) is essential for immune function, enzymatic activity and is widely used in topical preparations, including zinc oxide creams for skin protection and wound healing.

Fluorescence analysis

The light absorbed and emitted radiation by the various solvents can be used for the identification of the powdered drug (Table 5; Fig. 3).

Fluorescence analysis is a valuable pharmacognostic tool for the preliminary identification of crude drugs, especially powdered herbal samples. When observed under different wavelengths of light (UV 365 nm, UV 254 nm, and visible light), the color changes upon interaction with various reagents can give insights into the presence of certain

Table 5 Fluorescence analysis of powder

Sr. no	Test	Long wavelength (365 nm)	Short wavelength (254 nm)	Visible light
1	Powder + Methanol	Dark yellow	Creamish White	Lemon yellow
2	Powder + Hexane	Fluorescence Pink	Parrot green	Pale green
3	Powder + 1 N NaOH	Black	Dirty green	Orangish brown
4	Powder + Benzene	Creamish whitish	Green	Yellowish green
5	Powder + Chloroform	Orange	Brown	Light brown
6	Powder + Con. H ₂ SO ₄	Black	Black	Orangish Yellow
7	Powder + Con. HNO ₃	Black	Dark green	Brown
8	Powder + 50% H ₂ SO ₄	Creamish white	Dirty green	Orangish yellow
9	Powder + Petroleum ether	Golden yellow	Parrot green	Lemon green
10	Powder + 50% KOH	Black	Black	Blackish brown
11	Powder + Toluene	White	White	Yellow
12	Powder + Glacial acetic acid	Yellowish white	White	Yellow
13	Powder + Ethyl acetate	Blue	Fluorescence blue	Creamish yellow
14	Powder + Picric acid	Green	Green	Yellow
15	Powder + FeCl ₃	Dark green	Black	Dirty green
16	Powder + Alcohol	Creamish white	Creamish white	Creamish white
17	Powder + Ethyl Formate	Creamish white	Lemon green	Lemon green
18	Powder + n-Butanol	Creamish whitish	Green	Yellow green
19	Powder + N-Propyl alcohol	Fluorescence blue	White	Dirty green
20	Powder + 50% FeCl ₃	Black	Black	Yellow

phytoconstituents such as alkaloids, flavonoids, phenolics, saponins, and tannins. These constituents often fluoresce or change color under UV light due to their conjugated systems and functional groups.

The observed distinct fluorescence reactions of the powdered drug with different solvents and reagents indicate the presence of various chemical groups:

Methanol and Ethanol treatments yielding yellow and Creamish fluorescence suggest the presence of flavonoids and phenolic compounds, which are known for their antioxidant, anti-inflammatory, and antimicrobial properties. The fluorescence pink and parrot green with hexane suggests the presence of non-polar compounds like terpenoids or fatty acids, which may have anti-inflammatory or anti-cancer activities. A black or dark green fluorescence with alkalis (NaOH, KOH) and sulfuric/nitric acid treatments implies the presence of tannins or lignin derivatives, which possess astringent, antimicrobial, and wound-healing properties. The fluorescent blue reaction with ethyl acetate and n-propyl alcohol indicates the presence of coumarins or certain alkaloids, which may be associated with anticoagulant, analgesic, or antimicrobial activity. Color reactions with FeCl₃ (ferric chloride) -notably dark green and black-point toward

phenolic and tannin-rich compounds, commonly known for their antioxidant, antibacterial, and haemostatic properties. Reactions with picric acid and petroleum ether producing green or yellow-green fluorescence may denote aromatic or resinous compounds, which often exhibit anti-inflammatory and antimicrobial roles.

Overall, the observed fluorescence patterns suggest that the powdered drug is rich in bioactive constituents such as flavonoids, tannins, phenolics, and possibly alkaloids or terpenoids. These findings support its potential medicinal uses in treating inflammatory conditions, microbial infections, oxidative stress-related disorders, and possibly gastrointestinal or dermatological issues.

Crude fibre Dutch method

Crude fibre of plant powder (Table 6).

All values in this table represent the mean $\pm$ SD (n = 12).

Phytochemical screening

See Table 7.

Fig. 3 Fluorescence analysis of leaf powder

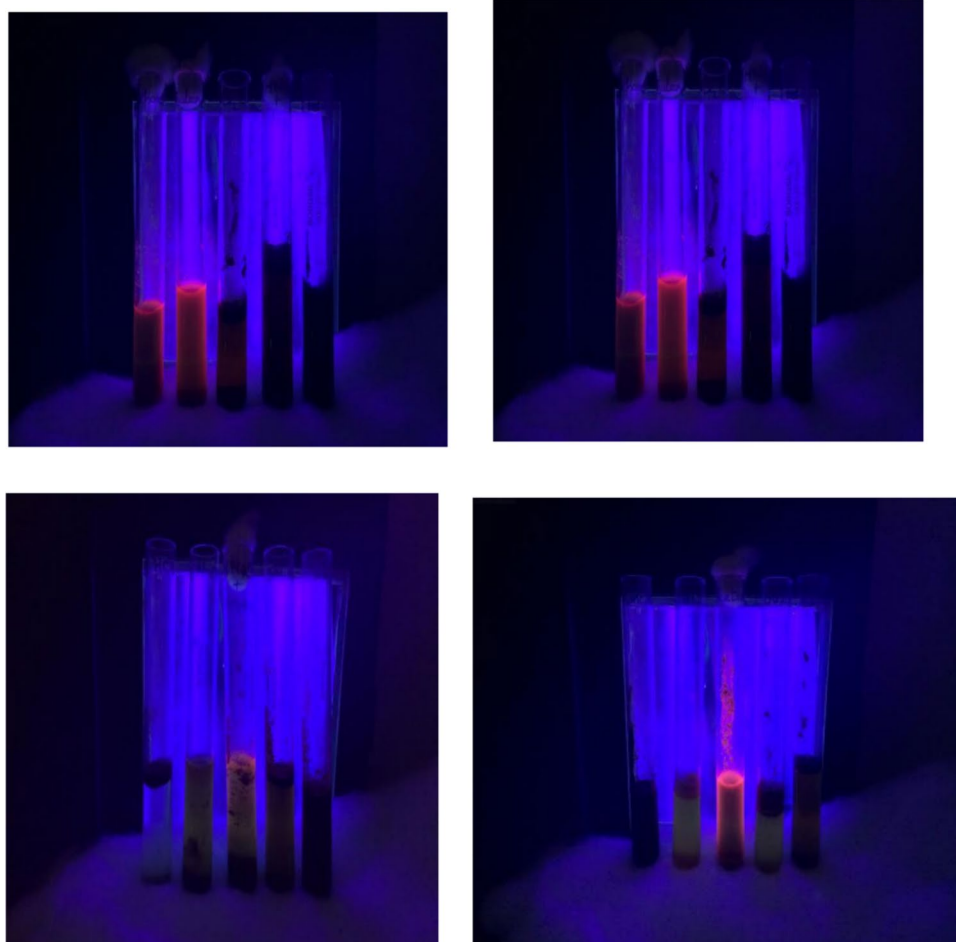


Table 6 Crude fibre of powder

Crude fibre	1.92 ± 0.002
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Table 7 Phytochemical analysis of powder

Sr. No	Secondary metabolites	Chemical test	HA
1	Alkaloids	Wagner's test	+
		Dragendorff test	+
		Mayer's test	+
		Tannic acid	+
2	Cardiac Glycoside	Keller-Kiliani test	+
3	Saponins	Foam test	—
4	Flavonoids	Alkaline test	+
		Shinoda test	+
5	Tannins	FeCl ₃ test	+
		Lead acetate	+
6	Terpenes	Salkowski test	+
7	Reducing sugar	Benedict's test	+
		Fehling's test	+
8	Carbohydrates	Barefoed test	+
9	Proteins	Ninhydrin test	+
10	Steroids	Lieberman-Burchard test	—

Presence (+) or absence (—) of phytochemicals

The preliminary phytochemical screening of the sample (HA) revealed the presence of a variety of secondary metabolites and primary biomolecules, which are indicative of its potential medicinal properties. The following interpretations can be drawn based on the tests performed:

Alkaloids were confirmed positive through multiple qualitative tests, including Wagner's, Dragendorff's, Mayer's, and Tannic acid reagents. The strong presence of alkaloids indicates potential pharmacological activities such as analgesic, antimicrobial, and antimalarial properties. Cardiac glycosides gave a positive result in the Keller-Kiliani test, suggesting the presence of steroid-based compounds with known cardiogenic properties, often used in managing heart failure or arrhythmias. Saponins tested negative in the foam test, indicating their absence, and thus reducing the likelihood of expectorant or immune-boosting effects, which are commonly associated with this group. Flavonoids were positively detected via the alkaline reagent test and Shinoda test, suggesting the presence of compounds with antioxidant, anti-inflammatory, and anticancer potential. Tannins showed positive results with both ferric chloride and lead acetate tests, confirming their presence. Tannins are known for their astringent, antimicrobial, and wound-healing properties, making them valuable in dermatological and gastrointestinal formulations. Terpenes, detected via the Salkowski test, are another important class of bioactive molecules with anti-inflammatory, anticancer, and antimicrobial properties. The presence of reducing sugars was confirmed

by both Benedict's and Fehling's tests, indicating the sample contains simple sugars, which may contribute to its energy-providing or nutritive value. The Barfoed test confirmed the presence of monosaccharides, supporting the reducing sugar findings and further indicating the presence of primary carbohydrates in the sample. Proteins were detected through the Ninhydrin test, indicating potential nutritional value and possible enzyme or biopeptide content. Steroids tested negative via the Lieberman-Burchard test, suggesting that steroidal compounds, often associated with hormonal or anti-inflammatory effects, are not significantly present in this extract.

The phytochemical profile of the extract (HA) indicates the presence of several pharmacologically significant secondary metabolites such as alkaloids, flavonoids, tannins, terpenes, and cardiac glycosides, along with primary metabolites like carbohydrates and proteins. These findings support the potential of the extract for use in traditional medicine and may justify its therapeutic applications in treating cardiovascular, inflammatory, infectious, or oxidative stress-related conditions. The absence of saponins and steroids narrows its scope for certain activities but does not diminish its medicinal value.

Quantitative phytochemical screening

1) Estimation of Alkaloids by Harborne's (1973) method

Name of the drug	Amount in gm (In 100 gm of material)
<i>Hydnocarpus pentandrus</i> (Buch. -Ham)Oken	1.87 ± 0.002

All values in this table represent the mean ± SD (n = 12)

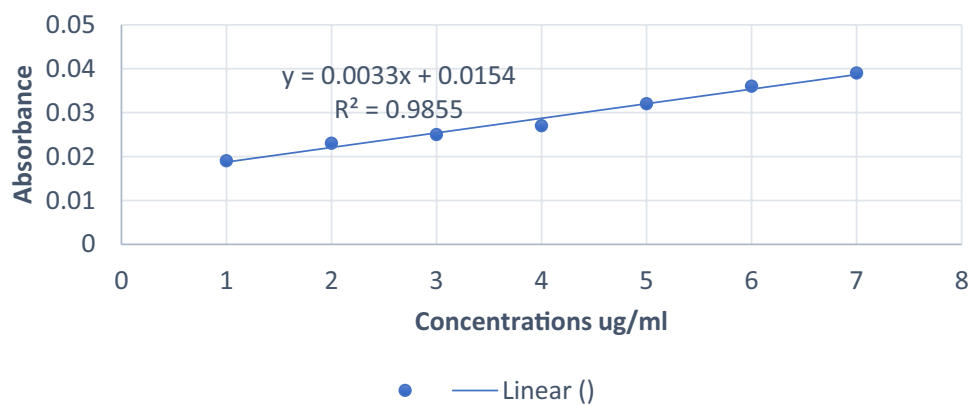
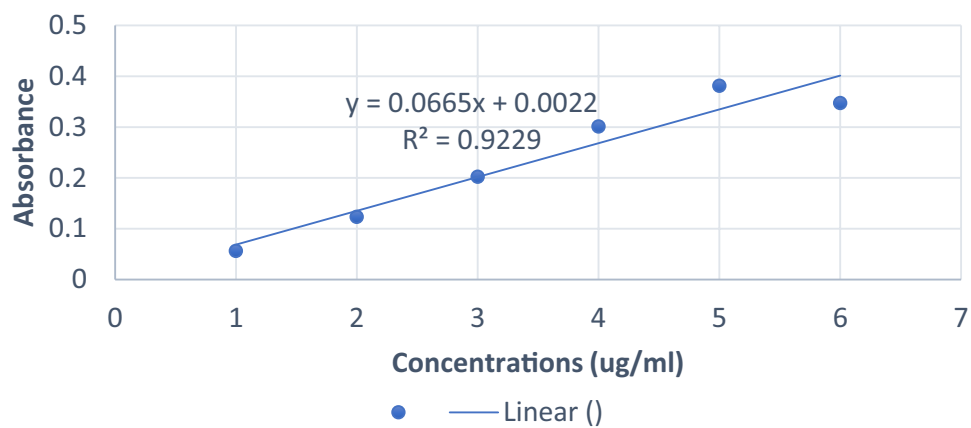
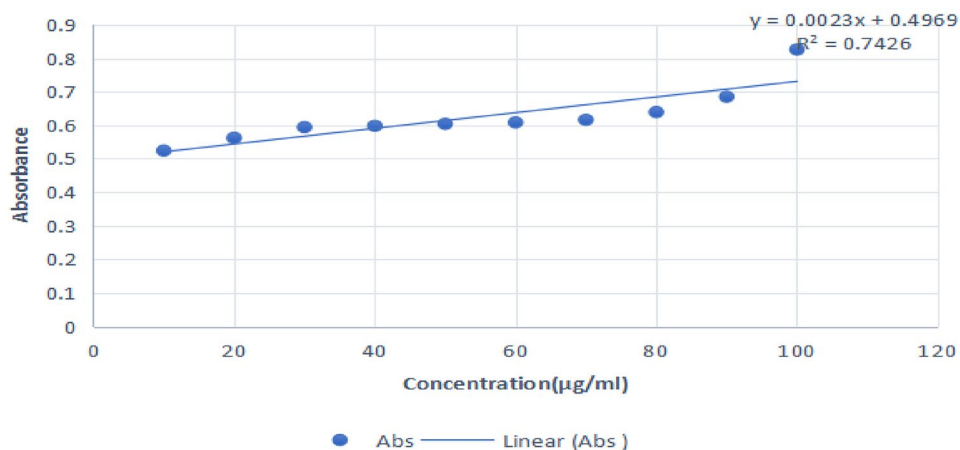
2) Estimations of total flavonoid content (TF) (Zhishen et al. 1999) (Table 8):

3) Estimation of Total Phenolic content by Folin–Ciocalteu reagent method (Shanmugam et al., 2013) (Table 9):

4) Estimation of Total Tannins Content (Table 10):

Thin layer chromatography (Table 11; Fig. 4)

The TLC analysis confirms the presence of diverse phyto-constituents such as alkaloids, phenols, flavonoids, tannins, and terpenoids in the extract. The varied R_f values reflect differences in polarity and mobility, while the fluorescence and color under UV light indicate functional groups and chemical structures typical of bioactive compounds. These findings support the potential medicinal value of the plant extract, especially in formulations aimed at treating infections, inflammation, oxidative stress, and cardiovascular issues.

Table 8 Calibration curve of Rutin**Table 9** Calibration curve of Gallic acid**Table 10** Calibration curve of Tannic acid

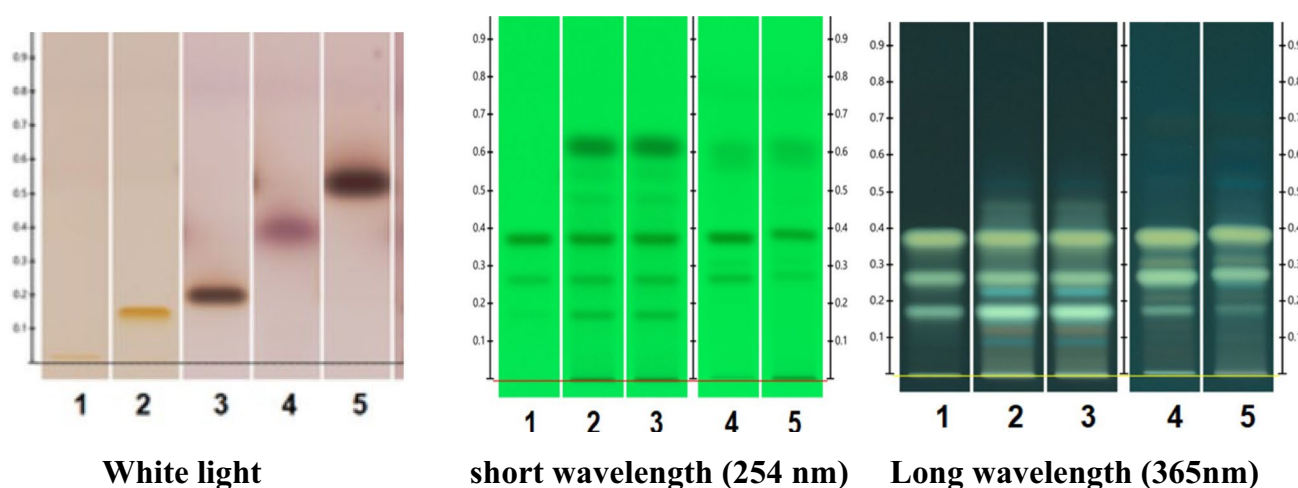
Conclusion

Hydnocarpus pentandrus (Buch. -Ham) Oken, a medicinally significant tree native to tropical regions of India, offers multiple sustainable uses that contribute to both healthcare and environmental conservation. Traditionally, various parts of the plant, including seeds, leaves, bark, and roots, are

utilized in folk medicine. The seeds are especially valued for their oil, which contains therapeutic fatty acids like hydnoarpic and chaulmoogric acids, historically used in the treatment of leprosy, skin diseases, and rheumatism. The leaves possess anti-inflammatory, antidiabetic, and wound-healing properties, while the bark and roots are known for their astringent and antimicrobial actions, used to manage

Table 11 Thin layer chromatography of powder

Phytoconstituents	White light	Short wavelength (254nm)	Long wavelength (365nm)	Rf value
Alkaloids	Yellow	Dark Blue	Yellow Green	0.76
Phenols	Orange Yellow	Royal Blue	Pine Green	0.83
Flavonoids	Pumpkin Orange	Cobalt Blue	Forest Green	0.86
Tannins	Purple	Denim	Sea Green	0.79
Terpenoids	Dark brown	Saphire Blue	Light Green	0.73

**Fig. 4** Thin layer chromatography of powder. (1) TLC Plate for detection of Alkaloids, (2) TLC Plate for detection of Phenols, (3) TLC Plate for detection of Flavonoids, (4) TLC Plate for detection of Tannins, (5) TLC Plate for detection of Terpenoids at Visible light, 254 nm & 365 nm

conditions like diarrhea and skin infections. Environmentally, the tree plays a vital role in agroforestry systems and reforestation efforts due to its adaptability to various climatic conditions, thereby aiding in soil conservation and biodiversity enhancement. Its integration into local healthcare practices and small-scale herbal industries supports rural livelihoods through sustainable harvesting methods, such as seed collection without damaging the plant. Furthermore, the plant's rich phytochemical profile holds potential for natural drug discovery and eco-friendly skincare products, aligning with the principles of green pharmacy and sustainable resource management. Thus, the whole plant of

Hydnocarpus pentandrus serves as a valuable resource in promoting health, supporting ecosystems, and empowering communities. Current studies show the presence of proximate analysis in the form of ash values & extractive value. Fluorescence analysis was also carried out for the first time. Preliminary phytochemical screening showed the presence of phenols, alkaloids, quinones, diterpenes, triterpenes, steroids, flavonoids, and tannins in the present study. The above tests revealed the following results. These indicate the presence of bioactive molecules, which are present in the plant & could be explored as a source of medicine in the future. The study analyses the elements present in medicinal

plants, revealing varying concentrations due to natural processes and differences in botanical structure, soil mineral composition, and climatic conditions. The data can be used to assess the potential of these plants. The findings can help determine the effectiveness of these plants in treating various diseases. Over the last few years, researchers have aimed at identifying and validating plant-derived substances for the treatment of various diseases. This revival of interest in plant-derived drugs is mainly due to the current widespread belief that “green medicine” is safe and more dependable than the costly synthetic drugs, many of which have adverse side effects. Recently, there has been a renewed interest in improving health and fitness through the use of more natural products. The researcher aimed to determine if the whole plant could be as effective as its leaves, thereby enhancing the utilization of the entire plant for medicinal purposes. This approach aligns with sustainable practices by reducing waste and expanding the sources of important phytochemicals. In the present study, the complete Phytochemical investigations were carried out in order to provide correct information about the presence of bioactive molecules in the crude drug, i.e., *Hydnocarpus pentandrus* (Buch. -Ham) Oken leaves. The standardization of the leaf drugs is of great significance in the authentication of the plant drug in whole as well as in powdered form. Thus, these Pharmacopeial standards are of utmost importance for the entry of the said plant part into the mainstream of herbal medicine. With this information, in the near future, plant extracts could be further exploited as a source of useful phytochemical compounds and may play a very important role in the modern system of medicine.

Acknowledgements The authors are grateful to the Department of Botany, Mithibai College (Empowered Autonomous) and to SAIF IIT-B for carrying out ESEM and EDS analysis of the sample and for providing the required facilities.

Data availability The datasets and materials used and/or analyzed during the current study are available from the authors on reasonable request.

Declarations

Conflict of interest The authors declare that there is no conflict of interest.

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