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Review Article

A Review On Phytochemical Investigations Of *Lawsonia Inermis* L. Bark

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ABSTRACT

Lawsonia inermis L., commonly known as henna, is a plant with a rich history of use in traditional medicine across various cultures. While the leaves of this plant are well-known for their dyeing properties, the bark has also been used for its medicinal benefits, though it has been less studied. This research focuses on the phytochemical investigation of Lawsonia inermis L. bark to identify its bioactive compounds and explore its therapeutic potential. The study employed solvent extraction, followed by chromatographic and spectroscopic techniques, to isolate and characterize the chemical constituents present in the bark. The analysis revealed a diverse range of phytochemicals, including flavonoids, tannins, saponins, alkaloids, and phenolic compounds. These compounds are associated with various pharmacological activities, such as antioxidant, antimicrobial, anti-inflammatory, and hepatoprotective effects. The presence of these bioactive compounds suggests that the bark of Lawsonia inermis L. holds significant potential for therapeutic applications, particularly in the development of natural remedies and pharmaceuticals. The findings of this study contribute to the growing body of knowledge on the medicinal properties of Lawsonia inermis L., emphasizing the importance of the bark as a source of valuable phytochemicals. Further research is recommended to explore the mechanisms of action of these compounds and to assess their efficacy in clinical settings. This investigation underscores the relevance of phytochemical studies in discovering new natural compounds with potential health benefits.

INTRODUCTION

Plants biosynthesize the chemicals that are useful for the preservation of health and the health of humans and other animals. Many of them are secondary and are at least 12,000 have been assigned to a host is to reach at least 10% of the total. In many cases, these substances (particularly the start to be seen) to serve as the defense mechanisms of plants, insects, and herbivores. Many of the herbs and the type is being used as sources to provide a positive connection to the medicines. Medicinal plants are part and parcel of human society to combat diseases, from the dawn

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of civilization1 . There exists a plethora of knowledge, information and benefits of herbal drugs in our ancient literature of Ayurvedic (Traditional Indian Medicine), Siddha, Unani and Chinese medicine. According to the World Health Organization, 2003 about 80 % of the population of developing countries being unable to afford pharmaceutical drugs rely on traditional medicines, mainly plant based, to sustain their primary health care needs 2 . Herbal medicines are in great demand in the developed as well as developing countries for primary healthcare because of their wide biological and medicinal activities, higher safety margins and lesser costs.[1] Herbal medicine, also known as botanical medicine, involves using herbs for their therapeutic or medicinal properties. Herbs are plants or parts of plants valued for their medicinal, culinary, or aromatic qualities. Plants produce a variety of chemicals that affect the human body, many of which are secondary metabolites. These chemicals often serve as defense mechanisms against pests, herbivores, and environmental stressors. An example of an herb rich in beneficial chemicals is the chemical plant, which contains compounds such as glycosides, saponins, resins, gum-resins, sesquiterpene lactones, and essential oils. There is growing interest in the organic mixtures found in herbal medicine, with some biologically active components being isolated and studied for their pharmacological effects. Many early medicines were derived from observing animals and through trial and error. Over time, different cultures developed their own knowledge of herbal remedies and compiled extensive pharmacopoeias. Even in the 20th century, much of modern pharmacopoeia has roots in traditional herbal medicine. Today, approximately 25% of prescription medications in the United States contain at least one active ingredient derived from plants, with some being synthesized to mimic natural plant compounds. The World Health Organization (WHO) estimates that around 80% of the global population uses plant-based medicines as their primary form of treatment. Ayurveda, traditional in India, is a major component of holistic medicine and integrates various aspects of life, including physical, metaphysical, and spiritual health. It emphasizes balance and harmony in mind, body, and emotions. Ayurveda, Siddha, Unani, and tribal medicine systems all play significant roles in local healthcare practices. Among these, Ayurveda is the most developed and widely practiced in India. It dates back to between 1500 and 800 BCE and is a key part of Indian philosophy. The term "Ayurveda" comes from Sanskrit, where "Ayur" means life and "Veda" means knowledge. Despite the historical roots of Ayurveda, there are still many plant species and their medicinal properties that have not been thoroughly studied. Some notable medicinal plants include vinblastine, vincristine, taxol, podophyllotoxin, camptothecin, and others. Both traditional and modern scientific approaches are crucial in exploring and understanding these plants' therapeutic potentials. Scientific studies help isolate active compounds and determine their mechanisms of action, which is essential for developing effective treatments. Herbal drugs refer to medicinal products derived from plants.

MATERIAL AND METHODS

Plant profile:

Lawsonia inermis, commonly known as henna, the henna tree, or the mignonette shrub, is a flowering plant and the sole species in the genus Lawsonia. It has various medicinal properties, including the treatment of renal lithiasis (kidney stones), jaundice, wound healing, and skin infections. Traditionally, the bark of Lawsonia inermis has been used to treat jaundice, spleen enlargement, kidney issues, leprosy, and other persistent diseases. The species is named in honor of Isaac Lawson, a Scottish doctor and a close friend of Carl Linnaeus.[2]

Plant of Lawsonia inermis

Leaves of Lawsonia inermis

Seeds of Lawsonia inermis

Bark of Lawsonia inermis

Synonym: Lawsonia Alba L.

Vernacular names:

English: Henna

Sanskrit: Mendhi, Mendika, Timir.

Arabic: Alhenna, Hinna.

French: Alcana d' orient.

Greek: Kypros.
Gujrat: Medi.
Hindi: Hena, Mehndi.
Marathi: Mendhi, Mendi.

Systematic arrangement:

Kingdom: Plantae
Subkingdom: Viridae-plantae
Division: Tracheophyta
Subdivision: Spermatophytina
Class: Magnoliopsida
Order: Myrtales
Family: Lythraceae
Genus: Lawsonia
Species: inermis

Chemical constituents:

Lawsonia inermis contains various bioactive compounds, including carbohydrates, phenolic compounds, flavonoids, terpenoids, saponins, alkaloids, quinones, coumarins, and xanthones. Notably, it also contains 2-hydroxy-1,4-naphthoquinone, along with other naphthoquinone derivatives such as 1,3-dihydroxy-naphthalene, 1,4-naphthoquinone, and 1,2-dihydroxy-4-glucosyl-naphthalene.

Description:

Lawsonia inermis is a highly branched, deciduous, glabrous shrub or small tree that can grow between 2.4 to 5 feet in height. It has grey-brown bark and may sometimes have thorns. In India, it is cultivated both as an ornamental shrub and for its commercial value, particularly in the art of painting with henna. The leaves are ovate or broadly shaped, ranging from 1.3 to 3.2 cm in length and 0.6 to 1.6 cm in width. They can be thin, with sharp or blunt tips, and are often slimy at the base. The petioles are relatively small. The flowers are numerous and smaller than 1.3 cm wide, with a sweet fragrance and can be white or pink. The calyx is broad and bell-shaped, measuring 3 to 5 mm in length. The petals are 2.5 to 3 mm long, suborbicular or subrenoid, and exhibit a waving pattern. The flower structure includes 8 stamens arranged in pairs within a tubular cup. The capsules are spherical, slightly veined, and measure around 6 mm or more in diameter. They are supported by a short, stout style.

Traditional Uses:

1. Lawsonia inermis has been traditionally used in the treatment of epilepsy and jaundice. It is also employed for coloring gray hair.
2. It is believed to have protective qualities and is used as a remedy for conditions perceived as "evil eye" or malevolent influences.

Medicinal Importance:

1. It is used to address diarrhea.
2. The plant is used in the preparation of liver tonic syrups.
3. Lawsonia inermis acts as an astringent, helping to tighten tissues and reduce secretions.

PHARMACOGNOSTIC INVESTIGATION:

This systematic pharmacognostic study of Lawsonia inermis aims to provide detailed documentation and assist the scientific community in further research. The study involves the following efforts:

1. Collection and Authentication of the Whole Plant:

Ensuring accurate identification and verification of the plant material used in the study.

2. Morphological Characters:

Detailed examination and description of the plant's physical characteristics.

3. Microscopic Characters:

Transverse Section of the Bark:

Analysis of the bark’s structure through microscopic examination.

Powder Microscopy:

Examination of powdered plant material to identify cellular components and structures.

4. Proximate Values:

Extractive Values:

Alcohol Soluble Extracts Assessment:
Measuring the amount of extractable substances soluble in alcohol.

Water Soluble Extracts Assessment:
Measuring the amount of extractable substances soluble in water.

Petroleum Ether Soluble Extracts Assessment:
Measuring the amount of extractable substances soluble in petroleum ether.

Moisture Content Determination:
Quantifying the moisture content in the plant material.

Ash Assessments:

Total Ash:
Determining the total inorganic residue after combustion.

Acid-Insoluble Ash:
Measuring the residue remaining after treatment with acid.

Water-Soluble Ash:
Measuring the residue soluble in water.

Sulphated Ash:
Determining the residue obtained after sulfuric acid treatment.

Collection and Authentication of the Whole Plant:
Ensuring accurate identification and verification of the plant material used in the study.

Morphological Characters:
Morphology is the study of the shape and structure of an entity, while morphography involves documenting and illustrating these shapes and structures from the material. Morphological and organoleptic characteristics—such as color, smell, taste, shape, and size—are assessed through both experiential and scientific methods in botany.

Study of Microscopical Characters:

a. Transverse piece of the Bark
Microtomic sections of the cortex are examined under both low and high magnification using a microscope.

b. Powder microscopy
The dried bark of Lawsonia inermis L. was ground into a coarse powder and treated with chloral hydrate for 5-10 minutes. It was then stained with chloroglucin and other reagents in a 1:1 ratio. The stained samples were observed under high power (40x) for various diagnostic features, including the identification of connective cells with crystal structures, medullary rays with attached tools, and bark cells using grain analysis.

Proximate Values
The values are directly defined for the bark of Lawsonia inermis L.

a. Extractive values
The extractive value is an important measure that determines the amount of soluble substances in medicinal plants. This involves extracting raw plant materials with various solvents to obtain solutions containing different phytoconstituents. The extraction process helps identify and quantify the specific soluble compounds present in the plant material.

Alcohol-soluble extractive value:
Four grams of shade-dried Lawsonia inermis L. bark residue are soaked in 100 ml of 95% ethanol in a closed bottle. The mixture is shaken regularly for 6 hours and then allowed to stand for 18 hours. After this period, the mixture is quickly filtered, taking care to minimize ethanol loss. Twenty-five milliliters of the filtrate are evaporated to dryness in a flat-bottomed dish, and the residue is dried at 105°C. The dry residue is then weighed to calculate the percentage of extractable material soluble in ethanol, which reflects the extractive value of the dried bark powder.

Water-soluble extracts assessment:
Four grams of shade-dried Lawsonia inermis L. bark residue are soaked in 100 ml of water in a closed bottle. The mixture is shaken frequently for the first 6 hours and then allowed to stand for 18 hours. After this period, the mixture is quickly filtered. Twenty-five milliliters of the filtrate are evaporated to dryness in a flat-bottomed dish and

dried at 105°C. The residue is then weighed to determine the extractive value, taking into account that the bark powder was dried in the shade.

Petroleum Ether soluble extracts assessment:

Four grams of shade-dried Lawsonia inermis L. bark powder were soaked in 100 ml of petroleum ether in a closed flask. The mixture was shaken frequently for the first 6 hours and then allowed to stand for 18 hours. After this period, it was quickly filtered. Twenty-five milliliters of the filtrate were evaporated to dryness in a flat-bottomed dish, and the residue was dried at 105°C. The dried residue was then weighed to determine the extractive value, considering that the bark powder was dried in the shade.

b- Moisture content

A measured amount of shade-dried Lawsonia inermis L. bark residue is placed in a tared crucible, and the initial mass is recorded. The crude drug is then heated in an oven at 105°C until a constant mass is achieved. This process is repeated until the weight remains constant, indicating that all moisture has been removed.

c- Ash assessment:

Total ash

Two grams of shade-dried, coarsely ground Lawsonia inermis L. bark are placed in a pre-weighed silica crucible and burned at a temperature not exceeding 450°C until all carbon is completely burned off. The remaining ash is then weighed. The percentage of ash residue is calculated based on the original weight of the dry bark.

Acid insoluble ash

The resulting ash residue is heated for 5 minutes with 25 ml of dilute hydrochloric acid (HCl). The insoluble substances are then collected on ash-free filter paper, washed with warm water, and burned. The residue is subsequently weighed. The percentage of acid-insoluble ash is calculated based on the original weight of the shade-dried bark powder.

Water-soluble ash:

The resulting ash residue is heated for 5 minutes with 25 ml of purified water. The insoluble substances are then collected on ash-free filter paper, washed with warm water, and then ignited for 15 minutes at a temperature not exceeding 450°C. The weight of the remaining ash is recorded. The percentage of water-soluble ash is calculated based on the original weight of the shade-dried bark residue.

Sulphated ash:

A silica crucible is heated to red heat (approximately 800°C) for 10 minutes, then allowed to cool and weighed. One gram of air-dried Lawsonia inermis L. bark powder is placed in the crucible and moistened with concentrated sulfuric acid. The mixture is carefully dried, and then the crucible is heated at a temperature around 800°C. After burning, the crucible is cooled, weighed again, and the sulfated ash is determined. The percentage of sulfated ash is calculated based on the original weight of the air-dried bark powder.

PHYTOCHEMICAL INVESTIGATIONS

Lawsonia inermis L. bark was subjected to the following phytochemical investigations:

A. Extraction:

Successive extraction with different solvents in increasing order of their polarity.

B. Preliminary Phytochemical Analysis:

Initial screening to identify the presence of various classes of phytochemicals.

C. Chromatographic Studies:

Utilization of chromatographic techniques to separate and analyze the components of the extracts.

D. Separation of Phytoconstituents:

Isolation of individual phytoconstituents from the extracts for further study.

E. Description of Separated Phytoconstituents:

Detailed characterization and description of the isolated phytoconstituents.

A. Extractions:

Successive Extractions:

The bark of Lawsonia inermis L. was shade-dried at room temperature and ground into a coarse powder. Two hundred grams of the powdered bark were sequentially extracted with methanol and water, respectively, in ascending order of polarity. The extracts were concentrated under reduced pressure using a rotary evaporator, and the remaining residue was dried in a desiccator over sodium sulfate. After drying, the percentage of yield was calculated for each extract. All obtained extracts were subjected to preliminary phytochemical screening to identify the presence of various chemical constituents.

B. Preliminary phytochemical analysis:

All extracts of Lawsonia inermis L. bark were analyzed, and despite the quality of chemical tests revealing the presence of various phytoconstituents, their effects were also evaluated.

Tests for Carbohydrates

Molisch's Test:

The extract solution is mixed with a few drops of alcoholic α-naphthol. Gradually add 0.2 ml of concentrated sulfuric acid (H_2SO_4) along the wall of the test tube. A purple ring should form at the interface, indicating the presence of the compound being tested.

Benedict Test:

In the process, a few drops of Benedict's reagent (an alkaline copper citrate complex) are added to the solution. The mixture is then boiled, and a red-brown precipitate indicates the presence of reducing sugars.

For the general monosaccharide test:

Heat a glass test tube containing 1 ml of Benedict's reagent and 1 ml of the solution. Boil the mixture, and observe for the formation of red copper oxide after two minutes. This indicates the presence of monosaccharides. Disaccharides may also give a positive result after longer heating (around 10 minutes) due to hydrolysis into monosaccharides. The process of extracting a few drops of reagent benedict (alkaline solve copper citrate complex) into the solution, and boiling the water produces a red-brown feeling to restore the sugar level.

In a general monosaccharide test:

Heat a glass that has 1 ml of reagent, and 1 ml of solution in the eye, bottle hot; red copper oxide occurs after two minutes, this is the monosaccharide that is available. Disaccharides when longer-term warm-up (10 min) can also cause a decrease due in part to hydrolysis to monosaccharides.

Selvinoff Test:

A solution of hydrochloric acid (HCl) reacts with a ketose sugar to form a furfural derivative, which then reacts with resorcinol to produce a red-colored compound.

To perform the test:

1. Add 5 ml of the reagent to the extracted solution.
2. Heat the mixture to boiling.

Fructose will produce a red color within 30 minutes. This test is sensitive to concentrations as low as 5.5 mmol/L. If glucose is present, it may not produce a distinct red color and requires a larger amount of glucose to achieve a similar color change. A solution of HCl reacts with a ketose sugar in the form of a furfuraldehyde derivative, which gives a red color to the compound they are bound to resorcinol. Add to it the extracted solution of nearby 5ml reagents, and heat to a boiled. Fructose produces a red-color, after half an hour and minutes. Test subtle to 5.5mmol/l. if glucose is lacking. If glucose is present it is fewer sensitive and on addition of big quantity of glucose it gives similar color.

Fehling's Test:

Mix equal volumes of Fehling's Solution A and Fehling's Solution B together in a test tube. Add a few drops of the solution to be tested. Heat the mixture. The appearance of a brick-red precipitate

indicates the presence of reducing sugars, which reduce the copper ions in the reagent to form a copper(I) oxide precipitate. Together with the volume of Fehling A and Fehling B, the reagents are mix together with a some droplets in hot mud, in a brick-red ad non-fulfillment occurs the lively color of copper reducing sugar.

Caramelisation:

When carbohydrates are treated with strong sulfuric acid, they undergo a process of carbonization, resulting in the formation of charcoal along with a characteristic burnt sugar odor. Carbohydrates when they are processed by strong sulfuric acid passes to the burning of coal with drying, along with the smell of burnt sugar.

Tollen's Test:

Add 100 mg of the sample to a test tube and mix with 2 ml of Tollen's reagent. Heat the mixture gently. If an aldehyde sugar (aldose) is present, a silver mirror will form on the inner walls of the test tube. 100 mg sample, add 2ml reagent tollen get a silver mirror, the inner wall of the tube made the operation expressed in that aldose sugar.

Tests for Sterols and Triterpenoids:

Libermann Burchard examination:

The skin is preserved through a few drops of acetic anhydride, brought to a boil and cooled add a concentrated can of acid since the side of the pipe, The brown circle at the intersection of the binary coatings, and the top coating, displays that the color is green, it shows the presence of sterols, and during the formation of dark red shows the presence of triterpenoids.

Salkovsky Test:

After treating the skin in chloroform, using little droplets of collected sulfuric-acid, mix well and let stand for a while, the red-color shown on the inferior coating shows the occurrence of sterols, during the development of yellow color, the inferior coating shows the occurrence of triterpenoids

Tests for Glycosides

Examination I:

Taken 200mg of sample and 5ml of diluted (10%) H_2SO_4 in a test tube, heated with 2 mins on water bath at 1000 C. centrifuged or filtered, pipetted supernatant or filtered. To defuse the acidic solution, use a 5% sodium hydroxide solution (note that an additional volume of NaOH. Add 0.1 ml of Fehling's solution (A) and (B). Alkaline (pH test paper) and for heating water in the bath for 2 minutes. Pay attention to the amount of accumulated red sediment and compare it with what appeared in Test (II).

Test II:

Then 200mg of the sample is mixed with 5ml and boiled on water bath. Later steaming, add the same amount of H_2O, volume of NaOH, to the test used above. Add 0.1 ml of Fehling A and B until alkaline (red button changes blue) and heat in a water bath for two minutes. Note that there is a large amount of red sediment. Compare sediment Examination II, Examination I. If the precipitate in examination (II) are larger than the Test, and glycoside I can participate. But Test-II is the sum of the free reducing sugar that is already available in the drug, while Test-I is a glycoside, after acid hydrolysis.

Tests for Alkaloid:

Mayer's Test:

Extract solution + few droplets of Mayer-reagent and a creamy-white precipitous appears.

Dragendroff's Test:

Extract sample + Dragendroff's reagent, a red-brownish precipitous is formed.

Wagner's Test:

Extracts sample, add a few drops of Wagner reagent, a red-brownish precipitate is formed.

Hager's Test:

Extracts solution, mixed with few droplets of reagent Hager, a yellowish precipitous is formed.

Examinations for Phenolic Compounds

Ferric chloride Test:

Few drops of Fecl3 + extracts solution produce blue-greenish colour.

Shinoda Test :

The extracts contains additionally several fragments of magnesium tape and concentrate salt, yellow-orange, sometimes it is orange, the color opens within a few minutes.

Tests for Flavonoids

Shinoda Test:

Extracts sample + some drops fragments of magnesium tape and add hydrochloric acid drop by drop, pink, red, crimson-red, sometimes green-blue color, it seems for several minutes.

Zinc-Hydrochloride reduction Test:

To the extract solution to, add the zinc dust mixture and grind until weighed. A solution of hydrochloric acid. It gives a red color for a few minutes

Alkaline-reagent examination:

In the sample you can add a some droplets of sodium hydroxide solution, the development of strong yellow color, creates conditions for up to, add a some droplets of dilute acid shows the presence of flavonoids

Tests for Tannins:

Gelatin Test

Taken sample along 1% gelatin solution having 10% sodium chloride whites precipitate is produce.

Ferric chloride Test:

Extract solution mixed with FeCl3, green color precipitate is produce.

Vanillin Hydrochloride Test:

Extract mixture while react along hydroxide solution, purple-red color are produce.

Alkaline reagent Test:

When sample solutions react with hydroxide solution, yellow-red precipitate are produce.

Test for anthraquinone glycosides

Modified Borntrager Test:

In the 5ml extract solution, add 5 ml of 5% chloride, iron and 5 ml of dil. ETO. Heat for 5 minutes the hot water in the bath gives cool, add benzene or organic solvents. mixing proper. Disable gradual layers; add an equivalent amount of dil. The most. The ammonia layer shows a pinkish-red color.

Test for Steroidal glycosides:

Kedde's test:

Extract the leaf residue through chloroform, vaporize to dryness and add a droplet of 90% alcohol and two droplets of two % 3,5-dinitrobenzoic acid (3,5-dinitrobenzenecarboxylic acid, Kedde reagent) 90% alcohol. Be alkaline through a 20% sodium hydroxide solution. It turns out that the color is pink. The color of the reaction with dinitrobenzoic acid is due to the presence of α,β unsaturated γ – lactone in the aglycone.

CHROMATOGRAPHIC STUDIES:

The ethyl acetate-soluble mass of the methanol eye was evaluated by TLC to govern the presence of a number of phytoconstituents in the extracts using a specific solvent system and found a reagent that was found to provide the appropriate distance. TLC mode chromatography, which shows the substrate as small spots, or a thin layer of sorbent supported by glass, plastic, or metal. The mobile phase moves with the stationary arm, seeing that the stage is influenced by the kapilyarların function, sometimes involving weight or pressure. Split TLC premier inventory each component together, a common migration process, but different migration distances. A mobile phase consisting of a solvent or a combination of solvents. Very hard sorbents have been used, among others, silica gel, cellulose, polyamide, aluminum oxide, resins, and silica gel-related chemicals. This to avoid the appearance of cracks on the surface of the adsorbent. Once it is set, the stove is kept in the oven at a temperature of 100-1200c each for 1 hour. TLC active plates is nothing more than to remove water, moisture and other adsorbed substances from the surface of the

adsorbent by heating at high temperature, so that the adsorbent business is left behind.

Rf values were determined using formula:

$$\text{Resolution Factor } (R_f) = \frac{\text{Distance traveled by the solute from the origin}}{\text{Distance traveled by the solvent front from the origin}}$$

Isolation of phytoconstituent from ethyl acetate fraction:

Isolation of the phytoconstituent was carried out on the ethyl acetate soluble fraction of methanol solution, these fractions were found to have three bonds, respectively. These mixtures were separate through column chromatography. Column chromatography is the maximum suitable method for separating, purifying both solid, liquid substances. Column chromatography is additional method of solid-liquid chromatography of two phases-solid (motionless phase) and liquid (movable phase). The concept of column chromatography is similar to the theory of thin-layer chromatography. The maximum commonly used adsorbents are silica gel and alumina. The extracts is melted in a minor volume of solvent (eluent) and applied to the top of the column. The eluate, instead of rising by capillary activity, up, TLC, it flows down through a column filled with adsorbent. Establish a balance between the dissolved harsh, adsorbed on silica gel or aluminum oxide and the eluting solvent by over the columns. Column chromatography is usually used as a purification method, in which it is impossible to remove the necessary bonds from the mixture.

Adsorbent :

Silica gel activated for column chromatography

Dimensions of the column :

L - 45cm, Diameter- outer -2.2 cm, inner -2 cm

Length of adsorbent packed: 26cm

Rate of elution 15 rops/min.

Volume of elute collected : 500 ml

Type of elution : Isocratic elution.

Preparation of sample:

3 g of the soluble ethyl acetate portion of methanol solution is mixed with 3 g of silica gel and dry in a vacuum furnace at a temp of 45 ° C. The adsorbed material is then deposited in the column.

Packing column:

In 100 g of silica gel, which activate the hot air in the furnace at a temperature of 1100 ° C-1 hour. These bottles are placed at the bottom of the column. It is activated silica gel suspension represents Toluene: Acetone: Formic Acid (60:60:10), and the load will be in the column in small portions by pressing the "open soft essential oil" button for each structure to ensure uniform packaging. A small amount of solvent is stored at the top of the column to avoid drying out or temperature. The crack of a column. One of the early steps is to prevent the formation of an air bubble in the column above the nozzle, which otherwise may interfere with separation. The column did not hold out all night. The columns were running very fast, at any time, using a movable stage to remove any possible raises. The sample was then loaded into a column and given results. For example, a small tank was placed, which was used to avoid mixing particles with the examples. At this time, it was eluted as a mobile phase to collect fractions. Additional attention is being paid to the factions. Each group was evaluated by TLC to determine the amount of available phytoconstituents. It fractions shows that the same amount of compounds of the same value, Rf, combined, were concentrated and evaporated to zero. This percentage yield rate, physical properties and chemical tests

Data on column eluents of the ethyl acetate fraction.

TLC Research Fractions

Toluene: Acetone: Formic acid (60:60: 10)

Quantity

Spots of Color, Rf, Costs

From 1 To 6 Without A Website ---- ----

From 7 to 12 are 2 yellow spots of light brown, 0.88,0,63
13-47 1 Brown 0.63
48-52 2 yellow spots of light brown, 0,56,0,44
From 53 To 60 Without A Website ---- ----
TLC Eluate Columns:
Surfacing: Silica Gel (L)
Solvent System: Toluene : Acetone : Formic Acid 60:60:10 ratios
Visualize reagent chloride, iron agent
Columns of eluate with the same meaning of Rf according to TLC data and the results are on the side.

Table 1: Characterization of isolated Phytoconstituent (COMP-A).

Sr. No.	Phytoconstituents	Type of spectra	Make of the Instrument	Results are as on page
1.	COMP-A	UV Spectra	JASCO UV- Visible Spectrophotometer.	29
2.	COMP-A	FT-IR Spectra	Thermo Nicolet IR 200 Spectrometer	30

The characterizations of the isolated compounds were reported. Results are as on COMP- A.

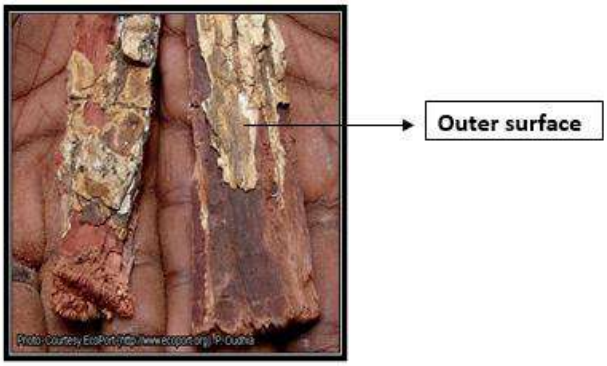
Purification:

The isolated compounds (COMPUTER (A) are dissolved in pure methanol, distinguished and evaporated in a boiling water bath. 90% of the evaporation to be filtered out to separate the dust and come up to it.

RESULT AND DISCUSSION

PHARMACOGNOSTIC INVESTIGATIONS

A. Morphological Evaluation of Lawsonia inermis L bark.



Photograph of Lawsonia inermis L bark

Table No- 2 Morphological Evaluation Of Lawsonia Inemis L Bark.

SL.NO.	FEATURES	OBSERVATION
D1.	Outer surface	Grey to black.
2.	Inner surface	Reddish brown
3.	Taste	Bitter.
4.	Oduor	Odorless.
5.	Shape	Quilled.
6.	Fracture	Fibrous.
7.	Size	Length: 10-12cm, width: 3-5cm. Thickness : 0.5-1.5cm

Figure No. 1 T.S. Of Lawsonia Inemis L. Bark (A, B).

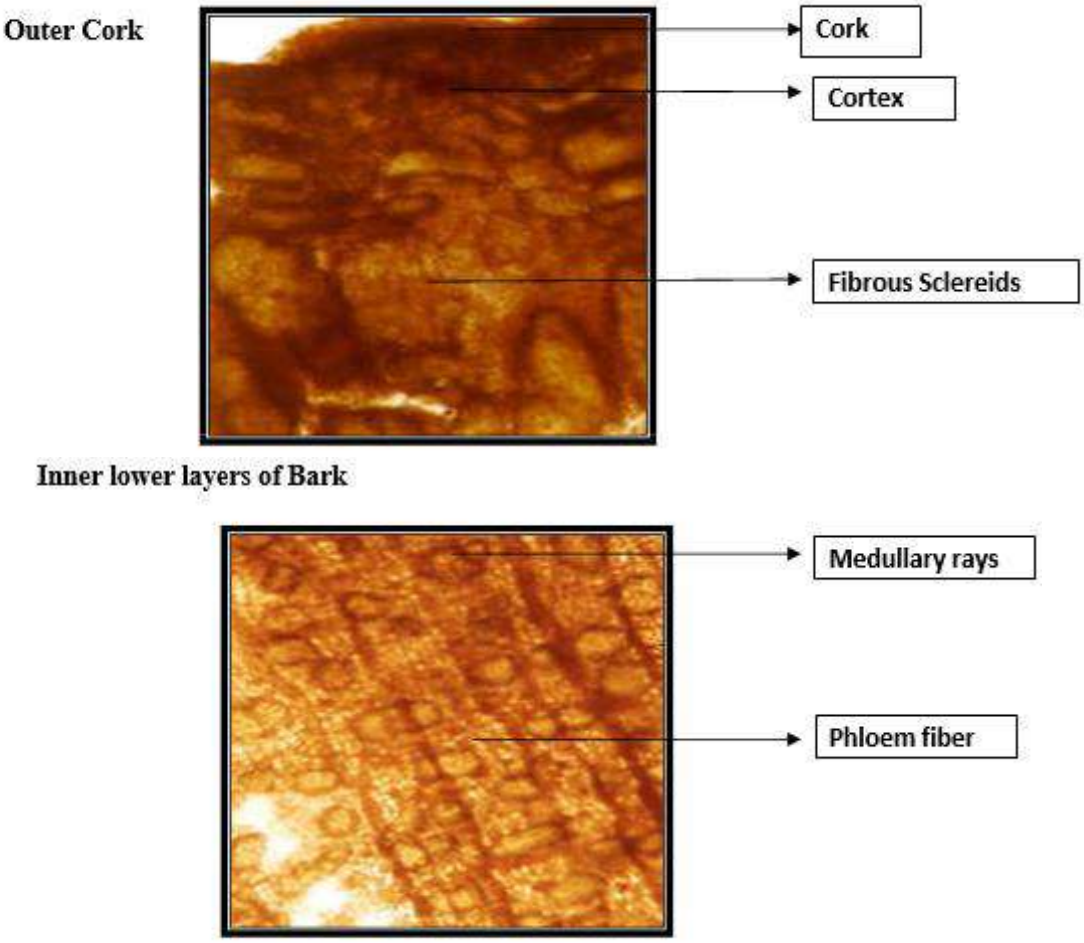


Figure No: 2 Photographs Of Prominent Microscopic Characters Of Coarse Powder Of Lawsonia Inemis L Bark

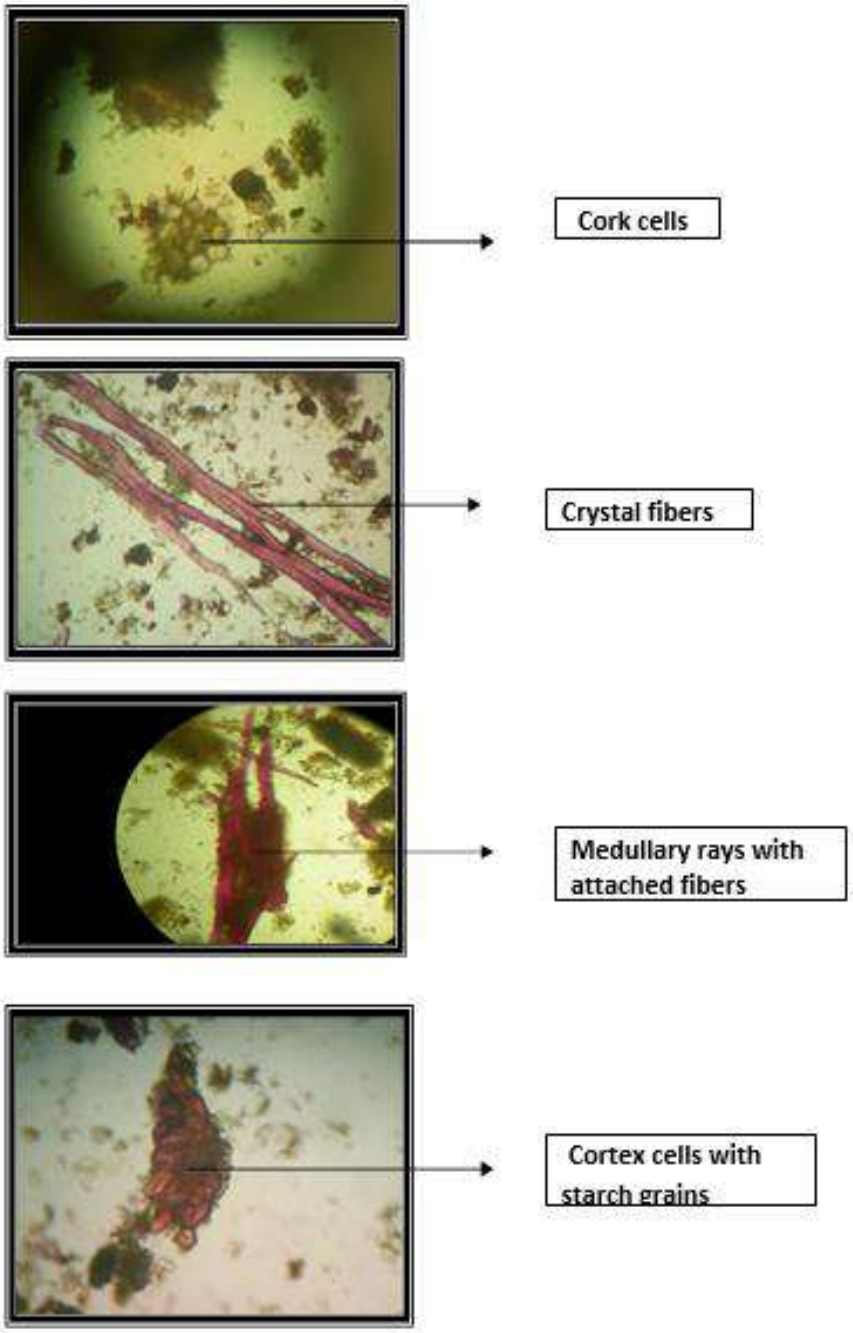


Table No: 3 Proximate values of Lawsonia inemis L bark.

Sl. No.	Parameter	Determined Value % w/w
A	**Extractive values**	
1	Alcohol soluble extracts value	22.00
2	Water soluble extracts value	28.00
3	Pet ether soluble extracts value	1.00
B	**Moisture content**	12.75
C	**Ash Values**	
1	Total ash	6.5
2	Water soluble ash	1.00
3	Acid insoluble ash	1.5
4	Sulphated ash	4.5

PHYTOCHEMICAL INVESTIGATIONS

Table No: 4 Percentage Yield And Physical Characteristics Of Various Extracts Of Lawsonia Inemis L Bark.

Extract	% Dry wt. in gms.	Colour	Oduor	Consistency
Chloroform	2.85	Brownish black	Characteristic	Sticky mass
Methanol	23.9	Brown	Characteristic	Powder
Aqueous	3.35	Reddish brown	Characteristic	Powder

Table: 5 Methanolic extract was further fractionated with ethyl acetate

Fraction	% Dry wt in gms.	Colour	Oduor	Consistency
Ethyl acetate	4.00	Reddish brown	Characteristic	Powder

Table No: 6 Preliminary phytochemical tests of various extracts of Lawsonia inemis L bark

Nature	Successive Extraction.			
	CHCl3	MeOH	AQ	EA
Alkaloids	Positive	--ve	--ve	Positive
Steroids	Positive	--ve	--ve	Positive
Carbohydrates	Positive	--ve	--ve	Positive
Phenolic compounds	Positive	+ve	+ve	Positive
Flavonoids	Positive	+ve	--ve	Positive
Glycoside	Positive	--ve	--ve	Positive
Triterpenoid	Positive	--ve	--ve	Positive
Tannins	Positive	+ve	+ve	Positive

Keywords: CHCl3 = Chloroform. MeOH = Methanol. AQ = Aqueous.

EA=Ethyl acetate. +ve = Present --ve = Absent

Chromatographic Studies.

Evaluation of Ethyl acetate fraction by TLC

Stationary Phase : Silica gel G

Mobile Phase : Toluene: Acetone: Formic acid

Proportion : 60:60:10

Detection : Ferric chloride reagent

Solvent front : 10cm

No of spots : Three (8.8cm, 6.3cm, 5.6cm)

Rf Values : 0.88, 0.63, 0.56

Figure No: 3 TLC of ethyl acetate soluble fraction of methanolic extract.

EA Fraction

Table No: 7 Yield of isolated COMP-A

Isolated Compounds	Yield from column
COMP-A	107 mg

Table No: 8 Physical parameters of isolated COMP-A

Parameters	COMP-A
Physical State	Solid
Colour	Brown
Oduor	Characteristic
Solubility	Methanol, DMSO

Table No: 9 Chemical examination of isolated COMP-A

Chemical Tests	Observation	Inference
FeCl3	Greenish ppt	Flavonoids may be present
Shinoda Test	Pink Color	Flavonoids may be present
Zinc-HCl test	Red Color	Flavonoids may be presents

Table No:10 TLC evaluation of isolated COMP-A

Mobile phase : Toluene:Acetone:Formic acid
Proportion : 60:60:10
Detection : UV-254

Isolated Compound	Evaluation of the Chromatogram	
COMP-A	**Under UV range**	**R_f value**
	Whitish spot	0.63

EACOMP- A

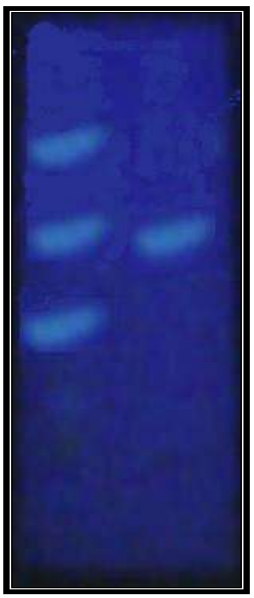


Figure No: 4 TLC Photograph of Ethyl acetate extract and Isolated COMP-A

Spectra and their Characterization:

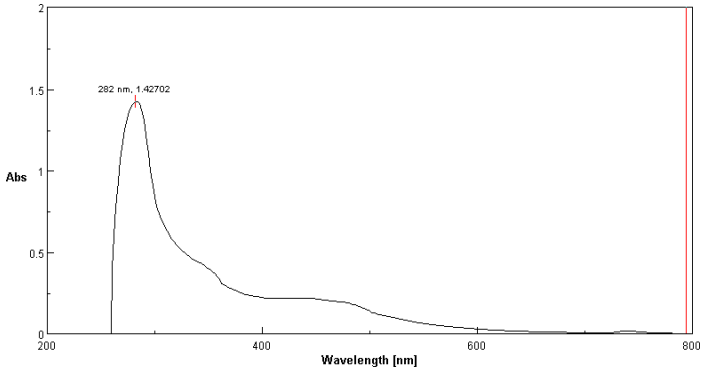


Figure No: 5 UV spectra of isolated COMP-A

Date: 30.01.2021 Time: 2:35PM
Model: V-530
Serial No: B054360568 Response: Fast
Measurement range: 200-800 Data pitch: 1nm
Scanning speed: 1000nm/min No of cycle: 1
SAMPLE Name: COMP-A
Peak found at: 282n

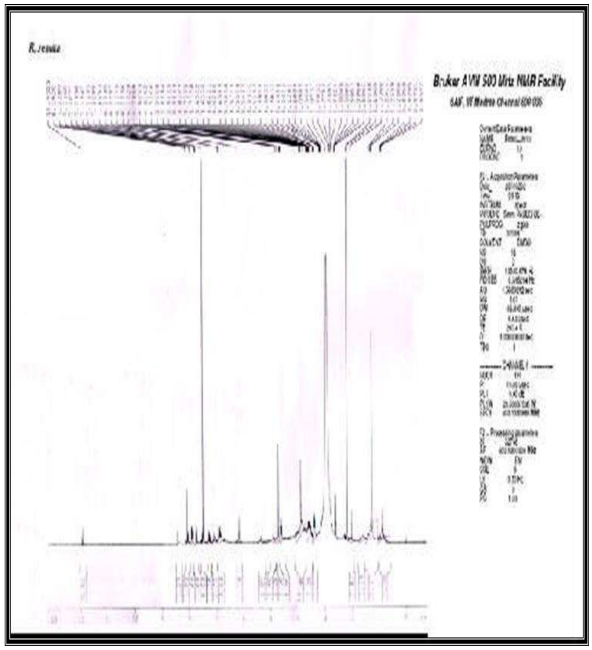


Figure No: 6 FT- IR spectr

Table No: 11 Characterization of Isolated COMP-A

Spectra	Characters
UV	One peak with λ_{max} at 282 nm
FT-IR	Peaks at following wave number are observed Wave number 3369.77 – OH Stretching 2924.07 – C-H Stretching (Aromaticity) 2855.13 – C-H Stretching 2363.93– OH Stretching

DISCUSSION

This study includes Pharmacognostic, Phytochemical, studies of Lawsonia inemis L. bark.

Pharmacognostic Contributions :

The bark was from up to black to red-brown, not with each other, pencil with mirror, round surface to surface. Cross section and powder microscopy of the cortex showed the presence of corks, cortex, and brain gives phloem fibers, fiber, sclerotic, crystal fibers, brain rays of the sun with attached instrument and crying cells composed of starch, grains.

Proximate values:

Close values of the bark of Lawsonia inemis L. These are: Alcohol-soluble extractive value (22.00%), Water-soluble extractive value (28.00%), Pet-ether-soluble extractive value (1.00%), Drying losses (12.75%), Total ash (6.5%), Acid-soluble ash (1.5%), Water-soluble ash (1.00%) and its sulfated ash (4.5%). These are the criteria that determine the identity and quality of the raw drug.

Phytochemical Investigations:

Initial phytochemicals studies of chloroform, methanol and water for the skin and ethyl acetate fraction of methanol for the skin reveal the presence of alkaloidal, steroids, triterpenoids, tannins, flavonoids and phenolic compounds. Add to the performance of liquid chromatography based separation is decided in the ethyl acetate fraction of methanol solution. It was found that the ethyl acetate fraction contains three TLC spots; an attempt was made to isolate this compound by column chromatography with isocratic elution. The isolated compounds were further characterized by physico-chemical testing, chromatography, and spectral analysis, including UV & FT-IR.

The isolated COMP-A:

The following analytical data are exposed.

UV spectra: a vertex with a limited lambda frequency at 282 nm.

IR spectra: wave number ten, 3369.77 O-Stretch, 2924.07 C-H-Stretch (Aromaticity), 2855.13 C-H-Stretch, 2363.93 O-Stretch.

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