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THERAPEUTIC POTENTIAL OF *THESPESIA POPULNEA* SEED EXTRACT IN THE TREATMENT OF ANDROGENETIC ALOPECIA: A PHYTOCHEMICAL AND PHARMACOLOGICAL PERSPECTIVE

Anjan Gowda B. H.*, Hemalatha K. V. and Gagana M. L.

Department of Pharmacognosy, KLE College of Pharmacy, Bangalore, India.

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***Corresponding Author**
Anjan Gowda B. H.
Department of Pharmacognosy, KLE College of Pharmacy, Bangalore, India.

ABSTRACT

Thespesia populnea, a tree native in India's coastal forest and tropical regions, which has demonstrated various therapeutic properties, like hepatoprotective, antibacterial, anti-inflammatory, and antioxidant effects. This study aims to further investigate the potential of *T. populnea* seed extract in treatment of alopecia, highlighting its rich chemical profile and possible applications in dermatological treatments. The present study explores the medicinal potential of *Thespesia populnea* (L.) seed, particularly in the treatment of androgenetic alopecia. Using the Soxhlet method of extraction, various bioactive compounds were extracted from the seeds. A wide variety of pharmacologically significant compounds, like β-sitosterol, thespone, thespesone, kaempferol, herbacetin, quercetin, and rutin. In particular β-sitosterol has shown to promote hair growth, making it a promising for treating hair loss conditions like alopecia. The results suggest that the active compounds in *Thespesia populnea* could provide a novel, plant-based approach to promote hair growth and treating alopecia.

KEYWORDS: *Thespesia populnea*, Alopecia, Soxhlet extraction, β-sitosterol.

INTRODUCTION

India, renowned as the "Medicinal Garden of the World," has a rich tradition of utilizing plants for both preventive and curative purposes. Medicinal plants are integral to traditional Indian healing systems like Ayurveda, Siddha, and Unani, and they continue to play an essential role in modern medicine. The demand for herbal medicine has grown significantly

worldwide, driven by its therapeutic potential and safety profile, which are essential for addressing health care challenges.[1,2,3]

Thespesia populnea (L.) Soland ex Correa, commonly known as the Portia Tree, is a small evergreen tree that typically grows to a height of 6–12 meters with a short, often crooked stem and a broad, dense crown. It belongs to the family Malvaceae and is native to warm coastal regions, extending from Africa to Southeast Asia, Melanesia, and Polynesia. Its ability to grow in sandy and saline soils and its resistance to wind and salt spray make it an effective coastal windbreaker. The name "Thespesia" is derived from the Greek word *thespesios*, meaning divine or sacred, emphasizing its significance.[4,5]

Medicinal plants possess a spectrum of active components and are useful as remedies for various human and animal diseases. The continuing use of herbs in medicine reveals their functional value and necessity in modern healthcare. Thespesia populnea, commonly referred to as *Hibiscus populnea*, belongs to the family Malvaceae and is an evergreen tree.[6]

Thespesia populnea is recognized for its numerous medicinal properties. Various parts of the tree, including its bark, leaves, seed, and flowers, have been used in traditional medicine to treat conditions such as scabies, psoriasis, ringworm, and liver disorders. Its bark decoctions are used for skin ailments and arthritis, while the fruit juices are applied for rheumatic sprains and insect bites. The plant also exhibits significant antibacterial, antioxidant, and anti-inflammatory properties.[5,7]

The phytochemical constituents of Thespesia populnea include compounds like gossypol, tannins, acacetin, and lupeol, along with quinones such as thespone and mansonone-D. These bioactive compounds contribute to the plant’s pharmacological activities, including its antifertility, hepatoprotective, and purgative effects. These diverse applications underscore the importance of the tree in traditional and modern medicinal practices.[7,8]

This study aims to explore the chemical properties of *Thespesia populnea* and its potential applications in treating various health conditions, with a particular focus on its use in the treatment of alopecia. The plant’s therapeutic potential highlights its importance in both traditional and modern medicine.

MATERIAL AND METHODS

MATERIALS

Chemicals: All Chemicals used in the entire study were of analytical grade obtained from the KLE college of Pharmacy Laboratory.

Plant Material: Dry seeds of Thespesia populnea (L) collected in April 2024 from the rural areas surroundings of Doddaballapur, Bengaluru. The plant material was authenticated at K.L.E. Society's S. Nijalingappa College in the Botany Department, Rajajinagar, Bengaluru, India.

METHODS

Preparation of plant extract

The dry powdered material of *Thespesia populnea* (L.) was sieved through a 100 µ mesh. A coarse powder of 125 g was then extracted using a Soxhlet apparatus with *n*-butanol for 28 hours at a temperature range of 40–50 °C. The *n*-butanol extract was concentrated under reduced pressure using a rotary evaporator at a temperature below 55 °C, yielding a semi-solid sticky residue of 15 g, which was then stored for further studies.[7]

Evaluation of crude drugs

Determination of moisture content

Approximately 1g (W_2) of powdered seed material was accurately weighed and placed in a silica crucible (W_1). The crucible was then heated in an oven at 105°C until a constant weight (W_3) was achieved the sample was placed in a desiccator. The loss of weight was recorded and expressed as the moisture content (w/w %).

$$\% \text{ of moisture content} = \frac{(W_1+W_2)-W_3}{W_2} \times 100$$ [9]

Ash Value

The total ash, acid-insoluble ash, and water-soluble ash values were measured from air-dried samples, following the procedures outlined as per the Indian Pharmacopoeia (IP).

Procedure

Total Ash Value

Approximately 2 g of powdered drug was accurately weighed and placed into a pre-weighed silica crucible. The sample was incinerated at 450°C in a muffle furnace until all

carbonaceous material was eliminated. After cooling, the crucible was weighed, and the percentage of total ash was calculated based on the weight of the air-dried sample using the below mentioned formula.

Determination of total ash value formula:

$$\text{Total ash value of the sample} = \frac{z-y}{x} \times 100\ \%$$

X= weight of empty dish

Y= weight of the drug taken

Z= weight of the dish + ash (after complete incineration)

Acid-Insoluble Ash

The total ash residue was boiled with 25 ml of 2N hydrochloric acid for a few minutes and filtered through ashless filter paper. The filter paper, along with any retained residue, was transferred to a pre-weighed silica crucible and incinerated at 450°C until all carbon traces were removed. After cooling, the weight of the crucible was recorded, and the percentage of acid-insoluble ash was calculated relative to the air-dried sample.

Water-Soluble Ash

The total ash residue was boiled with 25 ml of distilled water for several minutes and filtered through ashless filter paper. The filter paper was then placed in a pre-weighed silica crucible and incinerated at 450°C to eliminate carbon. After cooling, the crucible was weighed, and the percentage of water-soluble ash was determined with reference to the air-dried sample.

Thus ash values help assess the purity and quality of the drug by detecting inorganic impurities.[10]

Phytochemical investigation was performed to ensure the presence of different phytoconstituents in the obtained extract

1. **Test for Alkaloids**

a. Mayer's Test: A small quantity of the extract is combined with a few drops of Mayer's reagent (potassium mercuric iodide). The formation of a cream-colored deposits indicates the presence of alkaloids.

b. Wagner's Test: Adding Wagner's reagent (iodine dissolved in potassium iodide) to the extract can indicate alkaloids if a reddish-brown precipitate is observed.

c. Hager's Test: To 1 ml of the extract, a few drops of Hager's reagent (saturated picric acid solution) are introduced. A yellow precipitate confirms the presence of alkaloids.

d. Dragendorff's Test: A small sample of the extract is treated with Dragendorff's reagent (potassium bismuth iodide solution). An orange or yellow deposit indicates alkaloids.[11]

2. Test for Carbohydrates

a. Molish test: A few drops of napthol (20% in ethyl alcohol) was added to 2 ml of alcoholic extract. Then, about 1 ml of concentrated H_2SO_4 was applied down the side of the test tube. presence of carbohydrates shows a reddish violet ring at the junction of the two layers.

b. Fehling's Test: Take 0.5 g of the extract and heat it with dilute hydrochloric acid to break down polysaccharides. Neutralize the resulting solution by adding sodium hydroxide (NaOH), then mix it with Fehling's solutions I and II. The formation of a red precipitate indicates the presence of reducing sugars or carbohydrates.

c. Benedict's Test: Add 1 ml of the sugar solution to 2 ml of Benedict's reagent. Heat the mixture in a boiling water bath for a minute. If carbohydrates are present, a precipitate will form, appearing green, yellow, or red depending on the sugar concentration.

d. Barfoed's Test: Mix 1 ml of the test solution with 1 ml of Barfoed's reagent and heat the mixture in a water bath. The presence of monosaccharides is indicated by the formation of a red cupric oxide precipitate. Prolonged heating (about 10 minutes) may also cause disaccharides to partially hydrolyze into monosaccharides, resulting in a similar reaction.[12]

3. Test for Glycosides

a. Keller-Killiani Test: Add 1 ml of the extract to 1 ml of glacial acetic acid containing ferric chloride traces, followed by 1 ml of concentrated sulfuric acid. A brown ring at the interface, occasionally with a violet ring below, indicates glycosides.

b. Legal's Test: Mix 1 ml of the extract with pyridine, freshly prepared sodium nitroprusside, and sodium hydroxide (1 ml each). A pink to red color confirms glycosides.

c. Borntrager's Test: Combine 1 ml of the extract with 1 ml of benzene and 0.5 ml of dilute ammonia solution. A reddish-pink color suggests the presence of glycosides.

d. Baljet Test: To 1 ml of the extract, add 1 ml of sodium picrate solution. A yellow to orange color indicates glycosides.[13]

4. Test for Tannins

a. Test with Ferric Chloride Solution: Add 5 drops of ferric chloride solution in methanol to 1 ml of the extract in a test tube. The formation of a green to black precipitate confirms the presence of tannins.

b. Test with Gelatin Solution: Mix 1 ml of the extract with 1 ml of a 1% gelatin solution and sodium chloride. The presence of tannins is indicated by the formation of a white precipitate.

c. Test with Ferrous Sulphate Solution: To 1 ml of the extract, add 2 ml of 0.1% ferrous sulfate solution and 0.5% sodium potassium tartrate. A violet color formation indicates tannins.[14]

5. Test for Flavonoids

a. Alkaline Reagent Test: Treat the extract with a few drops of sodium hydroxide solution. The formation of an intense yellow color, which turns colorless upon adding dilute acid, indicates flavonoids.

b. Lead Acetate Test: Add a few drops of lead acetate solution to the extract. The presence of flavonoids is confirmed by the formation of a yellow precipitate.

c. Aluminum Chloride Test: Apply a drop of the test solution on filter paper, let it dry, and spray with aluminum chloride reagent. The appearance of a yellow spot or yellow-green fluorescence under UV light indicates flavonoids.

d. Ammonia Test: Mix the extract with alcohol or water and add a few ml of ammonia. Observe under UV and visible light; the presence of fluorescence indicates flavonoids. Alternatively, fumigate a filter paper containing the extract with ammonia. A yellow or yellow fluorescence under UV light confirms flavonoids.[15]

6. Test for Proteins and amino acids

a. Biuret Test: Mix 2 mL of the filtrate with 1 drop of 2% copper sulfate solution, 1 mL of 95% ethanol, and a few potassium hydroxide (KOH) pellets. The formation of a pink-colored solution in the ethanolic layer indicates the presence of proteins.

b. Millon's Test: Add a few drops of Millon's reagent to 2 mL of the filtrate. The presence of proteins is confirmed by the formation of a white precipitate.

c. Ninhydrin Test: Combine 2 mL of the filtrate with 2 drops of a ninhydrin solution (prepared by dissolving 10 mg ninhydrin in 200 mL acetone). The development of a purple-colored solution indicates the presence of amino acids.

d. Xanthoproteic Test: Add a few drops of concentrated nitric acid to the plant extract. The appearance of a yellow-colored solution confirms the presence of proteins or amino acids.[16]

7. Test for Terpenoids

a. Salkowski Test: Mix 5 ml of the extract with 2 ml of chloroform in a test tube. Carefully add 3 ml of concentrated sulfuric acid (H_2SO_4) along the sides of the tube. The formation of a reddish-brown color indicates the presence of terpenoids.

b. Test for Sesquiterpenes: Add 1 ml of concentrated sulfuric acid to 2 ml of the extract and mix well. A reddish-brown color confirms the presence of terpenoids.[17]

8. Test for Phytosterols

a. Salkowski's Test: Add a few drops of concentrated sulfuric acid (H_2SO_4) to the filtrate, shake well, and let it stand. The appearance of a red color in the lower layer indicates the presence of sterols or terpenoids.

b. Libermann-Burchard's Test: Dissolve 50 mg of the extract in 2 ml of acetic anhydride and carefully add 1-2 drops of concentrated sulfuric acid along the side of the test tube. A series of color changes confirms the presence of sterols.

c. Acetic Anhydride Test: Mix 0.5 ml of the plant extract with 2 ml of acetic anhydride and 2 ml of concentrated sulfuric acid. A color change from violet to blue or green indicates sterols or triterpenoids.

d. Hesse's Response: Combine 5 ml of the aqueous extract with 2 ml of chloroform and 2 ml of concentrated sulfuric acid. The formation of a pink ring or red color in the lower chloroform layer suggests the presence of sterols or terpenoids.[15]

Thin Layer Chromatography

Thin Layer Chromatography (TLC) analysis of *Thespesia populnea* (L.) revealed an Rf value of 0.55. This was determined by reconstituting the extracts of crystals in chloroform and spotting them onto the TLC plate. The mobile phase used consisted of a mixture of toluene, chloroform, and methanol in a ratio of 4:4:1 (v/v).[18]

TLC plates were prepared using silica gel 'G'. A suspension of 30 grams of silica gel and 60 ml of distilled water was homogenized for two minutes, applied evenly to plates, and air-dried until transparency disappeared. The plates were further dried in a hot air oven at 110°C for 30 minutes and then stored in a dry environment for use. Crude extract was diluted with chloroform, and 1–10 µl of each sample was applied to the origin of the prepared TLC plates, 1 cm above the bottom, using capillary tubes. The samples were spotted on the pre-coated silica gel plates and placed in a saturated chamber containing a solvent system of toluene, chloroform, and methanol (4:4:1 v/v). The solvent was allowed to rise until it reached three-fourths of the plate. This TLC analysis was primarily conducted to detect phytosterol components.

The retardation factor was calculated using the below formula

$$\text{Rf value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$ [19,20]

Powder Microscopy

An authenticated crude drug sample was collected, powdered, and passed through a sieve No. 60-mesh size. The powdered material was cleared using a chloral hydrate solution and mounted in a mixture of chloral hydrate and glycerol to prevent crystal formation during microscopic examination. Lignification was identified by treating the sample with a solution of phloroglucinol and hydrochloric acid. Glycerin was used to mount the coverslip, effectively restricting cell movement for clearer observations. Additional preparations were made using different stains like iodine, Sudan III, ruthenium red and sulfuric acid to highlight specific cells or cellular contents. Pre-cautional Care was taken to eliminate air bubbles. Using a compound microscope, diagnostic features and cellular dimensions were

meticulously recorded, and the unique characteristics of the powder such as trichomes, vittae, oil glands, vascular bundles etc. were captured and documented.[21]

Fourier Transform Infrared spectroscopy (FTIR)

Fourier Transform Infrared (FTIR) spectroscopy was employed to identify the characteristic functional groups present in the plant. For the analysis, 10 mg of dried seed extract powder was mixed with 100 mg of potassium bromide (KBr) to form a translucent pellet. The prepared sample was then analyzed using an FTIR spectrometer (Shimadzu 8000 series) over a scan range of 800 to 4000 cm^{-1} with a resolution of 4 cm^{-1}, and the corresponding peak values were recorded.[20]

RESULT AND DISCUSSION

Preliminary characterization of powdered drug was carried out for various tests such as moisture content, ash value, acid insoluble ash value and water soluble ash value as shown in the Table 1.

Table 1: Ash values and moisture content of *Thespesia populnea* (L.) powdered seed.

Characterization	Percentage
Moisture content value	4%
Ash value	5.05%
Acid insoluble ash	0.48%
Water soluble ash value	0..62%

After the successful processing of the sample and Hot Soxhlet extraction of the seed extract in the investigation, the preliminary phytochemical study revealed that n-Butanol extract of T.populnea (L.) contains carbohydrates, proteins, flavonoides, tannins, glycosides, phenols, terpenoids, alkaloids, Phytosterols and saponins were present in the extract as shown in Table 2.

Table 2: Qualitative Phytochemical screening of *Thespesia populnea* (L.) powdered seed.

SI.No	Chemical test	Result
1	**Test for alkoloids**	
	Dragendroff's test	+
	Mayers test	+
	Hagers test	+
	Wagners test	+
2	**Test for carbohydrates**	
	Molish test	+
	Fehlings test	+
	Benedicts test	+

	Barfoed's test	+
3	**Test for glycosides**	
	Killer killani test	+
	Legal's Test	+
	Borntrager's test	+
	Baljet Test	+
4	**Test for Tannins**	
	Test with Ferric Chloride Solution	+
	Test with Gelatin Solution	+
	Test with Ferrous Sulphate Solution	+
5	**Test for flavanoids**	
	Alkaline reagent test	+
	Lead acetate test	+
	Aluminum Chloride Test	+
	Ammonia Test	+
6	**Test for protens amino acids**	
	Biuret test	+
	Millon's test	+
	Ninhydrin test	+
	Xanthoproteic Test	+
7	**Test for Terpenoids**	
	Salkowski Test	+
	Test for Sesquiterpenes	+
8	**Test for Phytosterols**	
	Salkowski's Test	+
	Libermann-Burchard's Test	+
	Acetic Anhydride Test	+
	Hesse's Response	+

The Thin Layer Chromatographic analysis of the T. populnea (L.) n-Butanol extract of seed was carried out as explained. The chromatogram revealed 0 bands under UV and 3 bands in visible light as seen in Figure 1.

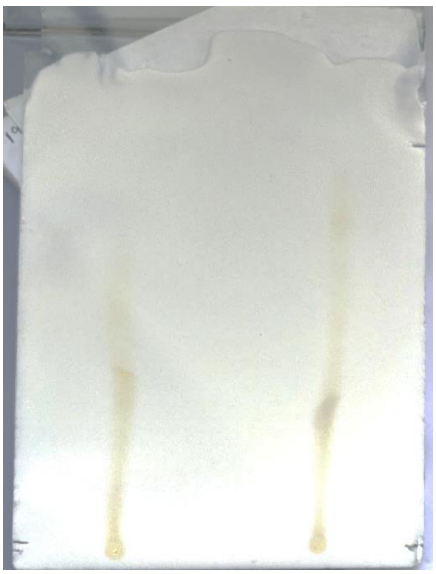

Fig. 1: TLC of *Thespesia populnea* (L.) powdered seed extract.

The Rf values calculated for each band obtained in TLC plate as shown in (Table 3).

Table 3: Rf value calculation of each separated bands.

Band NO	Rf values
Band No.1	0.45
Band No.2	0.57
Band No.3	0.84

The pharmacognostic characters of the seed were studied with the help of different parameters. Organoleptic study of the seed indicated characteristic colour, odour and taste (Table 4).

Table 4: Basic characterization.

Characters	Observation
Colour	Brown
Odour	Aromatic
taste	Bitter
Shape	ovulated
Size	0.8 cm avg

The microscopic examination of the powder showed Endosperm (Fig. 1), calcium oxalate (Fig. 2), vittae (Fig. 3), oil glands (Fig. 4), Vascular bundle (Fig. 5), Trichomes (Fig. 6) Powder character.

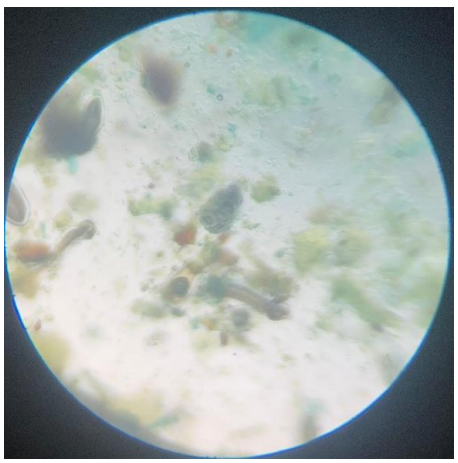

Fig. 1: Endosperm.

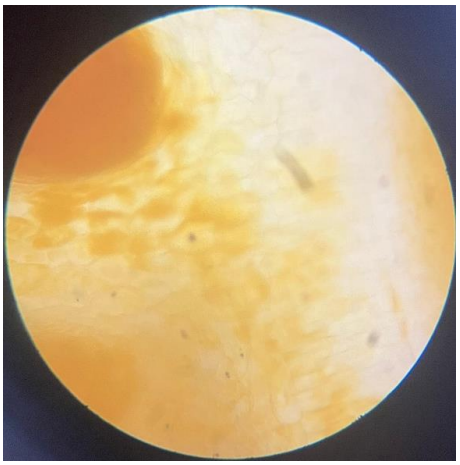

Fig. 2: Calcium oxalate crystals.

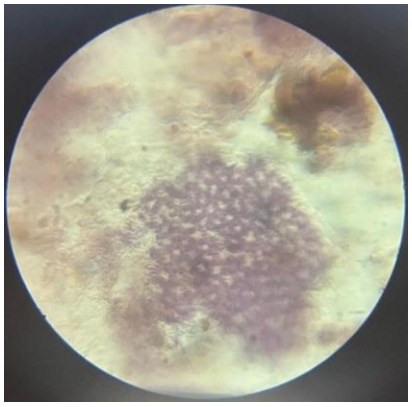

Fig. 3: Vittae.

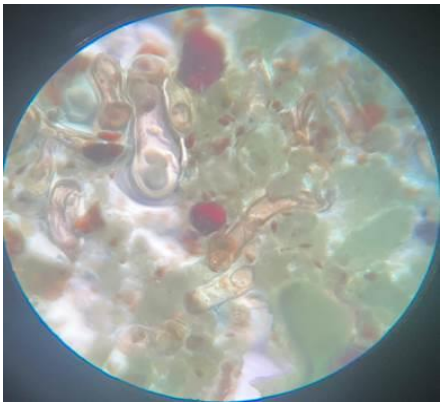

Fig. 4: Trichomes.

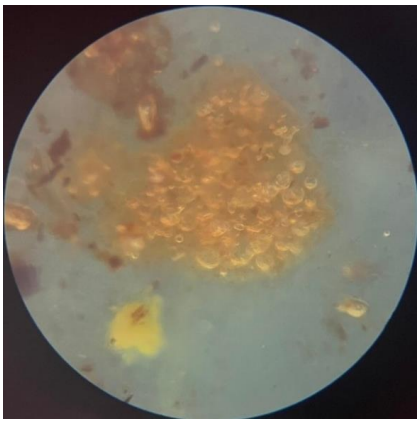

Fig. 5: Oil globules.

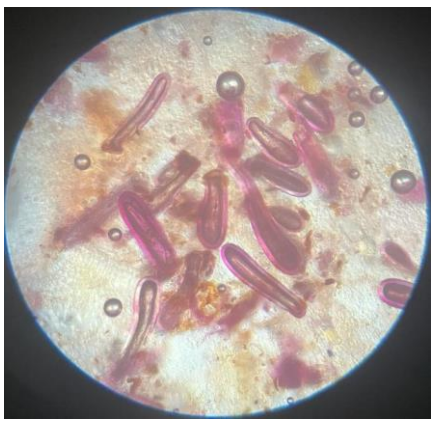

Fig. 6: Vascular bundles.

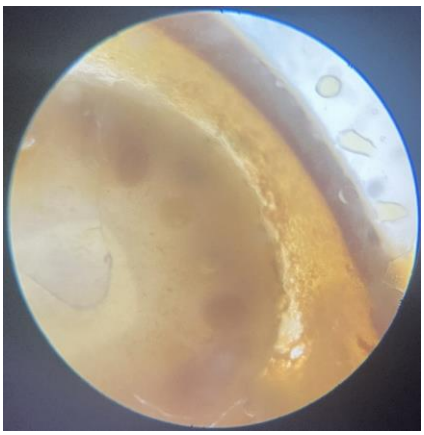

Fig. 7: T.S of Seed in 10x.

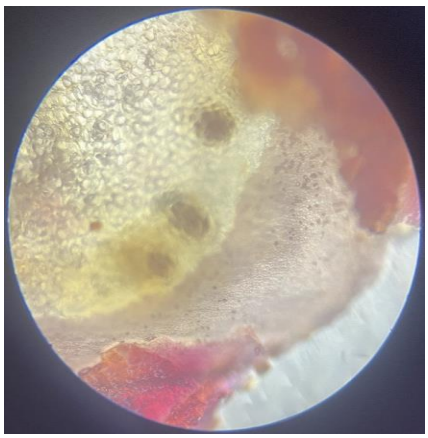

Fig. 8: T.S of Seed in 45x.

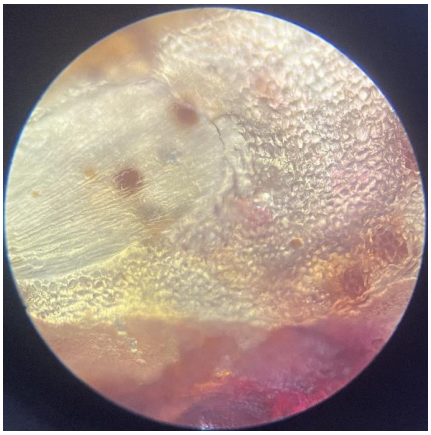

Fig. 9: T.S of Seed with Endosperm.

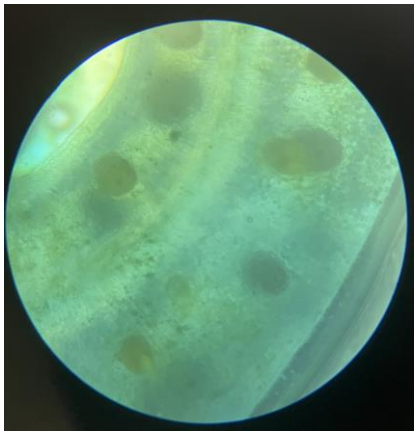

Fig. 10: Staining of T.S using Methylene Blue Stain.

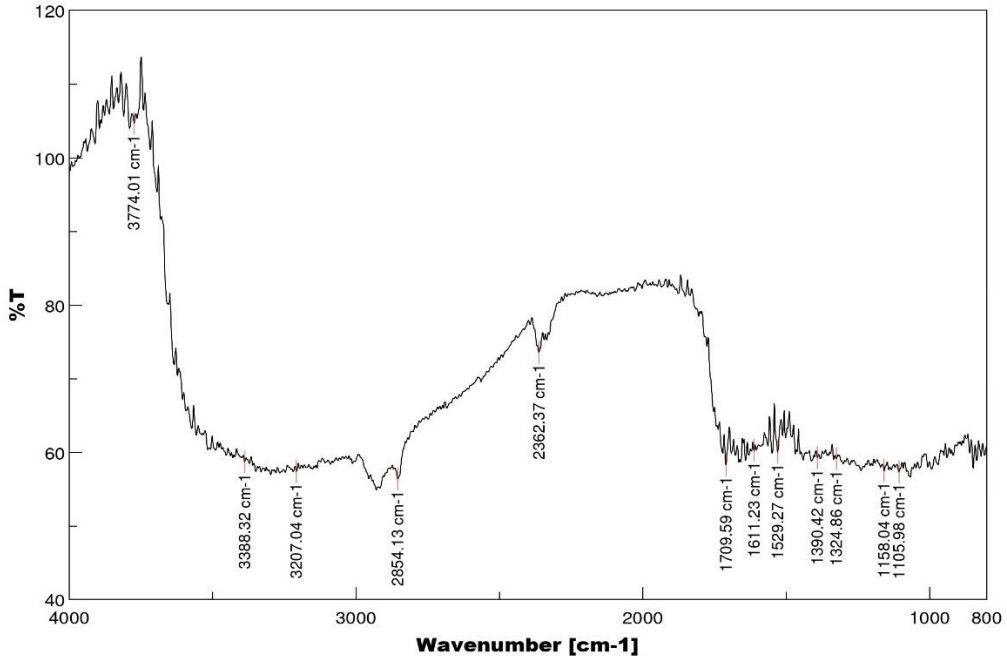

Fig. 11: FTIR result analysis of powdered crude drug.

The FTIR results provided show absorbance peaks at specific wavenumbers (cm^{-1}). To determine the presence of Beta-sitosterol, we need to correlate these peaks with functional groups typically present in its structure. Beta-sitosterol has the following functional groups:

1. **Hydroxyl group (-OH)**: Typically observed in the range of 3200–3600 cm^{-1}.
2. **Aliphatic C-H stretching**: Found around 2800–3000 cm^{-1}.
3. **C=C stretching (from aromatic or unsaturated structures)**: Appears near 1600–1680 cm^{-1}.
4. **C-O stretching**: Seen around 1050–1150 cm^{-1}.

Observations from the FTIR Data

- **3388.32 cm^{-1} (%T: 58.728)**: This corresponds to the hydroxyl (-OH) group, suggesting the presence of alcohol functionality in beta-sitosterol.
- **2854.13 cm^{-1} (%T: 56.4074)**: Indicates aliphatic C-H stretching, consistent with the aliphatic chain in beta-sitosterol.
- **1611.23 cm^{-1} (%T: 60.3053)**: May be attributed to C=C stretching in unsaturated structures, aligning with the sterol ring system.
- **1158.04 cm^{-1} (%T: 57.5324)** and **1105.98 cm^{-1} (%T: 57.379)**: These are in the range of C-O stretching, supporting the presence of a hydroxyl group attached to the sterol nucleus.

The FTIR spectrum analysis shows characteristic peaks that align well with the functional groups present in beta-sitosterol. These include evidence for the hydroxyl group, aliphatic chains, and the sterol structure. Based on the observed data, it is likely that the sample contains beta-sitosterol. This confirms the presence of beta-sitosterol, supporting its use in further herbal hair oil formulations for treating alopecia.

CONCLUSION

Thespesia populnea (L.) seed extract has shown promoting results in the treatment of androgenic alopecia. Soxhlet extraction yielded bioactive substances such as β-sitosterol, thespone, thespesone, kaempferol, herbacetin, quercetin, and rutin that promote hair development. The plant extract's content of β-sitosterol, which inhibits DHT, implies it might be a natural option for treating hair loss.

Furthermore, phytochemical and pharmacognostic analysis verified the presence of important bioactive ingredients, that promote hair follicle stimulation. Thin Layer Chromatography (TLC) and FTIR studies confirmed the chemical composition and supported the medicinal claims.

In conclusion, *Thespesia populnea* seed extract has enormous potential as a natural therapy for alopecia, suggesting additional clinical research and formulation development for dermatological uses.

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