Formulation and Evaluation of herbal ointment to treat psoriasis with *Indigofera aspalathoides*

Ch. Vidyulatha*¹, N. Lathesh², Y. Govardhan Reddy², Ch. Dharani², G. Sri Vidya², Hasanur Rahaman²

¹Department of Pharmaceutics, Nimra College of Pharmacy, Nimra Nagar, Ibrahimpatnam, Vijayawada, Krishna District – 521456, Andhra Pradesh, India

²Nimra College of Pharmacy, Nimra Nagar, Ibrahimpatnam, Vijayawada, Krishna District – 521456, Andhra Pradesh, India

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**Abstract**

The current study aims to prepare an herbal ointment using several extracts of the complete Indigofera aspalathoides plant and assess the extracts' antimicrobial effectiveness for psoriasis. The entire plant's morphological and physiochemical characteristics were evaluated. Different extracts of powdered whole plant were made, and these extracts underwent phytochemical analysis. Alkaloids, carbohydrates, flavonoids, steroids, and other phytoconstituents were all present in the ethanolic extract. The extract's antimicrobial activity was examined throughout. Even at low concentrations, the ethanol and acetone extracts demonstrated promising efficacy and a maximum zone of inhibition. The antifungal activity of the ethanolic extract was assessed at 4%w/w and 2%w/w concentrations in an ointment formulation. The antifungal activity of the ointment made with ethanolic extract was on par with that of regular clobetasol cream. The ethanol extract cream's activity was shown to outperform that of the typical ointment.

**Keywords:**

Herbal ointment, psoriasis, ethanolic extract, antimicrobial, *Indigofera aspalathoides*

**INTRODUCTION**

Around 80% of the world’s population receives primary healthcare from herbal remedies, mostly in developing nations. They have endured over time because of their safety, effectiveness, cultural acceptability, and lack of adverse consequences. In addition, herbal remedies for age-related illnesses such as memory loss, osteoporosis, osteoarthritis, diabetes, immune system, liver, and other ailments are mentioned in ancient literature. For these conditions, palliative care is the only treatment available. Since their chemical components are involved in the physiological processes of live plants, they are thought to be...
more suited to human health. More than 1.5 million people use medicinal herbs for therapeutic, promoting, and preventive purposes as part of a traditional medical system [1]. Plants are prospective sources of herbal formulations, and their use has drawn interest from experts in a variety of medical specialties as well as scientific communities from other fields. The number of companies producing herbal treatments has increased as a result of the growing use of these drugs due to the toxicity and adverse effects of allopathic medicines. The number of non-prescription users of herbal drugs has increased during the last few decades. These medications have held up well to thousands of years of human testing and real-world testing. Some medicines have been withdrawn because they are too toxic, while others have had their side effects adjusted or balanced by adding more herbs [2].

**MATERIAL AND METHODS**

**Collection of Plant Material**
The whole plant of *Indigofera aspalathoides* was collected in January 2023 at Tirupati.

**Macroscopic Evaluation**
The morphological feature of the plant was evaluated and recorded.

**Microscopic Evaluation**

**Fixation of Leaf, Stem, and Root**
Whole plant sliced and fixed in FAA solution (5 milliliters of formalin, 5 milliliters of acetic acid, and 90 milliliters of 70% ethanol). The specimen was fixed for 24 hours, and then it was dehydrated. Following the usual protocol, the entire plant was graded using a succession of tertiary butyl alcohol.

The process involved progressively adding melting paraffin wax at 58 to 60º C until the Tertiary butyl alcohol (TBA) solution reached supersaturation. The samples were poured into blocks of paraffin. Using a Rotary Microtome, the specimens embedded in paraffin were divided into sections. The sections were between 10 and 12μ thick. The parts were waxed using conventional methods. I used a hematoxylin stain on the sections. Under a microscope, the stained slices were examined [3].

**Powder Microscopy**
The entire plant was dried in the shade and crushed into powder for microscopic analysis. To distinguish the numerous microscopical distinctive characteristics, several staining reagents were utilized in conjunction with observing the organoleptic characters. Under a microscope, the powder was dyed with strong hydrochloric acid and 1% phloroglucinol in 90% ethanol. The identification of distinctive features and different cell components is done via powder analysis [4].

**Quantitative Microscopy**

**Linear Measurement of Fibres**
Under a microscope, the length and width of the fibers found throughout the entire plant were measured. This quantitative analysis will aid the drug’s identification. The stage micrometer calibrates the eyepiece micrometer as the initial step in this process. The eyepiece micrometer is inserted into the ridge after the lens is unscrewed, and the eyepiece is taken out of the microscope to get the calibration factor [5]. Next, the lens is changed. After that, the stage micrometer is put on the microscope’s stage and focused at high power using an eyepiece that lines up with each division of the stage micrometer, and the calibration factor is determined by applying the standard formula. This slide holds the powdered drug instead of the stage micrometer. A drop of phenolphthalein and a hydrochloric acid solution is added to the whole plant powder on the slide, which is then ready to be examined under a microscope. By concentrating them on the lines of the eyepiece micrometer, fibers can be measured for length and breadth. The number of divisions in the fibers’ length and width cover should be noted [6].

**Physiochemical Analysis**
The powdered, shade-dried *Indigofera aspalathoides* were utilized to analyze several physiochemical parameters that help determine the quality and purity of crude pharmaceuticals. According to the standard WHO recommendations, total ash, extractive values, loss on drying, foaming index, swelling index, and foreign organic materials were measured. This information is beneficial in determining the quality and purity of crude pharmaceuticals [7].
**Determination of Ash Values**

The residue that remains after burning is called the drug's ash content, and it simply refers to the inorganic salts that are either naturally present in the drug, adhere to it, or are purposefully added to it to degrade it.

**Total Ash**

After 30 minutes of red-hot heat, the silica crucible was allowed to cool in desiccators. A precise weight of about 2 grams of the powdered material was added to the crucible and spread uniformly. Dried for one hour at 100–105ºC, then ignited at 600±25ºC to constant weight in a muffle furnace. In a desiccator, the crucible was allowed to cool. The formula was then used to determine the percentage yield of ash concerning the air-dried material [8].

**Water Soluble Ash**

Using 25 milliliters of water, the entire amount of ash was brought to a boil for five minutes. A filter paper without ash was then used to gather the insoluble material. Following a 15-minute ignition at a temperature not to exceed 450ºC, it was cleaned with hot water. It was possible to compute the percentage of water-soluble ash in air-dried substances by subtracting the weight of the insoluble matter from the weight of the ash and using the weight difference as a proxy for the water-soluble ash [9].

**Acid Insoluble Ash**

The residue left over after burning the remaining insoluble material and boiling the entire ash in diluted hydrochloric acid is known as acid-insoluble ash. This gauges the concentration of silica, particularly in the form of sand and siliceous earth.

**Sulfated Ash**

Around 3 grams of an air-dried material in a crucible was initially slowly burned until it was completely blackened. After that, the residue was allowed to cool, moistened with one milliliter of sulfuric acid, heated gradually until no more white fumes were produced, and then burned at 800 ± 25ºC until all black particles vanished. A few drops of sulfuric acid were added to the crucible and heated after cooling. It was then lit again, let to cool, and weighed. Next, the air-dried substance's sulfated ash percentage was computed [10].

**Loss on Drying**

A precise weight of the materials was added to a silica crucible that had been ignited and cooled and then gently distributed throughout. The materials were then heated to a constant weight for a predetermined time. Before being weighed, the crucible was covered with its lid and let to cool at room temperature in a desiccator. The crucible was weighed in the end to determine the drying loss for the air-dried material [11].

**Determination of Foaming Index** [12]

After weighing about 1g of the coarsely ground drugs, it was added to a 500ml conical flask containing 100ml of boiling water. The flask was kept between 80 and 90 degrees Celsius for almost half an hour. After cooling and filtering, enough water was poured through the filtrate to fill the volume to 100 milliliters in a volumetric flask. Ten stopper test tubes (diameter 16 mm, height 16 cm) were filled with the decoction in incremental volumes of 1 ml, 2 ml, 3 ml, and 4 ml, up to 10 ml. The liquid volume in each tube was then brought down to 10 ml with water. After stopping, the tubes were shaken twice a second for 15 seconds in a longitudinal motion. The foam was measured after being left to stand for fifteen minutes. Each tube has a foaming index more significant than 1000 and a foam height greater than 1 cm. To get a result in this scenario, repeat the determination using a fresh set of decoction dilutions.

Utilizing the formula below, get the foaming index.

**Foaming index = 1000/a**

**Determination of swelling index**

The swelling index is the volume in milliliters, which means that one gram of plant material will swell under specific circumstances. Method A predetermined amount of the plant material was weighed precisely and then put into a measuring cylinder with a glass stopper, a capacity of 25 milliliters. The graded portion should be around 125mm, with divisions of 0.2 ml from 0 to 25 ml oriented upwards. The cylinder's internal diameter should be approximately 16 mm. Add 25 milliliters of water and shake the mixture well every 10 minutes for an hour unless otherwise specified in the test protocol. Let it remain at room
temperature for three hours. The amount of plant material, including any sticky mucilage, was measured in milliliters (ml). Determine the individual determination's mean value with 1gm of plant material [13].

RESULTS AND DISCUSSION

The results of pharmacognostic studies are as follows.

Organoleptic character
Leaf – pale green
Stem – dark brown
Root – brown color

Microscopy of leaflet, stem, and root

T.S. of Leaflet

The leaflet has a V-shaped appearance when it is folded or laid flat adaxially. The midrib section has a thickness of 200μm horizontally and 170μm vertically. There are rectangular cells and a smooth surface along the adaxial groove. The xylem components are compact, thick-walled, angular, and narrow. In sectional view, the leaf margin is 130μm thick, semicircular, and slightly dilated [Figure 1]. The leaf margin has a thick layer of the epidermis. Similar to the central portion of the leaflet, palisade and spongy parenchyma are produced from the mesophyll tissue. PM - Palisade Mesophyll, SMSpongy Mesophyl, AdS: Adaxial side.

Figure 1 T.S of Leaflet

T.S. of Stem

In sectional view, the stem has a circular shape, smooth and even surfaces, and a thickness of 900 μm. The thickness of epidermal cells is five μm. The width of the cortical zone is 100 μm. It is heterogeneous, consisting of a broad isolated group of two or three cells and four or five layers of small, compact cells in the outer section; in the middle are noticeable, distinct, circular masses of mucilage or gelatinous fibers grouped in a ring around the stem [Figure 2]. Four or five layers of quite wide, compact parenchyma cells have different diameters in the inner cortex. A continuous sheath of phloem surrounds the xylem. The vessel lines run from the cylinder’s inner to outer limits.

Figure 2 T.S of Stem
Ep - Epidermis,
Co – Cortex, Ph – Phloem, X: Xylem, Pi: Pith.

T.S. of Root

Ph – Phloem, X: Xylem, PE: Periderm,

Figure 3 T.S of Root
The tubular, suberized phloem cells that comprise the thin root are arranged in sheets or layers. An outside, dark cylinder of sclerenchyma and crushed phloem makes up the secondary phloem. Radial files of elements without sclerenchyma cells within the inner phloem are non-collapsed. The xylem in question is round. 50 μm is the diameter of the broad vessel in the outer zone. One may distinguish between the inner non-collapsed phloem and the outer collapsed phloem in the secondary phloem. With a considerably wider distribution, the collapsed phloem comprises crushed phloem elements and triangular cone-shaped dilated rays that alternate [Figure 3]. Gelatinous in nature, the fibers have both lignified outer walls and gelatinous inner unlignified walls.

Powder microscopy

The following structures were found using powder microscopy.

Epidermal cells: These cells were found in the outer layer and may have straight, brownish-colored walls. Non-lignified fiber: Sclerenchyma region (middle rib region) included them. They have pointed tips, thin walls, and narrow lumens.

Trichomes: There were two different kinds of trichomes [Figure 4]. The trichomes covering the uncellular layer were seen to be long, thin, curved at the base, and pointed at the tip. Knee-shaped trichomes: these are found in the lamina region and are also found in vasaka leaves. Spiral xylem vessels. Calcium oxalate: the oxalate prism.

QUANTITATIVE MICROSCOPY

Linear measurement of fibers

The entire powdered plant of Indigofera aspalathoides was tested for fiber length and width [Table 1]. The fibers were measured to be between 84 μ and 398 μ meters in length and between 3.1 μ and 12.04 μ meters in width.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Minimum (μm)</th>
<th>Average (μm)</th>
<th>Maximum (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length</td>
<td>84</td>
<td>214</td>
<td>398</td>
</tr>
<tr>
<td>Width</td>
<td>3.1</td>
<td>8.64</td>
<td>12.04</td>
</tr>
</tbody>
</table>

Physicochemical Parameters

Numerous physiochemical constants were examined, including total ash (10.59±0.54), acid-insoluble ash (8.6±0.31), water-soluble ash...
(9.6±0.36), extractive values (6.9±0.41), loss on drying (11.54±0.34), swelling index (Nill), and foaming index (Nill).

To distinguish Indigofera aspalathoides from adulterants and related species, a thorough pharmacogenetic analysis of the entire plant will help identify the drug both in crude and powder form.

PHYTOCHEMICAL STUDIES

Preparation of extracts

Using a soxhlet apparatus, the entire plant was harvested, let to dry in the shade, coarsely ground, and then extracted using a series of solvents with varying polarities, such as ethanol, ethyl acetate, and chloroform, in a continuous percolation process. Each extract was concentrated using a rotary vacuum evaporator following extraction. After drying, the yield % was computed. The extract’s consistency and appearance were also observed.

Preliminary phytochemical screening

A qualitative chemical analysis was performed on the whole plant powder and extracts to identify the active ingredients in each extract and the powdered whole plant.

DETECTION OF ALKALOIDS

Dragendorff's reagent

After adding 5 milliliters of 2M HCl and 1 milliliter of Dragendorff’s reagent to the sample, it was seen that an orange-red precipitate formed immediately.

Mayer's reagent

A small amount of diluted hydrochloric acid and Mayer's reagent were added to the material, and it was then observed to see if a white precipitate formed.

Wagner’s reagent

Wagner’s reagent was used sparingly on the test material, and the formation of a reddish-brown precipitate was monitored.

DETECTION OF GLYCOSIDES

Borntrager’s test

One milliliter of sulfuric acid and the powdered substance were cooked in a test tube for five minutes. Hot filters were cooled and shaken with an equivalent amount of chloroform. Half of the solvent’s capacity in diluted ammonia was added, and the lower layer was agitated. Anthroquinone glycosides are indicated by a rose pink to red color formed in the ammonical layer.

Modified Borntrager’s test

The test sample was brought to a boil using two milliliters of diluted sulfuric acid. This was treated for five minutes with two milliliters of freshly made 5% aqueous ferric chloride solution, and then it was agitated with an equal amount of chloroform. Half the diluted ammonia was added to the lower solvent layer and shaken. The ammonical layer produces a rose-pink to crimson color.

Legal’s test

Test sample after methanolic alkali and pyridine treatment with sodium nitroprusside. When cardiac glycosides form, a pink-red color is present.

DETECTION OF STEROIDS AND TRITERPENOIDS

Liebermann Burchards Test

After adding a few drops of acetic anhydride, the powdered medication was heated, boiled, and cooled. Following the addition of concentrated sulfuric acid from the test tube's sidewalls, a brown ring developed at the intersection of two layers, the upper layer turning green to indicate the presence of steroids and the creation of a deep red color indicating the presence of tri terpenoids.

Salkowski Test

After adding a few drops of strong sulfuric acid to the extract, the lower layer turned red, signifying the presence of steroids, and the bottom layer turned yellow, indicating the presence of triterpenoids.

DETECTION OF FLAVONOIDS

Shinoda’s test

After dissolving a small amount of extract in alcohol, drops of strong hydrochloric acid were added to the magnesium and heated. The magenta color appearance indicates the presence of flavonoids.
After dissolving a small amount of extract in alcohol, drops of strong hydrochloric acid were added to the magnesium and heated. The magenta color appearance indicates the presence of flavonoids.

**Alkaline reagent test**

When a small amount of the extract is dissolved in aqueous sodium hydroxide, flavonoids are present because of the emergence of the yellow color.

**DETECTION OF CARBOHYDRATES**

**Molisch's test**

A purple to violet color ring forms at the junction when a few drops of conc. Sulfuric acid and alcoholic alpha naphthol are added to the test solution via the tube's walls. This confirms the presence of carbs.

**Fehling's test**

Fehling’s I and II were combined with the test solution and heated, and the presence of sugar was detected by looking for the appearance of red coloration.

**DETECTION OF TANNINS**

**Lead acetate test**

A basic lead acetate solution was combined with the test solution, and a white precipitate was developed.

**Test for ferric chloride**

Two milliliters of the drug’s aqueous extract were mixed with a few drops of a 5% aqueous ferric chloride solution, and the mixture was then tested for the formation of a bluish-black color.

**PROTEIN DETECTION**

**Biuret test**

After applying 5-8 drops of a 10% w/w copper sulfate solution to the sample, violet color was noted.

**MEASUREMENT OF SAPONINS**

The sample was mixed with a drop of sodium bicarbonate solution, shaken briskly, and allowed to sit for three minutes. The formation of any foam resembling a honeycomb was studied.

**DETECTION OF GUMS AND MUCILAGE**

The test component was dissolved in 5 to 10 milliliters of acetic anhydride using heat and chilled, and then 0.05 milliliters of concentrated sulfuric acid were added. The development of a vivid purplish-red hue signifies the existence of mucilage and gums.

**DETECTION OF FIXED OILS AND FATS**

Two filter papers were used to press little amounts of extracts between them. An oily stain on the filter paper indicates fixed oils and fats.

**QUANTITATIVE ESTIMATION OF TOTAL FLAVONOID CONTENT**

**Total Flavanoid content**

The total flavonoid content was ascertained by employing the calorimetric technique and quercetin as a standard. Each test sample was separately dissolved in DMSO. Next, 150 μl of the sample solution was combined with 150 μl of 2% aluminum chloride. A spectrophotometer was used to measure the absorbance of the supernatant at 435 nm following ten minutes of incubation at room temperature. Every test sample was created in three duplicates. The total flavonoid content was expressed in milligrams of quercetin equivalent per gram of extract (mg QRT/gm extract).

**FLUORESCENCE ANALYSIS**

When exposed to U.V. light, a lot of crude drugs exhibit fluorescence. Because of the general unreliability of the fluorescent effect, evaluation of crude pharmaceuticals based solely on fluorescence in daylight is rarely often used. Several crude drugs exhibit characteristic fluorescence, which is highly valuable for their evaluation. Fluorescent lamps have appropriate filters that exclude visible light from the bulb and transmit U.V. radiation of a specific wavelength.

**THIN LAYER CHROMATOGRAPHY**

**Principle**

When exposed to U.V. light, a lot of crude drugs exhibit fluorescence. Because of the general unreliability of the fluorescent effect, evaluating crude pharmaceuticals that only rely on fluorescence in daylight is rarely used. Several crude drugs exhibit characteristic fluorescence, which is highly valuable for their evaluation. Fluorescent lamps have appropriate filters that
exclude visible light from the bulb and transmit U.V. radiation of a particular wavelength.

**TLC Plate Preparation**

The Stahl TLC spreader was used to prepare the plates. To make a uniform suspension, 40g of silica gel G and 85ml of water were combined, and then the mixture was placed into the spreader. Plates of 0.25 mm thickness were made, air dried until the layer's transparency vanished, and then dried for 30 minutes at 110°C before being stored in desiccators.

**Selection of mobile phase**

The phyto-constituents included in each extract were considered when choosing the solvent mixture. The rate of constituent separation was examined for many factors, including polarity, stationary phase, and component nature. The best solvents that demonstrated good separation with the most significant number of components were chosen from the extensive analysis [Table 2, 3].

Rf value = Distance traveled by solute from the Basel / Distance traveled by solvent from the baseline

**Table 2 Percentage yield extracts of the Whole plant of Indigofera aspalathoides**

<table>
<thead>
<tr>
<th>Extract</th>
<th>Yield (% W/W)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform</td>
<td>6.4</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>6.1</td>
</tr>
<tr>
<td>Ethanol</td>
<td>7.8</td>
</tr>
</tbody>
</table>

**QUALITATIVE PHYTOCHEMICAL ANALYSIS**

Based on the qualitative analysis, The ethanol extract had the highest concentration of active ingredients, including flavonoids, steroids, carbohydrates, and alkaloids. The whole plant, powdered, also contained all of these chemicals. There were also more active ingredients present in the ethyl acetate extract. The chloroform extract demonstrated the presence of alkaloids, fat, fixed oil, and glycoside. Every extract exhibited the presence of flavonoids and steroids [Table 4].

**FLUORESCENCE ANALYSIS**

**CHROMATOGRAPHIC STUDIES**

**Thin layer chromatography**

All three extracts underwent thin-layer chromatography, and Table5 illustrates the Rf values of each extract [Figure 5].

**Figure 5 Thin Layer Chromatographic Studies on Extract**

**SELECTION OF ACTIVE EXTRACT**

**MATERIALS AND METHODS**

All the extracts underwent in-vitro antioxidant activity and antimicrobial testing to determine the best extract. These studies are utilized to choose the best extract before any further actions.

**IN VITRO ANTIOXIDANT ACTIVITY**

An antioxidant is a molecule that prevents other molecules from oxidizing and producing free radicals. These free radicals then set off a series of events that harm cells and result in the emergence of different diseases. By eliminating free radicals and preventing oxidative reactions, antioxidants put an end to these chain reactions. As a result, antioxidants that can scavenge free radicals will be more crucial for disease prevention and treatment.

**DPPH ASSAY (2, 2-DIPHENYL-1-PICRYLHYDRAZYL)**

When antioxidants interact with DPPH, they convert it to DPPH-H, which lowers absorbance. The level of discoloration reveals the antioxidant chemicals’ or extracts’ capacity to scavenge hydrogen by donating energy. Plant extracts in various volumes (1.25–10µl) were prepared up to 40µl using DMSO, and 2.96 ml of DPPH (0.1 mM) solution was added. The reaction mixture was incubated at room temperature under dark conditions for twenty minutes. The mixture's absorbance was measured at 517 nm after 20 minutes. The control was 3 millilitres of DPPH.
ANTIMICROBIAL STUDIES

Antibacterial activity

Antibacterial investigations were conducted on several extracts of the whole plant of Indigofera aspalathoides against both gram-positive and gram-negative organisms.

PREPARATION OF THE BACTERIAL SUSPENSION FOR INOCULATION:

The pathogenic strains were isolated into small colonies and injected into 4 milliliters of peptone water. A bacterial suspension was created in these tubes by incubating them for two to five hours. Then, if needed, the suspension was diluted with saline solution to reach density, which is visually comparable to the standard by mixing 99.5 ml of 1% sulfuric acid solution with 0.5 ml of 1% barium chloride. An inoculum of bacteria was subsequently created using this solution.

Preparation of mueller hinton agar (M.H. agar):

A sensitivity test was conducted using 38g of Muller Hinton agar that had been weighed, dissolved in 100ml of distilled water, and adjusted to pH 7.3±0.2. The sample was autoclaved at 121°C and 1516 pressure for 60 minutes.

Preparation of agar plates:

The extracts were added to the Muller Hinton agar medium to achieve an extraction concentration equal to the original volume.

PROCEDURE:

Minimum inhibitory concentration:

Agar was used to make the plates and several extracts at varying dilutions, which were then allowed to crystallize and dry.

Table 3 Preliminary Phytochemical Analysis

<table>
<thead>
<tr>
<th>Chemical Constituents</th>
<th>Powdered Drug</th>
<th>Chloro Form</th>
<th>Ethyl Acetate</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavanoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Proteins &amp; Amino acids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fats and fixed oils</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Guns &amp; Mucilage</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phenols</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Volatile oils</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: + - Indicates the presence and absence

Table 4 Fluorescence properties of extracts from the entire plant of Indigofera aspalathoides

<table>
<thead>
<tr>
<th>S. No</th>
<th>Treatment</th>
<th>Day Light</th>
<th>Short-Uv (254nm)</th>
<th>Long-Uv (365nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Powder</td>
<td>Pale brown</td>
<td>Black</td>
<td>Brown</td>
</tr>
<tr>
<td>2</td>
<td>Powder + Water</td>
<td>White viscus</td>
<td>Light black</td>
<td>Brown</td>
</tr>
<tr>
<td>3</td>
<td>Powder + Ethanol</td>
<td>Light yellow</td>
<td>Brown</td>
<td>Light brown</td>
</tr>
<tr>
<td>4</td>
<td>Powder + 1N HCL</td>
<td>Yellow</td>
<td>Yellow</td>
<td>Greenish brown</td>
</tr>
<tr>
<td>5</td>
<td>Powder + 1N H2SO4</td>
<td>Black</td>
<td>Black</td>
<td>Brown</td>
</tr>
</tbody>
</table>

Table 5 Thin layer chromatography extracts and Rf values

<table>
<thead>
<tr>
<th>Extract</th>
<th>Solvent system</th>
<th>No. of spot</th>
<th>Rf value</th>
</tr>
</thead>
<tbody>
<tr>
<td>chloroform</td>
<td>Chloroform : Methanol (9:1)</td>
<td>5</td>
<td>0.85, 0.65, 0.57, 0.52, 0.47</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>Chloroform : Methanol (9:1)</td>
<td>4</td>
<td>0.87, 0.77, 0.70, 0.62</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Chloroform : Methanol (9:1)</td>
<td>7</td>
<td>0.85, 0.72, 0.67, 0.62, 0.57, 0.50, 0.45</td>
</tr>
</tbody>
</table>

% Inhibition = \( \frac{Abs (control) - Abs (test)}{Abs (control)} \times 100 \)
Table 6 The Dpph Assay’s Percentage Inhibition of Extracts

<table>
<thead>
<tr>
<th>Extract</th>
<th>% Inhibition at various concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>60.02</td>
</tr>
<tr>
<td>Chloroform</td>
<td>33.30</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>37.12</td>
</tr>
<tr>
<td>Ethanol</td>
<td>54.12</td>
</tr>
</tbody>
</table>

Table 7 The minimum inhibitory concentration of several Indigofera aspalathoides extracts

<table>
<thead>
<tr>
<th>Extract</th>
<th>Conc.µg/ml</th>
<th>Microorganism used</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Chloroform</td>
<td>100</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>-</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>100</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>300</td>
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<td>400</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>-</td>
</tr>
<tr>
<td>Ethanol</td>
<td>100</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>+</td>
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<tr>
<td></td>
<td>300</td>
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<td></td>
<td>400</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>500</td>
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</tr>
</tbody>
</table>

1. Staphylococcus aureus, Enterococcus - (+) Presence of colonies
3. E.coli, Pseudomonas aeruginosa, Proteus mirablis, Candida albicans - (-) Absence of colonies.
Inoculating the plate were suspensions of bacterial colonies. The plates were then incubated for 24 hours at 37°C, after which the findings were noted.

**Zone of inhibition:**

On the surface of a solid medium, a broth culture or broth suspension of the test bacterium or fungus at an appropriate dilution is flooded (M.H. agar). The plate is tilted to guarantee even distribution and pipette out any surplus broth. Swabs can also be used to disseminate the infection. Using a sterile syringe, antibiotics are injected into the well of the plates after drying at 37°C for 30 minutes [Figure 6]. Growth inhibition zones surrounding the well are measured to ascertain the degree of sensitivity during overnight incubation [Table 6].

**Standard Drugs:**

**Ciprofloxacin:**

It is a fluoroquinolone derivative that has a wide range of antibacterial action. The mutation causes the bacteria to become resistant to fluoroquinolones.

**Mechanism of action**

They work by blocking DNA gyrase (also known as topoisomerase II) and DNA topoisomerase IV. The former takes a direct action that stops DNA replication. The subsequent step also stops DNA replication, but it does so by preventing the enzyme from delinking the daughter DNA molecule as it usually does.
Gentamycin:
The bacteria Micromonospora purpurea produces this aminoglycoside antibiotic.

Mechanism of action:
Transported to the ribosome, it diffuses via the outer cytoplasmic membrane. The instructions from messenger ribosomes use RNA to produce enzymes. Once it attaches itself to 30S ribosomes, it prevents mRNA translation, delays the start of protein synthesis, and ends it early.

Cefotaxime:
These are cephalosporins; they work against both gram-positive and gram-negative microorganisms. Proteus, E. coli, and other gram-negative bacteria are among the vulnerable species for these drugs.

The DPPH experiment demonstrated the antioxidant chemicals' or extracts' capacity for scavenging. The inhibition percentages for ethanol, ethyl acetate, and chloroform were 79.53, 62.08, 67.31, and 58.76 at a maximum 100µg/ml concentration, respectively. It was found that the standard ascorbic acid inhibition percentage was 88.31. Comparable to the standard, the ethanol extract showed the highest inhibition rate. The minimum inhibitory concentration of several Indigofera aspalathoides extracts for antifungal and antibacterial properties [Table 7, Figure 7].

The antibacterial activity of Indigofera aspalathoides extracts was tested in vitro on agar plates against five bacterial strains and one fungus strain, and the results showed that different extracts of the whole plant inhibited microbial growth [Table 8].

Ethanol extracts had a minimum inhibitory concentration of 300µg/ml against tested microorganisms, making them more efficient than other extracts [Figure 8]. The plant's ethanolic extracts demonstrated superior antibacterial efficacy against Pseudomonas, Proteus, and Enterococcus, with nearly similar inhibition zones compared to the conventional medication Gentamycin. Plant extracts had effective antifungal action, with ethanol and chloroform extracts requiring a minimum inhibitory concentration of 300µg/ml. Antimicrobial tests on several extracts of Indigofera aspalathoides demonstrate its usefulness in treating disorders caused by these organisms.
CONCLUSION

The morphological and physiological features of the entire plant were assessed. Different extracts of whole plant powder were produced and phytochemically evaluated. The ethanolic extract contained a variety of phytoconstituents, including steroids, flavonoids, alkaloids, and carbohydrates. The extract was thoroughly tested for antimicrobial properties. Ethyl acetate and ethanol extracts demonstrated good action even at low doses and the largest zone of inhibition. The ethanolic extract was prepared into an ointment with concentrations of 2%w/w and 4%w/w and tested for antifungal efficacy. The ethanolic extract ointment had antifungal activity comparable to normal clobetasol cream. Ethanol extract cream had better activity than average ointment.

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Conflict of Interest: The Author declares that there is no conflict of interest.

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[12] Jurga Bernatoniene, Ruta Masteikova, Julija Davalgiene, Rimantas Peciura, Romualda Gauryliene, Ruta Bernatoniene,

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