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Studies on Antileucodermic Properties of Bark of *Dalbergia sissoo* Roxb

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Abstract: The medicinal plant *Dalbergia sissoo*, is most often called Indian Rosewood. The dry bark has astringent qualities and a dark hue. Aphrodisiac, abortifacient, expectorant, anthelmentic, antipyretic, appetiser, quells thirst, vomiting, burning feeling, heals skin ailments, ulcers, and other traditional uses are documented for diverse species. Leucoderma, leprosy oedema, and bladder disorders are some of the Ayurvedic conditions treated with bark. The bark contains acids, phenolic compounds, flavonoids, and other substances. In this study, the dried bark of *Dalbergia sissoo* was examined pharmacognostically. The evaluation involves macro and microscopic examinations, as well as the determination of physicochemical parameters.

Keywords: *Dalbergia sissoo*, Flavonoid, Physicochemical, Phytochemical

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Introduction

Over time, the world and its inhabitants have experienced several transformations, including the development of various inventions and advancements in technology. Additionally, the global population has encountered a significant rise in the prevalence of unfamiliar diseases, with some being localised and others affecting a substantial portion of the world's population (Vishal *et al*., 2004). Effective medications have been discovered for the majority of diseases and are currently being utilized. Synthetic medications, while providing rapid comfort and being readily accessible, can result in numerous side effects and unpleasant reactions. Additional medication is required to address these undesirable effects (Abhishek *et al*., 2006).

Herbal medicine is a scientific field that use plant-based compositions to treat diseases. The term "traditional" in the context of herbal medicine refers to items that have a long history of usage. Many traditional herbal remedies have been used for a significant period of time (Vaidya, and Devasagayam, 2007). A significant section of the population in numerous developing nations depends on traditional practitioners and their collection of medicinal plants to fulfil their healthcare requirements (Muthulakshmi, 2014; Talele *et al*., 2021).

Despite the coexistence of modern medicine, herbal medicines have consistently remained popular due to their historical and cultural significance (Rane *et al*., 2020a, b, c, 2023; Talele *et al*., 2021).

The objective of this study was to assess the antileucodermic activity of the bark of *Dalbergia latifolia* utilizing an *in vivo* technique. To the best of our knowledge, no Pharmacognostical research has been conducted on the bark of *Dalbergia sissoo*. Hence, an investigation was conducted to study the Pharmacognostical, Phytochemical, and antileucodermic properties of the bark of *Dalbergia sissoo*.

Materials and Methods

Materials:

The bark of *Dalbergia sissoo* was collected from the market and subsequently identified. Merck supplied the phenol reagent and sodium carbonate, while Qualigens was the source for the rutine and gallic acid.

Pharmacognostic study:

Microscopical investigation was performed on the shade-dried powdered plant material. Several staining reagents were employed to identify the distinct organoleptic traits that were found. Under the microscope, the powder was examined after being stained with 1% phenolglucinol in 90% ethanol, strong hydrochloric acid, and glycerin. Under polarized light, calcium oxalate crystals were seen (Gawkrodger *et al*., 2008).

Phytochemical studies:

Phytochemical evaluation involves the use of appropriate chemical assays to ascertain the type of phytoconstituents found in the plant. Methods such as thin-layer chromatography (TLC) and high-performance thin-layer chromatography (HPTLC) were used for qualitative investigation employing targeted reagents. Thus, in order to quantitatively and qualitatively characterize the phytoconstituents, a comprehensive examination was performed (Keservani *et al*., 2017).

Analysis of melanocyte melanin content:

We used the modified approach to test the effect of the extracts on melanocytes' ability to produce melanin. Cells were added to each well of 24 well plate. The cells were incubated at 37°C in a humidified atmosphere with 5% CO_2 for 48 h, and various amounts of bark extracts (hexane, ethyl acetate and ethanol) and kojic acid were added. The incubation period lasted for four days. Both DMEM and the extract vehicle were used to incubate the control group. Afterwards, the medium was discarded and the cells were subjected for 1 h at 80°C lysing with 500 µl of 1 N NaOH in DMSO. Using a plate reader to measure

absorbance at 490 nm allowed us to calculate the relative melanin content (Boone *et al*., 2007; Birlea *et al*., 2009).

Antioxidant Activity:

The Oyaizu method was used to determine the reducing power test. Each plant extract was combined with 1 ml of sodium phosphate buffers at a concentration of 200 mM/L and 1 ml of potassium ferricyanide at a concentration of 1%. The mixture was incubated at 50°C for 20 min. Then added 1 ml of 10% trichloroacetic acid and spun the mixture at 2000 rpm for 10 min. Deionized water and fresh ferric chloride, each measuring 2.5 ml, were added to the solution in the top layer. A greater absorbance value indicates a higher reducing power; the absorbance was measured at 700 nm (Muthulakshmi, 2014; Gautam *et al*., 2015).

Preparation of Gel:

Gels are semisolid or solid preparations that include one or more active substances in either a hydrophilic or hydrophobic phase. They can be transparent or translucent. They use a gelling agent in their production. Gels manufactured from highly cross-linked synthetic or semisynthetic polymers typically have low viscosity and a relatively high yield value, and they often have pseudoplastic flow characteristics. Due to the evaporation of water, products have a cooling effect and are typically sleek and beautiful. Additionally, they can be dried out into films. The films stay put on the skin and come off easily when washed. Semisolid systems known as gels are composed of liquid interpenetrated by big organic molecules or suspensions of small inorganic particles (Muthulakshmi, 2014; Kharkwal *et al*., 2014).

Evaluation of gel:

pH:

The pH of the gel was determined using a digital pH meter. The electrode was fully immersed in the gel to ensure total coverage. This is to verify that the pH of the formulation is closer to the pH of the skin (Upwar *et al*., 2011).

Viscosity:

The viscosity of the gel was measured using a Brookfield viscometer equipped with spindle 7 under controlled temperature conditions (Kaur *et al*., 2011).

Spreadability:

The spreadability was assessed using an instrument consisting of a wooden block, which was positioned at one end. The measurement of Spreadability was conducted based on the slip and drag properties of gels using this approach. A surplus amount of gel being investigated was applied onto this glass slide. The gel was subsequently placed between these slides for a duration of 5 min in order to remove any air and ensure a consistent layer of gel between the slides. The surplus gel was scraped away from the edges. The upper plate was subsequently exposed to a tensile force of 80 g (Niranjan *et al*., 2010).

Draize skin irritation test:

An assessment of the irritancy potential of the formed gel was conducted to determine its effect on human skin. This evaluation is a necessary requirement for all chemicals and formulations. To carry out this assessment, rabbits were used as test subjects. The procedure adhered to the guidelines outlined in the OECD TG 404. A hard copy of the protocol was submitted to the institutional animal ethics committee and received permission. The animals were acclimated to the typical daily and nightly cycles. The animals were provided with an unrestricted amount of green vegetables and water. The mean weight of rabbits was 2.5 kg. The gel that was prepared needed to undergo testing to determine its potential for causing irritation (Hugar *et al*., 2010; Pund *et al*., 2012).

In vivo studies:

The study was conducted with prior authorization from the institution's animal ethics committee. The antileucodermic action was investigated in melanistic mice. The mice were individually kept

in a polystyrene cage lined with rice husk bedding. They were provided with unlimited access to a regular laboratory meal and fresh water. A hard copy of the protocol was submitted to the institutional animal ethics committee and obtained permission. The black mice, with weights ranging between 20-25 g, were measured and separated into three groups. Each group consisted of six mice. Utmost care was taken to ensure that the animals were treated in a manner that prioritized their welfare and adhered to ethical standards (Sidana *et al*., 2012; Moreira *et al*., 2012).

Results and Discussion

Pharmacognostic study:

Pharmacognostical investigations are crucial for identifying and verifying the authenticity of herbal plant material. The plant's botanical identity was determined by the examination of its anatomical characteristics. *Dalbergia sissoo* Roxb belongs to the Fabaceae family and shares a close relationship with other species. The anatomical analysis of *Dalbergia sissoo* Roxb bark revealed significant microscopic features including cork, phloem, sclerides, phelloderm, starch grains, and calcium oxalate crystals. Physicochemical constant characteristics are employed to verify the purity and quality of the powdered medication. Ash readings are useful for evaluating the presence of inorganic salts or other foreign substance that may be attached to or intentionally added to plant tissue. The total ash is determined by oxidising the constituent of the powdered crude medication (Singh *et al*., 2017).

Morphology: Color- Brown; Odour- Odourless; Taste-Bitter; Size- 0.5–1 cm thickness; Shape-Flat; Surface-Rough and Hard; Fracture- Splintery.

Phytochemical study:

Herbal medications are derived from plants and are typically made using methods such as infusion, decoction, or extraction. In such instances, they are susceptible to contamination, resulting in the degradation of phytoconstituents and fluctuations in chemical composition. Therefore, prior to conducting pharmacological screening or clinical trials, it is required to verify the phytocomponents of herbal plants and determine their efficacy (Negi *et al*., 2012). Phytochemicals present in *Dalbergia sissoo* are illustrated in Table 1.

Analysis of melanocyte melanin content:

Table 2 illustrates the melanin content synthesis and the effects of various extracts.

In Vitro Study:

Cytotoxicity study:

Kojic acid and *Dalbergia sissoo* Roxb bark extracts were tested for cytotoxicity using the MTT assay technique in the B16F10 melanoma cell line. We used a cytotoxicity test to look for these chemicals. Cell viability was used to evaluate the cytotoxic effect of both the plant extracts and the standard. A lower concentration of the test chemicals was associated with an increase in cell survival. The findings are displayed visually and in tabular form (Table 3). Ethyl acetate outperformed hexane and ethanol in terms of cell survival among the tested chemicals (Jadhav *et al*., 2010).

Evaluation of gel:

Spreadability:

The gel was sandwiched for 5 min between the slides to remove air and create a consistent layer of gel between them (Table 4). Scraped out from the edges was the excess gel. After then, an 80 g pull was applied to the top plate. After doing a stability study, we also assessed the gel's spreadability, and we discovered that it was less variable than the first created gel. After conducting stability testing compared to the first generated gel, the extrudability was found to be outstanding. At a temperature of 25°C, the initial viscosities were noted. In addition, the preparation was shown to be stable under typical storage settings according to the stability analysis (Karthika, and Jayshree, 2013).

Viscosity:

For the purpose of determining the gel's viscosity

Table 1: Phytochemical evaluation

S. No	Phytoconstituents	Powder	Hexane	Ethyl Acetate	Ethanol
1	Carbohydrates	+	_	+	+
2	Flavonoids	+	_	+	+
3	Glycosides	+	_	+	+
4	Alkaloid	_	_	_	_
5	Saponin	+	_	+	+
6	Phytosterols	_	_	_	_
7	Phenolic compounds	+	_	+	+
8	Proteins	_	_	_	_
9	Fixed oils and fats	_	_	_	_
10	Tannins	+	_	+	+
11	Triterpenoids	+	+	_	_
12	Gums and mucilage	_	_	_	_

Table 2: Melanin content synthesis and the effects of different extracts

S. No.	Conc.	Hexane	Ethyl acetate	Ethanol
1.	Control	0.12	0.21	0.14
2.	Vehicle	0.2	0.2	0.2
3.	Standard	0.29	-	-
4.	0.01	0.045	0.061	0.060
5.	0.03	0.069	0.081	0.081
6.	0.1	0.087	0.225	0.231
7.	0.3	0.254	0.212	0.254
8.	1	0.32	0.30	0.31
9.	3	0.31	0.40	0.40

Table 3: Cytotoxicity Study

S. No.	Conc. (μg/ml)	Cytotoxicity study							
		Standard		Hexane		Ethyl acetate		Ethanol	
		Abs (O.D)	% Cell Viability	Abs (O.D)	% Cell Viability	Abs (O.D)	% Cell Viability	Abs (O.D)	%Cell Viability
1	1000	0.10	18.52	0.12	22.22	0.09	16.66	0.10	20.37
2	500	0.13	25.92	0.17	31.48	0.21	38.88	0.17	33.33
3	250	0.21	40.74	0.22	40.74	0.25	46.29	0.23	44.44
4	125	0.28	53.70	0.26	48.14	0.28	51.85	0.27	50
5	62.5	0.33	62.96	0.31	57.40	0.35	64.81	0.29	55.55
6	Cell control	0.54	100	0.54	100	0.54	100	0.54	100

Table 4: The Herbal Gel's Spreadability throughout the Trial

S. No.	Evaluation condition	Spreadability (g.cm/sec)
1.	Initial at 40˚C ±2˚C /75±5% RH	25.42±0.21
2.	At 1month at 40˚C ±2˚C /75±5% RH	25.41±0.29
3.	At 2month at 40˚C ±2˚C /75±5% RH	25.40±0.54
4.	At 3month at 40˚C ±2˚C /75±5% RH	25.31±0.41

Table 5: Herbal Gel's Viscosity Throughout the Assessment

S. No.	Evaluation month	Viscosity (cps)
1.	Initial	16000
2.	At 1 month	16000
3.	At 2 month	16000
4.	At 3month	15400

Table 6: Study on Draize Skin Irritation

Name of the Group	Animal numbers	Grading	
Group I	1	7^{th} day	14^{th} day
		0	0
	2	0	0
	3	0	0
Group II	1	0	0
	2	0	0
	3	0	0

at a temperature that was under control, a Brook field viscometer was utilized. The results are illustrated in Table 5.

After the herbal gel was made, it was tested using all the relevant metrics. The translucent herbal gel had a brownish-yellow hue, felt silky smooth when applied, and looked great. In addition to being non-irritant when applied to the skin, the gel's pH remained stable throughout the study, ranging from 6.5 to 6.8.

Study on Draize Skin Irritation:

According to the results of the Draize skin sensitivity test, there were no indications of irritation, such as redness or erythema, which indicated that the gel did not contain any irritating content (Table 6) (Rani *et al*., 2010).

Conclusion

This study used *Dalbergia sissoo* Roxb, a plant with purported medicinal uses in folk medicine and traditional Indian medicine. Melanin production was enhanced by all extracts. Various concentrations of hexane, ethyl acetate, and ethanolic bark extracts of *Dalbergia sissoo* were observed to increase melanin content. In comparison to hexane and ethanol, ethyl acetate produced the most melanin of the three test extracts. We recommend more research on isolating the active phytoconstituents. This study introduces a novel method for the gel-making process. The produced gel had properties that were favourable to its formulation as gel, making it an excellent vehicle for topical distribution.

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