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PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF LEAVES OF DALBERGIA SISSOO

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ABSTRACT

Nature, a vast repository of medicinal plants has played a pivotal role in shaping modern pharmaceuticals. Among these, *Dalbergia sissoo* stands out for its extensive use in traditional medicine globally. *Dalbergia sissoo* is also an herbal medicinal plant that belongs to the family Fabaceae (Leguminosae). This comprehensive review delves into the literature surrounding *Dalbergia sissoo*, elucidating its traditional applications, phytochemistry, and therapeutic uses. Various plant parts, from leaves to wood and bark, have found utility in treating diverse ailments, including gonorrhea and skin disorders. Research underscores the antimicrobial process of *Dalbergia sissoo*, showcasing potent antibacterial activity against both gram-positive and gram-negative bacteria. Notably effective against clinical isolates, the plant's extracts present Potential Avenue for crafting antiseptic formulations targeting chronic bacterial infections. Despite its multifaceted benefits, *Dalbergia sissoo* has yet to be developed into a pharmaceutical drug. This underscores the need for systematic studies, including identification, cataloging, and documentation, to preserve and propagate traditional knowledge of herbal medicinal plants. Plant part extracts from Dalbergia sissoo are reported on anti-inflammatory, anti-termite, anti-diabetic, analgesic and antipyretic, antihelminthic, antioxidant, antimicrobial, antinociceptive, osteogenic, antispermatogenic, gastroprotective, neuroprotective, anti-molluscicidal, antilarvicidal, antiulcer, immunomodulatory, antibacterial activity Explorations into the plant's antioxidant

properties reveal potential in free radical scavenging and lipid peroxidation inhibition. In summary, *Dalbergia sissoo* emerges as a versatile medicinal plant with a rich history and promising pharmacological activities. Future research holds the key to unlocking its full therapeutic potential and seamlessly integrating it into modern healthcare practices.

KEYWORDS: *Dalbergia sissoo*, Antimicrobial Activity, Lipid Peroxidation, Agar media, Methanol, Staphylococcus aureus, E.coli, and Candida Albicans.

INTRODUCTION

Globally, traditional methods employ herbal drugs derived from various medicinal plants, showcasing the triumph of diverse therapeutic options. The recent surge in plant-based health products has led to a substantial global growth in herbal remedies. The *Dalbergia* genus, comprising 300 species, including 25 in India, features timber trees like rosewoods, valued for fragrant wood and aromatic oils. Named after the Swedish brothers Nils and Carl Dalberg, the genus's native plant in India, the state tree of Punjab, holds reputed medicinal properties. Adaptable to temperatures from just below freezing to 50˚C, annual rainfall of 500-2000 mm, and droughts of 3-4 months, sisso can grow in diverse soils but is shade-intolerant during the seedling stage. Leaves: leathery, pinnately compound, alternate, petiolate, around 15 cm long. Each leaflet, widest at the base, measures up to 6 cm with a pointed tip. Flowers: fragrant, whitish to pink, nearly sessile, arranged in dense clusters. Seeds: 4-5 mm, kidney-shaped, thin, flat, light brown. Long taproot and surface roots with suckers. Sapwood is white to pale brown, while heartwood is dark brown.

MATERIALS AND METHODS

Aim and Objectives: *Dalbergia sissoo* has a long-standing traditional application as an antimicrobial agent. Extensive literature reviews have indicated the presence of phenolic compounds in the plant, which have demonstrated antimicrobial properties. Our primary objective is to establish the antimicrobial potential of *Dalbergia sissoo* leaves, an aspect that has not been conclusively demonstrated.

The identification process involved macroscopic studies of a sample (small twig), followed by a thorough examination of relevant literature. We collected 300 grams of *Dalbergia sissoo* leaves, cleaned them, and allowed them to air dry in the shade for a week. After the shade-drying process, the leaves were gathered and ground into coarse powder. Subsequently, 20 grams of these leaves were utilized for Soxhlet extraction.

Procedure

The leaves were dried in the shade for one week, after which they were ground into a coarse powder. The powder underwent extraction using a Soxhlet apparatus with 200ml of methanol solvent in two separate Soxhlet apparatus for approximately six cycles.

Chemicals and Reagents: Aqueous methanol (80%)**,** Ethanol**,** Dragendroffs reagent**,** Wagner's reagent**,** Mayer's reagent**,** Molisch reagent**,** Barford's reagent**,** Seliwanhoffs reagent**,** Lead acetate, GAA**,** Gelatin solution**,** Conc. Sulphuric acid**,** Acetic anhydride.

Preliminary phytochemical analysis

Investigating the phytochemical components in the ethanolic extract of $_2$ leaves: Standard procedures were employed to conduct a qualitative phytochemical analysis of the ethanolic extract of *Dalbergia sisso* leaves. Various chemical tests were utilized to evaluate the presence of different phytochemical constituents, including carbohydrates, glycosides, proteins, amino acids, phytosterols, saponins, flavonoids, alkaloids, and tannins in the bark of *D. sisso*.

1. **Test for reducing sugars (Fehling's test):** The ethanol extract (0.5g in 5ml of water) was introduced into a test tube containing boiling Fehling's solution (A and B). The mixture was then observed for a color reaction characterized by the appearance of a purple ring at the junction of the two liquids.

2. **Test for anthraquinones:** Boiling 0.5g of the extract with 10ml of sulfuric acid (H_2SO_4) was followed by hot filtration. The filtrate was then subjected to shaking with 5ml of chloroform. The chloroform layer was transferred to a different test tube, and 1ml of diluted ammonia was introduced. The resulting solution was examined for any observable color changes.

3. **Test for terpenoids (Salkowski's test):** 0.5g of the extract received an addition of 2ml of chloroform. Following this, a careful layering of 3ml of concentrated H_2SO_4 ensued. The solution was then scrutinized for the emergence of a reddish-brown coloration at the interface, signifying the presence of terpenoids.

4. **Test for flavonoids:** Three techniques were employed to assess the presence of flavonoids. Firstly, 5ml of dilute ammonia was introduced to a segment of an aqueous filtrate of the extract, followed by the addition of 1ml of concentrated sulfuric acid. The

appearance of a yellow coloration, which vanished upon standing, signified the existence of flavonoids. Secondly, a few drops of 1% aluminum solution were mixed with a portion of the filtrate, and the development of a yellow coloration indicated the presence of flavonoids. Thirdly, a segment of the extract was subjected to heating with 10ml of ethyl acetate over a steam bath for 3 minutes. After filtration, 4ml of the filtrate was combined with 1ml of dilute ammonia solution, and the presence of a yellow coloration indicated the presence of flavonoids.

5. **Test for saponins:** In a test tube, 5ml of distilled water was introduced to 0.5g of extract. The solution underwent vigorous shaking and was then examined for the presence of a stable, persistent froth. Subsequently, the froth was combined with three drops of olive oil and subjected to vigorous shaking again. The mixture was observed for the formation of an emulsion.

6. **Test for phenolic compounds:** Dissolving 50mg of extract in distilled water was followed by the addition of 3ml of a 10% lead acetate solution. The formation of a substantial white precipitate served as an indicator for the presence of phenolic compounds, as per the lead acetate test. Similarly, dissolving 50mg of extract in 5ml of distilled water and then adding 2ml of a 1% solution of gelatin containing 10% sodium chloride resulted in the observation of white precipitates, indicating the presence of phenolic compounds in accordance with the gelatin test.

7. **Test for tannins:** Approximately 0.5g of the extract underwent boiling in 10ml of water within a test tube, followed by filtration. A few drops of 0.1% ferric chloride were introduced, and the resulting solution was scrutinized for the manifestation of brownish-green or blue-black coloration.

8. **Test for alkaloids:** Diluting 0.5g of the extract to 10ml with acid alcohol, the mixture was then subjected to boiling and subsequent filtration. To 5ml of the resulting filtrate, 2ml of dilute ammonia was added, followed by the introduction of 5ml of chloroform. Gentle shaking facilitated the extraction of the alkaloidal base, and the chloroform layer was isolated and further treated with 10ml of acetic acid. The chloroform layer was divided into two portions, with Mayer's reagent added to one and Dragendorff's reagent to the other. The appearance of a cream (with Mayer's reagent) or a reddish-brown

precipitate (with Dragendorff's reagent) was considered indicative of the presence of alkaloids.

9. **Test for cardiac glycosides (Keller-Killiani test):** Diluting 0.5g of the extract in 5ml of water, a mixture was created to which 2ml of glacial acetic acid, containing a single drop of ferric chloride solution, was added. To form a distinct layer, 1ml of concentrated sulfuric acid was introduced, and the color at the interface was observed. The presence of a brown ring at the interface was indicative of a deoxy sugar characteristic of cardenolides. Additionally, a violet ring might appear below the brown ring, and in the acetic acid layer, a greenish ring could form just above the brown ring, gradually spreading throughout this layer.

Physico-chemical parameters

1. **Determination of ash value**

a) **Determination of total ash:** The determination of total ash involved the weighing of 2g of the air-dried crude drug in a pre-weighed platinum or silica dish. The incineration process occurred at a temperature not surpassing 450˚C, continuing until free from carbon. Subsequently, the dish was allowed to cool, and the final weight was recorded. The percentage of total ash value was calculated using the formula: % total ash value = (weight of total ash/weight of crude drug taken) * 100.

b) **Determination of acid insoluble ash:** The ash obtained from the preceding procedure underwent boiling with 25ml of 2M HCL for 5 minutes. The insoluble matter was gathered on ash-less filter paper, washed with hot water, ignited, cooled in a desiccators, and the weighed. The percentage of acid insoluble ash was calculated with respect to the air-dried drug using the formula: % acid insoluble ash value = (weight of acid insoluble ash/weight of crude drug taken) * 100.

c) **Determination of water-soluble ash:** The ash was subjected to boiling with 25ml of water for 5 minutes. The resulting insoluble matter was gathered on ash-less filter paper, washed with hot water, and ignited for 15 minutes at a temperature not exceeding 450˚C. The weight of the insoluble matter was then subtracted from the weight of the ash, representing the water-soluble ash. The percentage of water-soluble ash was calculated with respect to the air-dried drug using the formula: % water-soluble ash value = (weight of water-soluble ash/weight of crude drug taken) * 100.

2. **Determination of solvent extractive value**

a) **Determination of water-soluble extractive value:** Five grams of powdered drug were macerated with 100ml of water in a closed flask for 24 hours. The mixture was intermittently shaken every 6 hours and then left to stand for an additional 18 hours. After filtration, 25ml of the filtrate was evaporated to dryness in a pre-weighed flat-bottomed shallow dish. The residue was dried at 105˚C and weighed. The percentage of water-soluble extractive value was calculated with respect to the air-dried drug.

b) **Determination of alcohol soluble extractive value:** Five grams of powdered drug was macerated with 100ml of ethanol in a closed flask for 24 hours. The mixture was intermittently shaken over a 6-hour period and allowed to stand for an additional 18 hours. Following filtration, 25ml of the filtrate was evaporated to dryness in a pre-weighed flat-bottomed shallow dish. The residue was dried at 105˚C and weighed. The percentage of ethanol-soluble extractive value was then calculated with respect to the air-dried drug.

c) **Determination of moisture content:** Swiftly weigh 0.5g of finely powdered crude drug in a flat-bottomed dish. Dry the powder in an oven at 100-105˚C for 10 minutes, repeating the process until a constant weight is achieved. Each time the powder and watch glass should be cooled to room temperature before weighing. Calculate the percentage moisture content using the formula: % moisture content = (Initial weight-weight of the crude drug after drying)/initial weight.

3. **Antimicrobial activity by agar well diffusion method of assay:** Nutrient agar media composition: Meat extract – 2g, Peptone – 2g, Sodium chloride – 5g, Agar – 5g, Purified water – 100ml/µ, pH – 7.4

Procedure

a) Weigh the required quantities of ingredients and dissolve them in specified amounts of water.
b) If the broth is not clear, filter it through filter paper.
c) Adjust the pH to 7.4±0.1.
d) After pH adjustment, add the agar and heat the contents on a water bath until the agar dissolves. Distribute the melted agar medium into test tubes and flasks, and then plug them with non-absorbent cotton.

e) Sterilize the test tubes and flasks in an autoclave at 121˚C, 151 lbs. pressure for 15-20 minutes.

Assessment of antimicrobial activity using the agar well diffusion method

Assay using the agar well diffusion method: The assessment of antimicrobial potential in *Delonix regia* leaf extracts was conducted through the agar well diffusion assay, measuring inhibition zones at various concentrations. A uniform swab of 100 µl microbial suspension (10ˆ6 cfu/ml) was applied to Mueller-Hinton agar (HI media) on petri plates. Wells, 6 mm in diameter, were created with a cork corer, and varying concentrations (50 µl) of leaf extracts were introduced into each well. The concentrations were prepared by dissolving dried plant extracts in 1 ml of water, resulting in final concentrations of 300, 600, 900 µg/2400 µl/ml. Plates, inoculated with test samples, underwent incubation at 37˚C for 16 hours. As positive and negative controls, standard antibiotic ofloxacin (100, 200 µl/ml) and a control solvent were utilized. Following incubation, inhibition zones around the wells in both test and control plates were measured using the HI media zona scale. The reported results represented the mean values obtained from triplicate runs at each concentration.

RESULTS AND DISCUSSION

I. Collection of leaves	
Leaves	**Weight of leaves (Grams)**
Fresh leaves	308 grams
Shaded dry leaves	130 grams
Coarse powder	120 grams

II. Preliminary screening and phytochemical parameters						
Sl. No.	**Chemical constituents**	**Results**		**Sl. No.**	**Parameters**	**Results (%)**
		Methanolic extract	**Aqueous extract**			
1	Alkaloids	+	+	1	Total ash	5.21
2	Carbohydrates	+	+	2	Acid insoluble ash	4.20
3	Saponins	+	+	3	Water insoluble ash	1.84
4	Flavonoids	+	+	4	Water soluble extractive value	10.63
5	Carotene	+	+	5	Alcohol soluble extractive value	4.48
6	Tannins	+	+	6	Loss on drying	8.61
7	Anthraquinones	+	+	7	Moisture content	8.10
8	Coumarins	-	-			

9	Phenolic compounds	+	-			

III. Zone of inhibition for micro organisms					
Sl. No.	**Microorganism**	**Concentrations**	**Zone of inhibition with aqueous extract**	**Zone of inhibition with methanol extract**	**Zone of inhibition with standard drug**
1	Escherichia coli	300 µg/ml	12 mm	14 mm	16 mm
2	Staphylococcus aureus	600 µg/ml	10 mm	12 mm	14 mm
3	Candida albicans	900 µg/ml	7 mm	8 mm	12 mm

Dalbergia sisso, commonly known as Indian rosewood or Shisham, is a fast-growing deciduous tree belonging to the Fabaceae family, subfamily Faboideae. Widely distributed in various regions of the world, particularly in the Indian subcontinent and southern Iran, it is recognized for its hardiness and resilience. Characterized by leathery, alternate, pinnately compound leaves, *Dalbergia sisso* is valued for the active constituents found in its leaves and bark. The flowers of this tree exhibit insecticidal, would healing, and anti-helminthic properties. Preliminary phytochemical analysis of water extracts from *Dalbergia sisso* revealed the presence of sterols, triterpenoids, flavonoids, and phenolic compounds. The antimicrobial activity of the water extract is attributed to these bioactive antimicrobial agents, suggesting that the water extract possessed more potent antimicrobial properties compared to the methanol extract. The leaf extract of *Dalbergia sisso* demonstrated antimicrobial activity against multidrug-resistant clinical pathogens and exhibits membrane-stabilizing properties. Qualitative preliminary phytochemical tests detected various phytoconstituents such as flavonoids, carbohydrates, phenols, and tannins. Physicochemical parameters, including determination of foreign matter, ash value, solvent extract value, fluorescence analysis, and moisture content, were assessed using three samples. The obtained values for total ash, acid-insoluble ash, water-insoluble ash, water-soluble extractive value, alcohol extractive value, and moisture content were reported as 5.21%, 4.20%, 1.84%, 10.63%, 4.48%, and 8.10%, respectively. Furthermore, the antimicrobial activity of the leaf extract against microorganisms, including E. coli and Staphylococcus aureus, was investigated. The zone of inhibition for aqueous extracts at concentrations of 300 µg/ml, 600 µg/ml, 900 µg/ml, and 2000 µg/ml was compared to the standard antibiotic ofloxacin at concentrations of 100 µg/ml

and 200 µg/ml. The leaf extract exhibited moderate antimicrobial activity against E. coli and S. aureus.

CONCLUSION

Dalbergia sisso, commonly known as Indian rosewood, is found as an ornamental tree in various regions worldwide. Belonging to the flowering plant family Fabaceae, subfamily Faboideae, it is recognized for its distinctive appearance and is widely cultivated for its aesthetic value. The tree features leathery, alternate, pinnately compound leaves, contributing to its ornamental appeal. *Dalbergia sisso* holds significance in herbal medicine due to its diverse range of phytoconstituents and pharmacological properties. In this study, we focused on the phytochemical analysis of leaf extracts using both aqueous and methanol (80% solvent) methods. Leaves were collected from the MRIPS campus college garden. The preliminary phytochemical screening involved aqueous (water) and methanol (80% solvent) extractions, with the evaluation of identified phytoconstituents referring to standard literature. The extracts revealed the presence of alkaloids, carbohydrates, glycosides, tannins, flavonoids, and phenols. The findings suggest that the aqueous methanolic extract (80%) of *Dalbergia sisso* contains bioactive compounds of medicinal importance. Moreover, it exhibited a moderate antimicrobial effect against organism such as E. coli, Staphylococcus aureus, and Candida albicans. Interestingly, the alcohol extract demonstrated a greater zone of inhibition compared to the aqueous extracts, indicating stronger antimicrobial activity. Consequently, the alcohol extract of *Dalbergia sisso* holds potential for enhanced antimicrobial effects.

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