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Making and Assessing an Antibacterial Cream with Dalbergia Sissoo Leaf Extract

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Abstract:- Medicinal plants are still a valuable therapeutic resource for treating human illnesses. The plant Dalbergia sissoo is important to the health care system and includes several chemical constituents that have particular importance in traditional medical practices. This study's primary goal is to assess the ethanol extracts from D. sissoo leaves' antibacterial activity. Using ethanol, the dried leaves of Dalbergia sissoo are extracted. Additionally, the extract's phytochemical screening was completed. The ethanolic extract was then used to make the herbal antibacterial cream composition. Examine the cream's physical characteristics as well. Using the disc diffusion method with bioassay guidance, the assay was conducted on Staphylococcus aureus. Staphylococcus aureus bacteria, both gram positive (+) and gram negative (-), were utilised to measure the antibacterial activity of the cream.

Keywords:- *Dalbergia Sissoo, Staphylococcus Aureus, Triterpenoids, Phytochemical Screening, Leaves Extract, Ethanolic Extract, Wound Healing Activity, Antiinflammatory Activity, Antioxidant Activity.*

I. INTRODUCTION

India's plant-based medicine is increasingly popular globally, with approximately 50% of top-selling pharmaceuticals sourced from natural sources.[1] Herbal medicines, derived from various plants, have been used globally for centuries to treat various illnesses. Recently, the use of plant-based health products has grown exponentially, attracting both developed and developing nations.[2] Creams are semisolid dosage forms with medicinal or protective properties applied topically to the skin, eyes, or administered nasally, vaginally, or rectally. They treat localized effects of medicine by penetrating the skin or mucous membrane. Injuries brought on by physical, chemical, thermal, viral, microbial, or immunological stress have a detrimental effect on patients' quality of life, can be expensive, and leave a patient with lifelong scars.[3] Dalbergia sissoo, a plant species found in tropical regions, has been historically used by indigenous people for treating various illnesses. It contains antibacterial, antioxidant, antiinflammatory, and antidiarrheal properties. Its parts, including roots, bark, wood, leaves, and seeds, have been used for syphilis, blood disorders, skin conditions, stomach issues, dysentery, nausea, eye and nose disorders, and aphrodisiacs.[4] The Dalbergia sissoo tree, a 25-meter-tall Indian medicinal plant, is used in folk medicine for various conditions. Its leaves have five alternating leaflets and fragrant blooms. The study aims to prepare an ethyl acetate extract gel from the bark, assessing its pH, viscosity, spredability, and irritating activity.[5] Dalbergia sissoo Roxb. is a plant with various therapeutic properties, including eye, nasal, anthelmintic, digestive, scorching urine, scabies, and burning sensations. Its leaves and bark have been used to treat various ailments, and its extracts have been found to have analgesic, antipyretic, antidiabetic, antiinflammatory, and antidiarrheal properties. The plant bark also has antinociceptive, antioxidant, and antispermatogenic properties. The oil derived from wood scrapings has been shown to be effective against mosquitoes and has antiosteogenic properties. The plant extract has been used for termite control in structures and has been tested against helminthic infections. have antinociceptive qualities.[6] Dalbergia sissoo leaf extracts have various pharmacological actions, including antiparasitic, antiinflammatory, antidiabetic, skin, osteogenic, antimicrobial, antioxidant, antiulcer, antidiarrheal, heart, CNS, and reproductive effects. The anthelmintic activity of the leaf extracts was tested against Indian earthworms, and the hexane and methanol extracts were tested against paw oedema caused by carrageenan. It was discovered that the ethanol extract of Dalbergia sissoo bark increased melanin activity without being harmful. The bark extract also demonstrated antiresorptive and bone-forming properties, and its antioxidant activity was evaluated. The ethanol extract demonstrated significant and dose-dependent antidiarrheal activity. The bark extract also showed antispermatogenic properties in healthy, fertile men. In Swiss albino mice, the ethanol bark extract of Dalbergia sissoo was determined to be safe up to 3000 mg/kg body weight.[7]

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Fig 1: Plant of Dalbergia Sissoo

II. MATERIALS AND METHODS

A. Materials:

Dalbergia sissoo, bee wax, liquid paraffin, borax, methyl paraben, rose oil, distilled water, etc. were gathered from the local garden.

B. Methods:

➢ *Collection of Plant Materials:*

The botanical garden provided herbs for Dalbergia sissoo. Pharmaceuticals are collected when active ingredients are high, considering environmental conditions. Hiring a competent workforce is crucial for commercial gathering. Leaves with high chemical ingredients and reserve food are harvested.

➢ *Extraction of the Plant Materials Used to Make Cream:*

Dalbergia sissoo leaves were harvested, cleaned, and shade-dried for ten days. Pulverized into a powder, the extract was extracted using a Soxhlet device. The process took 96 hours at 70-80°C. The extract was concentrated, filtered, and stored in an airtight container.[8,9,10].

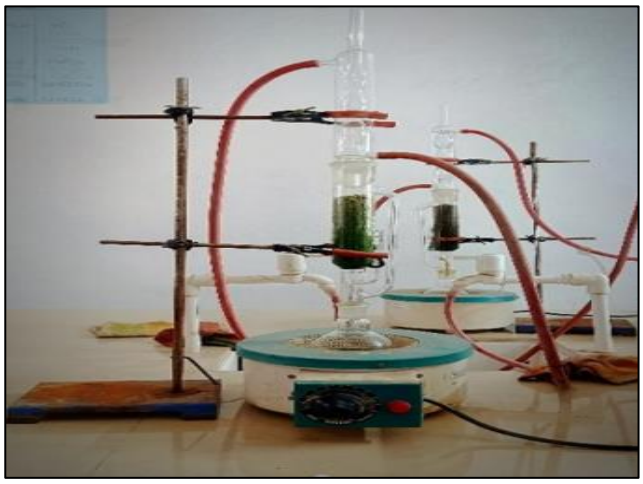

Fig 2: Extraction of Dalbergia sissoo

Fig 03: Extract of Dalbergia Sissoo

➢ *Formulation of Herbal Cream:*

Table 1: Ingredients used in Formulation of Herbal Cream

Sr. No.	Ingredients	Formulation (F1)	Formulation (F2)	Formulation (F3)
1.	Plant extract	0.5 ml	1 ml	2 ml
2.	Bees wax	7 gm	7 gm	7 gm
3.	Liquid paraffin	12 ml	12 ml	12 ml
4.	Borax	0.8 gm	0.8 gm	0.8 gm
5.	Methyl paraben	0.07 gm	0.07 gm	0.07 gm
6.	Rose oil	q. s	q. s	q. s
7.	Purified water	q. s	q. s	q. s
8.	Total	25 gm	25 gm	25 gm

➢ *Method of Preparation:*

- Pour liquid paraffin and beeswax into a beaker made of borosilicate glass. Raise the temperature to 75 °C and stay there the entire cooking process. (Phase of Oil)
- Dissolve borax and methyl paraben in distilled water, then heat the mixture to 75 °C in a different beaker to obtain a transparent solution. (Phase of water)

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- The heated oily phase should then be gradually added to the aqueous phase. Next, in order to create a smooth cream, add measured volumes of Dalbergia sissoo plant extract while stirring vigorously.
- After that, add some drop of rose oil for a nice aroma.
- Spread the cream around the slab.
- Next, blend the cream in a geometric fashion to give it a smooth texture and evenly incorporate all the ingredients.[11]

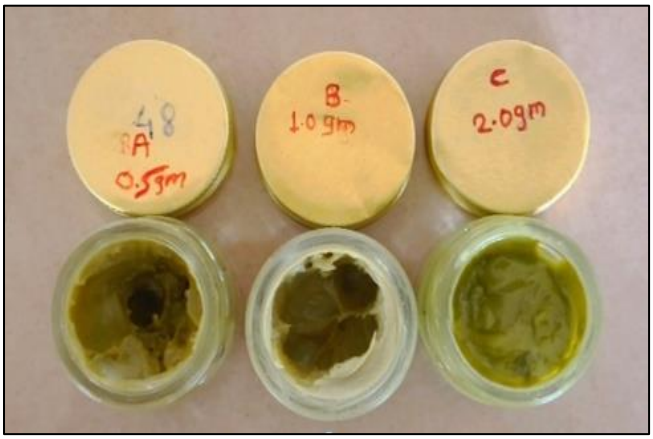

Fig 4: Herbal Cream Formulation

Fig 5: Formulation of Herbal Cream

➢ *Evaluation of Cream:*

The prepared creams were evaluated using a range of parameters in accordance with conventional protocols.

- Physical Evaluation: This test involved observing the cream's colour, odour, texture, and condition.
- Irritancy: Mark the posterior part of the left hand, apply cream, note the time, and monitor for a maximum of 24 hours to look for signs of irritation, edoema, or erythema.[12]
- Accelerated Stability Testing: Formulations underwent accelerated stability testing at room temperature for seven days, followed by 20 days at 37°C ± 1°C, obtaining comparable results on various parameters.
- Homogeneity: The uniformity of the formulations was assessed both visually and by touch.
- Appearance: The colour, pearlescence, and roughness of the cream's look were evaluated before it was graded.
- After feel: After applying a predetermined quantity of cream, the amount of residue left behind, slipperiness, and emolliency were assessed.
- Type of smear: The type of film or smear that formed on the skin after applying the cream was evaluated.[13]
- Washability: The hand was treated with a modest amount of cream and then cleansed with tap water.
- pH: A pH metre was calibrated using a standard buffer solution and a digital pH meter after 0.5g of cream was mixed with 50ml of distilled water.[14]
- Viscosity: The formulation's viscosity was assessed using a Brookfield viscometer at 27 °C and spindle number seven running at 100 revolutions per minute.
- Dye Test: The cream was combined with red and examined under a microscope. The colorless globules against a crimson background indicate a w/o type formulation.[15]
- Phase Separation: The cream was kept at 25-100°C, examined daily for 30 days, and any changes in phase separation were noted and verified.
- Spreadability: The amount of time it took for two slides divided by a cream layer to separate was used to gauge a cream's spreadability. Using glass slides of standard size, the cream was compacted into a thin layer. It was able to move freely because of the weight on the upper slide, and the duration of time it took for the upper slide to fall off was recorded.

$$\text{Spreadability} = m \times l/t$$

Where,
m= Standard weight which is tied to or placed over the upper slide (30g).
l= length of a glass slide (5 cm).
t= time taken in seconds.

- Greasiness: In this instance, the cream was applied to the skin surface as a smear, and its oiliness or grease-like consistency was assessed. [16]

III. CHROMATOGRAPHY

A. Thin Layer Chromatography:

" Their respective polarities, which are connected with the sort and quantity of functional groups on a hydrogen-bonding molecule."

$$\text{Rf Value} = \frac{\text{Distance travelled by solute front from origin line.}}{\text{Distance travelled by solvent front from origin line.}}$$

Where,
Rf =Retention factor

The ethanolic extract of Dalbergia sissoo leaves was subjected to thin layer chromatography tests to determine the presence of numerous compounds that were corroborated by chemical analyses. TLC spot colour and Rf value in a solvent system of benzene: ethyl acetate (1:9). Table 11 lists these

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TLC spots along with their hue and Rf value. The TLC plate from Fig. 09 is shown below.

B. Column Chromatography:

The basic concept of column chromatography is the adsorption of a component at the solid-liquid interface. To accomplish good separation, the components in the mixture should have different degrees of affinity for the solid support. The component with substantial adsorption for the column material is held up when the elute passes through the column, whereas the component with less affinity descends the column more quickly.

Fig 6: Column Chromatography of Dalbergia Sissoo

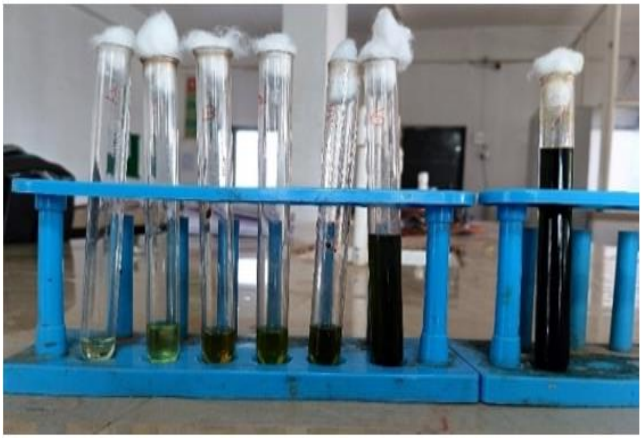

Fig 7: Elution of Column Chromatography

The way that the solvent descends the column distinguishes two types of column chromatography. The technique of letting the solvent go down the column by gravity or percolation is known as gravity column chromatography. When the solvent is forced down the column by air pressure, flash chromatography is employed.[17]

IV. BACTERIAL ACTIVITY

➢ *Antibacterial Activity Test:*

The antibacterial activity of D. sissoo leaf extracts was evaluated using the cup agar diffusion method.[18]

➢ *Test Organisms:*

Staphylococcus aureus was the test organism employed in the study; it was taken from the soil and grown in vital culture media.

➢ *Antibiotics:*

The study made use of antibiotic discs containing ampicillin and norfloxacin.

➢ *Disc Diffusion Method:*

Using the disc diffusion method, the antibacterial activity of a cream formulation and extract was assessed. Agar plates were uniformly swabbed with sterile cotton swabs, and a petri dish filled with varying concentrations of cream and extract was impregnated.[19]

➢ *Preparation of Culture Media:*

Nutrient agar was dissolved in distilled water, heated, wrapped, and autoclave-sterilized for 15 minutes at 121°C. The sterilized medium was then transferred to petri dishes, hardened, and refrigerated until needed.

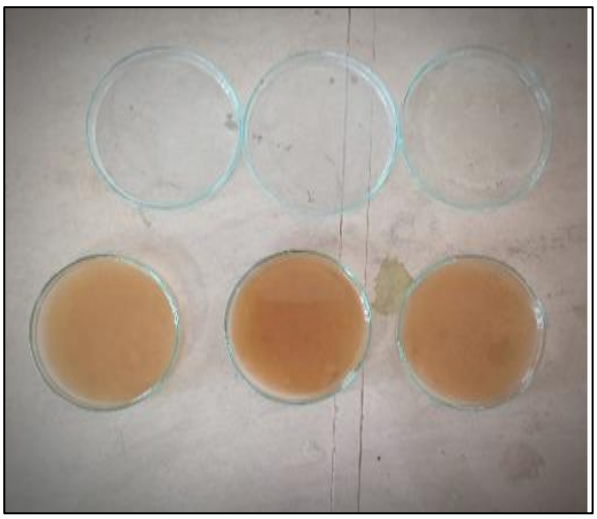

Fig 8: Preparation of Culture Media

➢ *Preparation of Solution of Extract:*

A 100 mg/ml methanol extract solution was prepared by dissolving 200 mg extracts in 2 ml methanol, shaking, and covering with aluminum foil. Stored at 4°C until needed.[20]

V. RESULT AND DISCUSSION

The current study aimed to manufacture and evaluate a herbal antibacterial cream using ethanolic extract in order to ascertain the cream's antibacterial properties. The ethanolic extract of Dalbergia sissoo was also evaluated by means of phytochemical screening, thin-layer chromatography, and column chromatography. The formulations were subjected to a variety of physical evaluation criteria, such as viscosity, emulsion type, spreadability, pH, phase separation, spreadability, and additional stability investigations. The results of the same are as follows:

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A. Phytochemical Analysis:

Table 2: Phytochemical Screening

Sr.No.	Component	Test	Inference
1	Alkaloids	a] Mayer's test b] Dragendroff test	+ + + + +
2	Protein and Amino acids	Ninhydrin test	+
3	flavonoids	Alkaline reagent test	+ + +
4	Carbohydrates	Molish's test	+ + +
5	Triterpenoids	Salkowski's test	+
6	Tannins	Gelatin test	+
7	Phenolic compounds	Ferric chloride test	+ + +
8	Phytosteroids	Salkowski's test	+ +
9	Steroids	Steroid test	+ +
10	Saponins	Saponin test	+

[+++-Strongly present,++-Moderate present, +Weakly present]

Alkaloids, protein and amino acids, flavonoids, carbohydrates, triterpenoids, tannins, phenolic compounds, phytosteroids, steroids, and saponins are all present in the ethanolic extract of D.sissoo, according to phytochemical screening.

B. Evaluation of Cream:

➢ *Physical Evaluation:*

Table 3: Physical Evaluation

Sr. No.	Parameters	F1	F2	F3
1	Colour	Light bottle green	Faint bottle green	Dark bottle green
2	Odour	Pleasant	Pleasant	Pleasant
3	Texture	Smooth	Smooth	Smooth
4	State	Semisolid	Semisolid	Semisolid

Based on visual observations, the created formulation cream F1, F2, and F3 were seen to have a smooth texture, pleasant smell, and a homogenous dim green colour.

➢ *Irritancy:*

Table 4: Irritancy

Sr. No.	Formulation	Irritant Effect	Erythema	Edema
1	F1	Nil	Nil	Nil
2	F2	Nil	Nil	Nil
3	F3	Nil	Nil	Nil

The developed formulation creams F1, F2, and F3 exhibit no erythema or edoema, nor any irritation impact.

➢ *Washability:*

Table 5: Washability

Sr.No.	Formulation	Washability
1	F1	Washable
2	F2	Washable
3	F3	Washable

When applied to skin, the created formulation creams F1, F2, and F3 can be removed with water, indicating that they are washable.

➢ *pH:*

Table 6: pH

Sr.No.	Formulation	pH
1	F1	6.80-7.00
2	F2	7.05-7.15
3	F3	6.90-7.10

The pH of all three of the formulations F1, F2, and F3 was determined to be between 6.80 to 7.10, which is a suitable range for skin pH, based on the results. All of the cream formulations had pH values between 5-7, which is closer to what the skin requires.

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➢ *Viscosity:*

Table 7: Viscosity

Sr. No.	Formulations	Viscosity
1	F1	21020
2	F2	11810
3	F3	18820

Cream's viscosity was measured using a Brookfield viscometer at 27 °C and spindle number seven running at 100 RPM. Based on the findings, the cream's viscosity fell between 11810 to 21020 Cps.

➢ *Phase Separation:*

Table 8: Phase Separation

Sr. No.	Formulation	Phase Separation
1	F1	Absence of phase separation.
2	F2	Absence of phase separation.
3	F3	Absence of phase separation.

The made cream was kept in a closed container at room temperature and kept out of sight. Phase separation was then investigated daily for 30 days. The results demonstrated that phase separation was not evident in any of the three formulations.

➢ *Spreadability:*

Table 9: Spreadability

Sr. No.	Formulation	Time(sec)	Spreadability (g×cm/sec)
1	F1	12	23.6
2	F2	07	29.2
3	F3	20	17.9

Out of the three formulations, F3 takes less time for the two slides to separate, which is good for spreadability since, as the evaluation test description states, less time is needed for the two slides to separate. In light of this, F3 demonstrated the expected Spreadability in comparison to F1 and F2.

➢ *Greasiness:*

Table 10: Greasiness

Sr. No.	Formulation	Greasiness
1	F1	Slightly-greasy
2	F2	Slightly-greasy
3	F3	Slightly-greasy

Upon observation, the created formulation creams F1, F2, and F3 have a little greasy nature.

C. Thin Layer Chromatography:

Table 11: Thin layer Chromatography of Dalbergia Sissoo

Mobile Phase	Extract	Rf Value	Color of Spot
Ethyl acetate: Benzene (1:9)	Ethanolic	0.35	Brown
		0.52	Light brown
		0.61	Yellow
		0.81	Light yellow
		0.88	Green
		0.94	Light green

The ethanolic extract of D. sissoo was subjected to thin layer chromatography, with ethyl acetate: benzene (1:9) serving as the mobile phase. The current study displays several coloured dots that have travelled from the origin. The displayed Rf value ranges from 0.35 to 0.9

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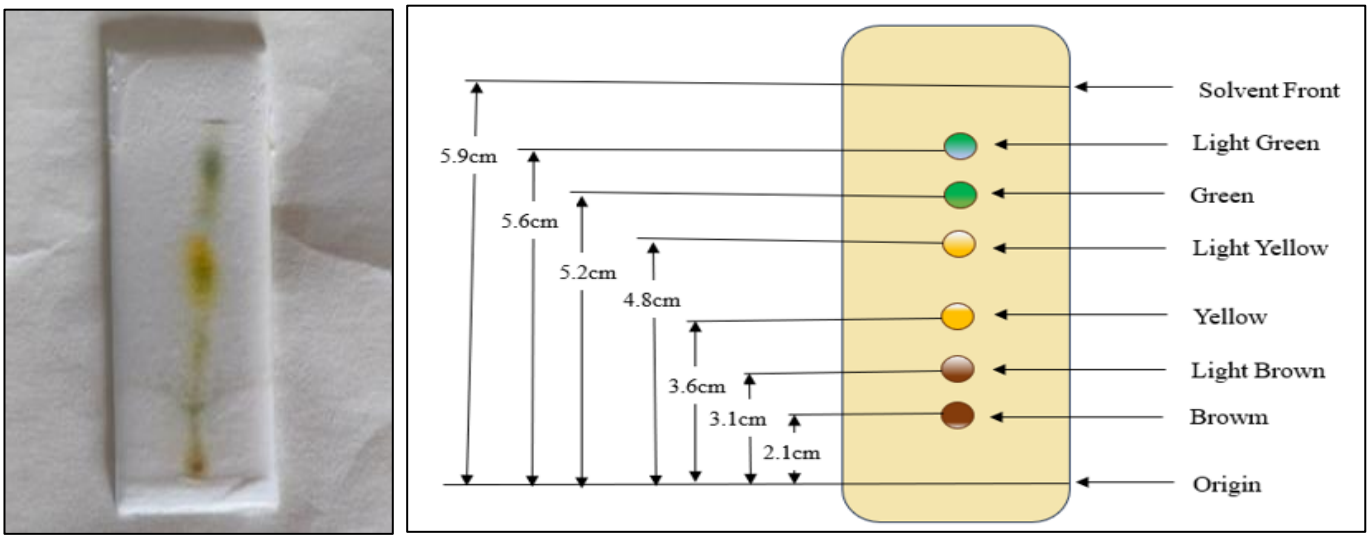

Fig 9: Thin Layer Chromatography

➢ *Column Chromatography:*

The ethanolic extract of D. sissoo was used in the column chromatography procedure. The silica gel was dissolved using methanol as a solvent. The herbal extract was added to the column of silica gel and let to run. Over time, the different components contained in the extract separated out. It indicates that the D. sissoo contains a variety of chemical components.

D. Antibacterial Activity:

The study investigated the antibacterial properties of Dalbergia sissoo plants against Staphylococcus aureus. Results showed strong antibacterial activity, with extracts showing significant resistance. Culture media were prepared using cup agar diffusion and disc diffusion methods. Staphylococcus aureus grew within five to seven days, and the ethanol extract, at a dosage of 10 mg/ml, produced an inhibitory zone of 25.9 + or -. The study concluded that Dalbergia sissoo plants have potent antibacterial properties.


Fig 10: Bacterial Activity of Dalbergia Sissoo

VI. CONCLUSION

In this research, we have shown that a plant sample has antibacterial action against several types of bacterial pathogens. Herbal medicine has potential benefits for the primary healthcare system. Certain plants possess antimicrobial properties. Due to the emergence of harmful bacteria that are resistant to many medications and the negative side effects of certain antibiotics, there is currently a lot of interest in the search for innovative plant-based antibacterial treatments. The antibacterial qualities of Dalbergia sissoo leaf extract. A variety of solvents are used in the extraction and formulation of antimicrobial cream, including as methanol, ethanol, and chloroform. The cream and herbal extract evaluation criteria were evaluated. The bacteria, Staphylococcus aureus, was grown in culture media under the appropriate conditions. The cup agar diffusion method revealed the extract's antibacterial activity against Staphylococcus aureus, as seen by the area of inhibition zone surrounding the wells. It has been shown that Staphylococcus aureus is highly sensitive to the ethanol extract. At 10 mg/ml, the ethanol extract was shown to create an inhibitory zone of 25.9 + or - against Staphylococcus aureus. Strong antibacterial activity was demonstrated by the ethanolic extract of D. sissoo leaves when tested against the identified microbes. This noteworthy result could be attributed to the active chemical components of the plant extract.

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