

Analysis of Phytochemical Constituents and Antibacterial Properties of *Dalbergia sissoo* Leaf Extracts from Rajasthan

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Abstract

The excessive use of chemical antimicrobial agents has resulted in serious environmental and human health concerns, particularly the rapid appearance of drug resistance microorganisms. Therefore, Medicinal plants have gained significant interest as natural sources of bioactive antimicrobial compounds. The current study aims to analyze the phytochemical properties and antibacterial activity of leaf extracts of *Dalbergia sissoo*, a medicinal plant frequently employed in folk herbal medicine. Leaves of the plant were subjected to extraction via decoction and maceration methods by using different solvents such as petroleum ether, hexane, chloroform, methanol, ethyl acetate, and water. The initial phytochemical study showed that the plant possessed flavonoids, phenols, alkaloids, tannins, glycosides, saponins, steroids, and terpenoids, and the methanolic extract demonstrated more phytochemical constituents. Antibacterial activity was determined using crude and fractionated extracts against four bacteria: *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* via well diffusion and disc diffusion. The extracts showed concentration-dependent antibacterial activity, producing low to moderate zones of inhibition, with methanolic and maceration extracts demonstrating comparatively higher efficacy. *S. aureus* showed maximum susceptibility while *E. coli* showed low susceptibility. Though it showed less effect than Kanamycin, the extract demonstrated some antibacterial effect; however, there was no inhibition effect seen in the DMSO control, indicating that the effect was due to the presence of active ingredients in the extract. This study validates the use of a locally available ethnomedicinal plant in the region through scientific research.

Keywords: *Dalbergia sissoo*, phytochemical analysis, antibacterial activity, medicinal plants, solvent extracts

1. Introduction

Medicinal plants are well recognized as natural sources of bioactive components that play an important role in the practice of medicine today. Herbal medicine is widely used in orthodox medicine for disease prevention and treatment owing to its medicinal properties and fewer adverse reactions. Specifically, herbal drugs are extensively used for chronic and age-related diseases such as inflammation, pain, digestive problems, skin infections, and microbe-related diseases, most of which are more complex to treat using only artificial drugs (Latif & Nawaz, 2025; Rizvi et al., 2022). About 80% of the population in the world relies on traditional herbal medicine for their basic medical needs as it is economical, easily

accessible, and culturally accepted (Balkrishna et al., 2024). As a result, there is scientific research conducted on medicinal herbs using phytochemistry and pharmacology, which has become highly significant in modern times (Kumar et al., 2023). *D. sissoo*, commonly known as Shisham or Indian rosewood, is a plant that falls under the Fabaceae plant family and is common on the Indian subcontinent due to its medicinal benefits. Historically, different parts of the *D. sissoo* have been widely utilized for various medicinal purposes such as relieving inflammation, pain, digestive complications, skin conditions, and microorganism-induced infections (Sen et al., 2023). For *D. sissoo* leaves, some of the secondary bioactive metabolites, which include phenolics, flavonoids, saponins, tannins, and glycosides, have been isolated from them. The importance of bioactive metabolites is mainly due to the wide range of biological activities associated with them, such as antioxidative and antibacterial activities (Chaddha et al., 2025). Several scientific studies have shown that the extracts of *D. sissoo* leaves exhibit anti-inflammatory, analgesic, antioxidative, and antibacterial effects (Sidana et al., 2012). Earlier studies have indicated that the leaf extracts of *D. sissoo* act against both Gram-positive and Gram-negative bacteria. However, in spite of all these positive results, there have not been many publications regarding the chemical composition and antibacterial activity of *D. sissoo* leaves from Rajasthan, whose environmental conditions could impact the chemical composition of its bioactive compounds. In order to confirm and investigate the medicinal value of traditional beliefs, more research needs to be done in this area. This is precisely what the present study aims to accomplish by evaluating the phytochemical and antibacterial properties of *D. sissoo* leaves from Rajasthan.

2. Materials and methods

2.1 Sample collection

D. sissoo is widespread in India and occurs in many ecological zones of Rajasthan. Healthy and disease-free leaves of *D. sissoo* were collected from the wildlife sanctuary of Bassi, Chittorgarh, Rajasthan, in October 2024. The collected samples of plants were identified botanically by Dr. Shahdab Hussain, Assistant Professor, Department of Botany, Sangam University, Bhilwara, Rajasthan. Herbarium vouchers were then prepared and preserved in the department of botany.

The collected leaves were taken to the laboratory and stored hygienically before being used for any further experiments.



Figure 1: *D. sissoo* (A) Whole plant and (B) Leaf

2.2 Leaf extract preparation

The newly harvested leaf samples were extensively washed under running tap water to remove the dirt, followed by sterilization with distilled water in order to achieve effective surface sterilization. The washed leaves were dried under shade at room temperature in order to maintain the thermally sensitive phytoconstituents present in them and further ground to fine powder.

Two extraction techniques, namely decoction and maceration, were employed for the preparation of crude leaf extracts.

Decoction method

For decoction extraction, 10 g of the powdered leaf samples were extracted individually using 100 mL of methanol and 100 mL of petroleum ether. Extraction was continued until the solvent became clear, indicating exhaustive extraction of soluble phytochemicals (Gul A. et al., 2022). The obtained extracts were filtered through Whatman No. 1 paper for clarification, after which the solvents were allowed to evaporate at ambient temperature. The obtained crude dried extracts were subsequently collected and preserved in airtight containers at 4°C until further analysis.

Maceration method

For maceration extraction, 10 g powdered leaves were taken and placed in a single sterile glass flask, and 20 mL each of hexane, chloroform, methanol and ethyl acetate were jointly added as a mixed solvent system. The sample was maintained at ambient conditions ($25 \pm 2^\circ\text{C}$) for 72 hours, with occasional shaking at intervals of 3–4 hours to facilitate effective extraction of phytoconstituents at room temperature. After completion of the extraction, the extract was clarified by passing through a Whatman No. 1 filter paper, followed by air drying at room temperature.

The dried extract was preserved at 4 °C for subsequent phytochemical and antimicrobial studies.

Standard extraction protocols reported by Jeffrey B. Harborne et al. (1998) and Azwanida Azwanida et al. (2015) were adopted with minor modifications in solvent volume and extraction time.

Percentage yield calculation

The extraction yield was calculated by applying the formula suggested by Parveen et al. (2014). Percent yield (%) = (Weight of dried crude extract / Weight of initial dried plant material) $\times$ 100

2.3 Phytochemical screening

The crude leaf extracts from decoction and maceration were then put through a qualitative phytochemical screening test using standard procedures as explained by Harborne et al. (1998), Gul A. et al. (2022), and Arora et al. (2023). The qualitative phytochemical tests analyzed different primary and secondary metabolites, such as carbohydrates, proteins, glycosides, amino acids, fats, alkaloids, flavonoids, saponins, terpenoids, tannins, phenolic compounds, steroids, coumarins, and anthraquinones. The qualitative phytochemical screening test gave initial information on the bioactive components contained in the leaves of the *D. sissoo* plant species.

2.3.1 Qualitative phytochemical tests

Primary metabolites were identified by applying standard biochemical tests such as the Molisch test, Barfoed test, Fehling test, Benedict test, Biuret test, Ninhydrin test, and saponification test.

Secondary phytochemicals were evaluated using the Ferric chloride test, Dragendorff's test, Foam test, Liebermann–Burchard test, alkaline reagent test, and anthraquinone detection methods according to established phytochemical procedures.

Modified from Harborne et al., 1998; Gul A. et al., 2022.

Table 1. Qualitative phytochemical screening of Dalbergia sissoo leaf for primary metabolites

Metabolites	Test	Procedure	Positive Result
Carbohydrate	Molisch test	1 mL extract + few drops of alcoholic α -naphthol solution + concentrated sulfuric acid	Violet ring formation
	Barfoed test	1 mL extract + 1 mL Barfoed reagent	Red precipitate
Reducing sugar	Fehling test	1 mL extract + 1 mL Fehling A + 1 mL Fehling B	Bricks' red colour appears.
	Benedict test	1 mL extract + a few drops of Benedict reagent	Red colour
Non-reducing polysaccharide	Iodine test	1 mL extract + a few drops of dilute iodine solution	Blue colour
Glycoside	Alkaline test	0.5 mL extract + NaOH + 1 mL distilled water	Yellowish colour layer formation
Amino acids and proteins	Biuret test	2 mL extract + 2 mL NaOH (4%) + few drops of CuSO_4 (1%)	Violet or pink colour
	Ninhydrin test	1 mL extract + 3 drops Ninhydrin solution	Purple-blue colour
Fat and fixed oil	Saponification test	1 mL extract + few drops alcoholic KOH + one drop phenolphthalein	Soap formation

Modified from Harborne et al., 1998; Gul A. et al., 2022; Arora et al., 2023

Table 2. Qualitative phytochemical screening of Dalbergia sissoo leaf for secondary metabolites

Metabolites	Test	Procedure	Result
Alkaloids	Dragendorff's test	2 mL extract + a few drops of Dragendorff's reagent	Orange-brown precipitate
Saponins	Foam test	1 mL extract + 10 mL distilled water, shaken for 15 min	Stable foam formation
Flavonoids	Alkaline reagent test	1 mL stock solution + 1 mL dilute NaOH	Yellow-green colour
Steroids	Liebermann–Burchard test	1 mL crude extract + 10 mL chloroform + concentrated sulfuric acid	Bilayer formation (upper red, lower greenish)
Terpenoids	Salkowski test	0.5 mL crude extract + 2 mL chloroform + 3 mL sulfuric acid	Reddish-brown colour
Phenols	Ferric chloride test	2 mL extract + a few drops of 5% ferric chloride	Blue-black precipitate
Coumarins	Alkaline reagent test	2 mL extract + 1 mL 10% NaOH, shaken for 5 min	Yellow-green colour
Anthraquinones	Ammonia test	2 mL extract + 0.5 mL chloroform + 1 mL ammonia	Pink colour

2.4 Antimicrobial assay

2.4.1 Bacterial strains

Reference bacterial cultures of *Staphylococcus aureus* (MCC2043T), *Escherichia coli* (MCC3671T), *Klebsiella pneumoniae* (MCC2451T), and *Pseudomonas aeruginosa* (MCC2080T). The bacterial strains were procured from the National Centre for Microbial Resources. The cultures were stored at 4 °C and periodically sub-cultured to maintain viability. For antimicrobial assessment, fresh inocula were developed through incubating the bacterial strains for 24 h at 37 °C to obtain actively growing cultures.

2.4.2 Preparation of experimental extract and control samples

The raw methanolic extract from the leaves of *D. sissoo* was suspended in 50% dimethyl sulfoxide (DMSO). Stock solutions were prepared in the concentration of 50 mg/mL. The commercially available kanamycin discs were used as the positive controls, while 50% Dimethyl sulfoxide was used as the solvent for the antibacterial experiments.

2.4.3 Determination of antibacterial activity using agar well diffusion

Antibacterial activity of crude plant leaf extract was evaluated on Mueller-Hinton agar using agar well diffusion method as described by Naushad et al. (2012). With some variations, sterilized Mueller-Hinton agar medium was cooled down to 45-50 °C and further inoculated with standardized bacterial suspension, followed by pouring into Petri plates to achieve a constant thickness of the medium. Solidified media were aseptically inoculated with three wells, having a diameter of 6 mm, with the help of a sterile cork borer. Separate wells were filled with 2.5 mg/mL and 5.0 mg/mL aliquots of the plant extract. Kanamycin was applied as the reference antibacterial agent at the same volume of 50%. The control was well-received, Dimethyl sulfoxide. The inoculated plates were allowed to stand at room temperature for 2 hours without disturbance to allow the extract to diffuse through the agar medium. Following diffusion, the plates were sealed and maintained at 37°C for a period of 24 hours for incubation. Antibacterial efficacy was assessed through measurement of the diameter of the inhibition zones observed around each well with an antibiotic zone reader as described by Sharmeen et al. (2012). The experimental procedures were conducted in triplicate to ensure reliable results.

2.4.4 Fractionation of crude methanol extract by liquid-liquid extraction

The crude extract prepared with methanol was fractionated through sequential liquid-liquid partitioning using solvents arranged in increasing order of polarity, following modified standard protocols of Parekh et al. (2005) and Harborne (1998). In the present study, solvent volume at each extraction step was standardized to 15 mL because of the limited extract quantity.

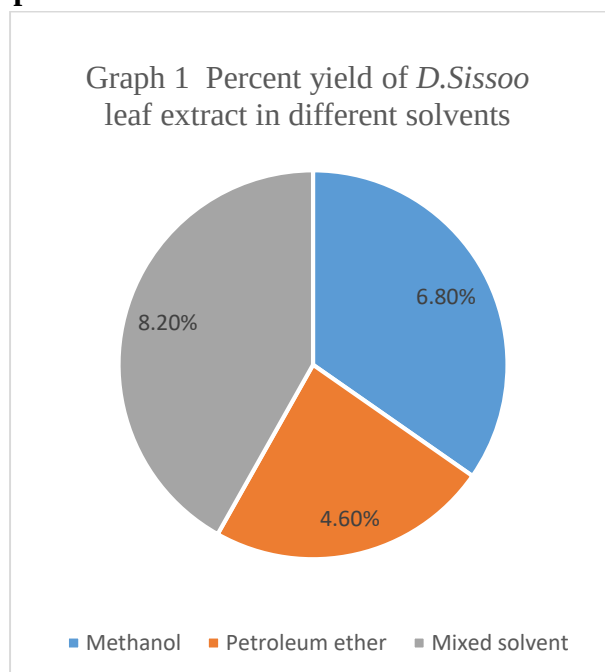
In the beginning, the crude extract was first suspended in sterilized water along with hexane in the separatory funnel. After thorough shaking, the upper layer was taken out and identified as the non-polar fraction. The water portion was then further extracted with fresh batches of hexane, and all the hexane fractions were pooled together. In order to prepare the medium-polar fraction, the leftover water phase was now extracted with chloroform. For the preparation of the semi-polar fraction, ethyl acetate was added to the water phase. Finally, for obtaining the polar fraction, methanol was added to the water phase.

2.4.5 Disc diffusion assay

The antibacterial potential was assessed using the disc diffusion method in accordance with Clinical and Laboratory Standards Institute guidelines (2018). Filter paper discs (6 mm in diameter), sterilized before use, were loaded with 50 µL of each extract fraction and carefully placed on previously inoculated Mueller-Hinton agar plates. After inoculation, the plates were maintained at 37°C for a period of 24 hours,

after which the clear inhibition zones surrounding the discs were recorded in millimeters. All experimental treatments were executed three times following the procedure described by Behera et al. (2013).

2.5 Statistical analysis of experimental data



Analysis of experimental data was done using SPSS version 16.0, where mean $\pm$ SD was used for triplicate. Comparison between treatments was performed using one-way ANOVA in CRD format, and the difference between means was done using Tukey's HSD. The level of significance was determined at $P < 0.05$. 3. Results

3.1 Percent yield of crude extract

Different extraction procedures produced varying yields of leaf extract from *D. sissoo*. The methanolic extraction yielded 6.8% crude extract with a light green appearance, while petroleum ether extraction produced 4.6% yield with similar coloration. Among the tested methods, maceration resulted in the highest extract recovery 8.2% and produced a greenish-brown extract. The variation in extract yield indicates that extraction efficiency was influenced by solvent characteristics and extraction technique (Graph 1).

3.2 Phytochemical constituents identified in the extract

Qualitative Evaluation of Phytoconstituents in *D. sissoo* leaf extracts revealed the occurrence of several primary and secondary metabolites in both methanolic and petroleum ether extracts. Among primary metabolites, carbohydrates, reducing sugars, glycosides, proteins, amino acids, and fats were detected in varying intensities in both extracts. Methanolic extract showed comparatively stronger reactions for proteins, carbohydrates, fat and amino acids. In contrast, the petroleum ether extract displayed comparatively lower intensity in most tests. Similarly, secondary metabolite profiling confirmed that the extract contained tannins, flavonoids, steroids, alkaloids, terpenoids and phenolic compounds. Methanolic extract demonstrated stronger positivity for tannins, steroids, and phenolic compounds compared with petroleum ether extract. Saponins, coumarins, and anthraquinones were absent in both extracts.

Table 3. Qualitative phytochemical analysis of primary metabolites in leaf extracts of *D. sissoo* (+) = Presence of phytochemicals, (-) = Absence of phytochemicals, M - Methanol, P- Petroleum ether

S.No.	Primary Metabolites	Test Name	Result	
			M	P
1.	Carbohydrate	Molisch	+++	+
		Barfoed	++	+
2.	Reducing Sugar	Fehling	+	+
		Benedict	-	-
3.	Non-reducing Polysaccharide	Iodine test	-	-
4.	Glycosides	Alkaline test	+	++
5.	Protein	Biuret	++	+
6.	Amino Acid	Ninhydrin	+	-
7.	Fat and fixed oil	Saponification	++	+

Table 4. Qualitative phytochemical evaluation of secondary metabolites in leaf extracts of *D. sissoo*, (+) = Presence of phytochemicals, (-) = Absence of phytochemicals, M -Methanol, P- Petroleum ether

S.No.	Secondary Metabolites	Test Name	Result	
			M	P
1.	Tannin	Ferric Chloride	+++	++
2.	Alkaloids	Dragendorff's test	++	+
3.	Saponin	Foam test	-	-
4.	Flavonoids	Alkaline reagent test	+	+
5.	Steroids	Liebermann Burchard	+++	+
6.	Terpenoids	Salkowski reaction	++	+
7.	Phenol	Ferric Chloride	+++	+++
8.	Coumarin	Alkaline Reagent	-	-
9.	Anthraquinone	Ammonia test	-	-

(A)



(B)



Figure 2: Qualitative phytochemical analysis of *D. sissoo* leaf for primary metabolites. (A) Methanol solvent (B) Petroleum ether solvent

(A)



(B)

Figure 3: Qualitative phytochemical analysis of *D. sissoo* leaf for secondary metabolites. (A) Methanol solvent (B) Petroleum ether solvent

3.3 Evaluation of antibacterial effect of methanolic leaf extract

The antibacterial activity of the leaf extract of *D. sissoo* was determined for various bacterial species using the zone of inhibition method. The antibacterial effect of the crude leaf extract was shown only for *S. aureus*. The extract showed zone of inhibition values of 12.50 ± 0.45 mm for 2.5 mg/mL and 15.75 ± 0.35 mm for 5.0 mg/mL, showing concentration-dependent antibacterial effect. In comparison, the commercial antibiotic kanamycin strip inhibited *S. aureus* up to 16.9 ± 0.45 mm. However, no antibacterial activity (NA) was observed against *E. coli*, *K. pneumoniae*, and *P. aeruginosa* at either concentration of the crude leaf extract tested. On the other hand, kanamycin strip showed clear inhibition zones towards *E. coli* (25.73 ± 0.53 mm), *K. pneumoniae* (22.03 ± 0.33 mm), and *P. aeruginosa* (18.32 ± 0.40 mm). No inhibition zone was seen on the control DMSO sample, which means that DMSO had no antibacterial activity (Table 5; Graph 2; Figures 4 and 5).

Table 5. Antibacterial properties of methanolic leaf extract of *D. sissoo* against selected bacterial strains.

Bacterial strains	Zone of inhibition (mm)			
	D. sissoo crude leaf extract		Kanamycin strip	DMSO
	2.5 mg/mL	5.0 mg/mL		
<i>S. aureus</i>	12.50 ± 0.45^b	15.75 ± 0.35^a	16.9 ± 0.45^a	NA
<i>E. coli</i>	NA	NA	25.73 ± 0.53^a	NA
<i>K. pneumoniae</i>	NA	NA	22.03 ± 0.33^a	NA
<i>P. aeruginosa</i>	NA	NA	18.32 ± 0.4^a	NA

Values are given as mean $\pm$ SD of three replicates, and distinct superscript letters denote significant differences ($p < 0.05$) as determined by Tukey's HSD test.

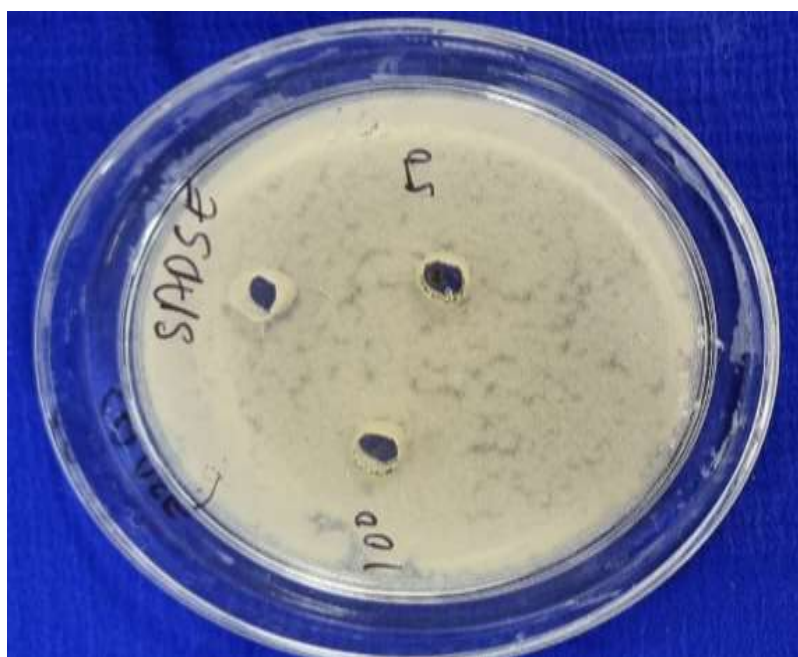


Figure 4. Antibacterial activity of hot extract of *D. sissoo* against *S. aureus*

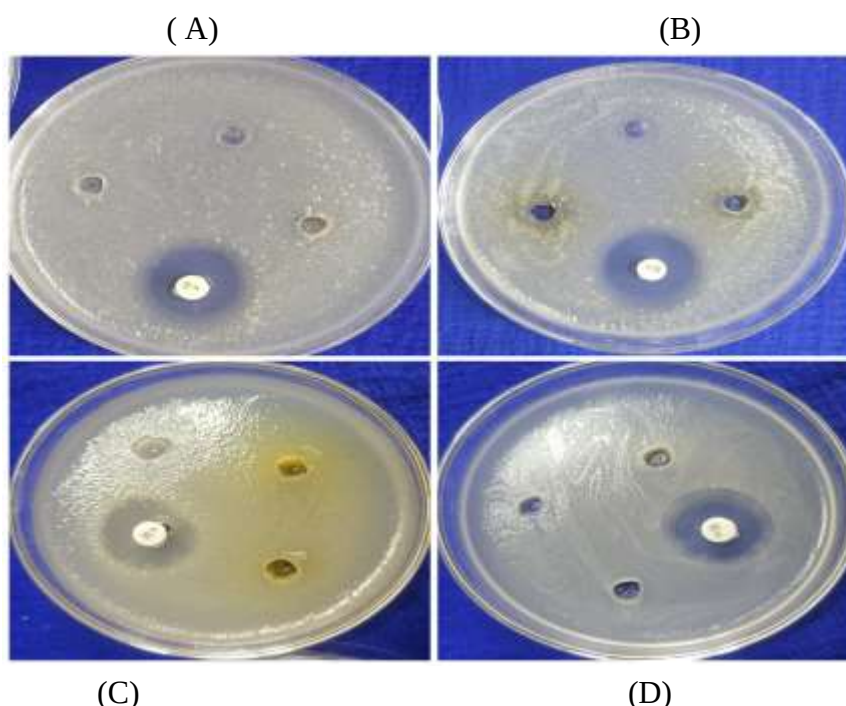


Figure 5: Zone of inhibition produced by Kanamycin (positive control) against (A) *S. aureus*, (B) *E. coli* (C), *K. pneumonia* (D), and *P. aeruginosa*.

3.4 Evaluation of antibacterial effect of maceration of crude leaf extract

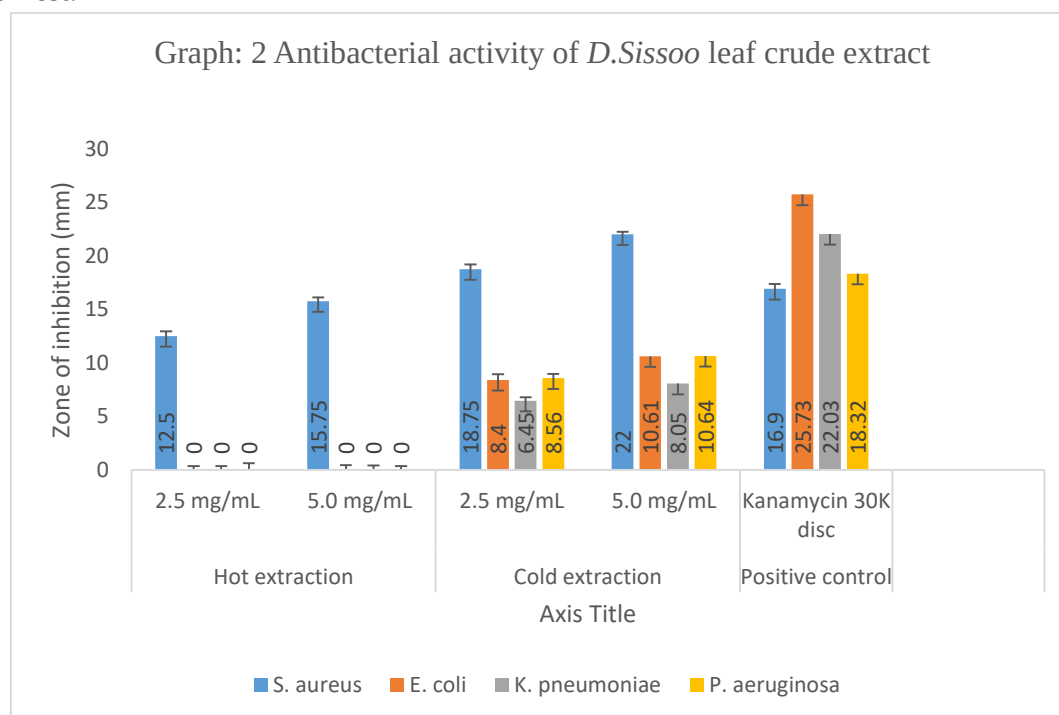
Antibacterial potential of *D. sissoo* leaves was studied by determining the zone of inhibition produced by it against different strains of bacteria. It was observed that the extract was effective against all tested strains of bacteria. As per the results obtained, the extract inhibited the growth of *S. aureus* with a zone of inhibition of 18.75 ± 0.45 mm at a 2.5 mg/mL concentration. However, when the concentration was raised to 5.0 mg/mL, the zone of inhibition produced was 22.00 ± 0.23 mm, which indicated a concentration-

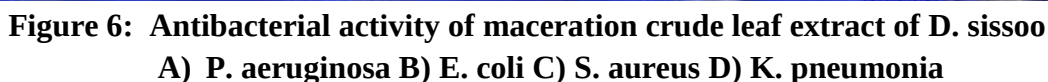
dependent antibacterial activity. On similar lines, when tested against *E. coli*, the extract showed an increase in inhibition zone from 8.40 ± 0.35 mm at 2.5 mg/mL to 10.61 ± 0.44 mm at 5.0 mg/mL concentration. When tested against *K. pneumoniae*, it produced inhibition zones of 6.45 ± 0.34 mm and 8.05 ± 0.42 mm at 2.5 mg/mL and 5.0 mg/mL concentrations, respectively. Moreover, on testing it against *P. aeruginosa*, its inhibitory effect increased from 8.56 ± 0.63 mm at 2.5 mg/mL concentration to 10.64 ± 0.35 mm at 5.0 mg/mL concentration. Thus, it was evident from the above results that the antibacterial activity was found to be concentration dependent for all strains tested. There was no inhibition in the case of DMSO, indicating the absence of antibacterial property of the solvents.

Table 6: Antibacterial activity of maceration crude leaf extract of *D. sissoo* against selected bacterial strains

Bacterial strains	Zone of inhibition (mm)		
	Dalbergia sissoo crude leaf extract		DMSO
	2.5 mg/mL	5.0 mg/mL	
<i>S. aureus</i>	18.75 ± 0.45^b	22.00 ± 0.23^a	NA
<i>E. coli</i>	8.4 ± 0.35^b	10.61 ± 0.44^a	NA
<i>K. pneumoniae</i>	6.45 ± 0.34^b	8.05 ± 0.42^a	NA
<i>P. aeruginosa</i>	8.56 ± 0.63^b	10.64 ± 0.35^a	NA

Results are expressed in terms of mean $\pm$ standard deviation from three replicates, whereas letters representing statistical significance at $p < 0.05$ were obtained using Tukey's Honestly Significant Difference Test.





Antibacterial activities of solvent fractions from *D. sissoo* were determined against several bacterial strains by the zone of inhibition method. It was noted that the antibacterial activity of the solvent fractions was selective in nature against *S. aureus* and *P. aeruginosa* but did not show any activity (NA) against *E. coli* and *K. pneumoniae*. Out of the studied solvent fractions, the chloroform fraction possessed high antibacterial activity against *S. aureus* with a zone of inhibition of 6 ± 0.8 mm, followed by the hexane and ethyl acetate fractions with an inhibition zone of 5 mm each. In contrast, the least active fraction was the aqueous fraction, having a zone of inhibition of 4 ± 0.6 mm. In case of *P. aeruginosa*, hexane fraction was most active with the zone of inhibition of 6 ± 0.5 mm, followed by ethyl acetate (4 ± 0.2 mm), chloroform (3 ± 0.3 mm) and aqueous fraction (2 ± 0.3 mm) respectively. Antibacterial activity was not found against *E. coli* and *K. pneumoniae* for any solvent fraction studied. Hexane and chloroform fractions displayed relatively higher activity against susceptible bacterial strains as compared to ethyl acetate and aqueous fractions. (Table no. 7; Figure no. 7)

S.No.	Plant Name	Separate liquid	EC	SA	PA	KP
1.	Dalbergia sissoo	Hexane	NA	5 ± 0.5 ^b	6± 0.5 ^a	NA
		Chloroform	NA	6± 0.8 ^a	3 ± 0.3 ^b	NA
		Ethyl Acetate	NA	5 ± 0.9 ^b	4±0.2 ^b	NA
		Aqueous	NA	4 ± 0.6 ^c	2 ± 0.3 ^c	NA

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4. Discussion

The results obtained in this study indicated that the leaves of *D. sissoo* contained various phytochemical compounds, exhibiting antibacterial properties against the tested bacterial species. The method of extraction was shown to exert a significant effect not only on the percentage of extraction yield but also on the biological activity of the extract. Of all the extraction techniques used, the maceration technique with a mixture of solvents gave the best extraction yield (8.2%). Meanwhile, the use of methanol and petroleum ether for extraction led to significantly lower yields. This could be explained by the presence of different polarities in the mixture of solvents that facilitated better extraction of phytochemicals from leaves. Similar findings were made by Babbar et al. (2014) and Kumar et al. (2023).

Another intriguing phenomenon noted in this study is the contrast between the antibacterial effects of hot and cold extractions. While the extract obtained from hot extraction possessed only antibacterial properties for *Staphylococcus aureus*, the extract obtained through cold extraction exhibited antibacterial action towards all the tested bacteria. From this result, we may conclude that cold extraction appears to be a more appropriate method in terms of preserving the antibacterial agents in plants. It seems likely that some of the biologically active substances, such as phenolic compounds and flavonoids, might be damaged or even partially degraded in the process of hot extraction due to their thermosensitivity. On the other hand, maceration is a much gentler process that preserves them. Such an assumption is supported by the results obtained by Azwanida (2015), according to whom overheating during the process of plant extraction leads to the loss of biologically active phytochemicals.

Phytochemical screening showed the presence of various primary metabolites like carbohydrates, reducing sugars, glycosides, proteins, amino acids, and fixed oils. Such an identification makes the significance of nutrition as well as medication in the use of this plant very clear. Such phytochemical results had been observed before in leaves of *D. sissoo* by researchers Bhattacharya et al. (2015) and Ali et al. (2016). Apart from the above-mentioned metabolites, some significant secondary metabolites like tannins, alkaloids, flavonoids, steroids, terpenoids, and phenolic compounds were also identified. It is a known fact that all of these secondary metabolites exhibit good antimicrobial, antioxidative, and anti-inflammatory activities. The findings obtained in the current study are corroborated by previous studies conducted on *D. sissoo*. In the antibacterial screening test, there is a dose-dependent effect; that is, higher concentrations produce bigger inhibition zones. In the bacteria tested, *Staphylococcus aureus* was found to be the most susceptible bacterium. This is consistent with studies by Chhabra et al. (2016) and Nayak (2021), who found more potent antibacterial effects of *D. sissoo* extracts against Gram-positive bacteria than Gram-negative bacteria. Less susceptibility displayed by *E. coli*, *K. pneumoniae*, and *P. aeruginosa* could be due to differences in cell wall composition. Bacteria of the Gram-negative type have an extra lipopolysaccharide layer in their cell wall, making them more resistant to antimicrobial agents because of increased restriction of their uptake (Shamsudin et al., 2022).

The antibacterial action seen in the current experiment may be attributed to the presence of phenols, flavonoids, tannins and alkaloids, whose presence was noted during phytochemical screening. It is worth noting that these secondary metabolites are capable of acting as effective antimicrobial agents by inhibiting cell membrane permeability, disrupting enzyme systems and interfering with metabolic processes (Shamsudin et al., 2022). There have been other reports of notable antimicrobial properties exhibited by extracts containing high amounts of phenols and flavonoids from *D. sissoo* (Chaddha & Gupta, 2025; Dahiya et al., 2025).

From the results of fractionation, antibacterial properties were found to be present in various fractions based on solvents. The chloroform fraction had the highest antibacterial activity against *S. aureus*, while the hexane fraction possessed the highest activity against *P. aeruginosa*. This suggests that different antibacterial constituents with different polarities are present in *D. sissoo* leaves. From the relatively high activity of the hexane and chloroform fractions, it is suggested that nonpolar and moderately polar compounds might contribute immensely to the antibacterial effect. Subedi et al. (2017) also reported similar results when they showed that antibacterial properties in *D. sissoo* exist in different fractions based on polarity. Furthermore, the presence of flavonoids, phenolics, and tannins, which contribute greatly to antimicrobial activity, has been noted in the fractions (Dahiya et al., 2025; Jyotsana & Gupta, 2025).

Results achieved in the current research scientifically prove the effectiveness of the medicinal usage of *D. sissoo* in Rajasthan. Climate, soil properties, temperatures, and water availability are considered ecological factors affecting the presence of secondary metabolites in medicinal plants. According to Li et al. (2022) and Kumar et al. (2023), ecological conditions play an important role in the phytochemical composition and biological activity of plants. Thus, the phytochemical composition and antibacterial activity found in the current research might be affected by the ecological conditions of the region from which the plants were collected.

Nevertheless, there are several limitations of the conducted research that should be addressed. First, the number of used bacterial strains was four, and MIC and MBC values have not been estimated in this research. Moreover, the bioactive compounds of the studied plant responsible for its antibacterial effects have not been identified. Further research is recommended to be conducted, where purification, isolation, and identification of active compounds will be performed.

5. Conclusion

The current research has indicated the presence of various significant phytochemicals such as flavonoids, phenols, tannins, alkaloids, steroids and terpenoids in *D. sissoo* leaves. The extraction method used had a great impact on the amount of extract obtained as well as the antibacterial property. Cold maceration was found to be the extraction method with high extract yield and superior antibacterial property when compared with hot extraction.

The extracts showed concentration-dependent antibacterial properties, and *S. aureus* was the bacterial strain with the highest sensitivity towards the leaf extracts. The findings reveal that the antibacterial activity of *D. sissoo* might be due to the synergistic action of various phytochemical components present in the plant leaves.

The current investigation is in line with the ethnobotanical usage of *D. sissoo* for medicinal purposes. Future studies on isolation of active compounds, minimum inhibitory concentrations and in vivo studies are essential to validate its medicinal uses.

Acknowledgements

The author is thankful to Sangam University, Bhilwara, and the staff of Bassi Wildlife Sanctuary, Rajasthan, for providing research support and laboratory facilities.

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