

Research Article

Phytochemical Analysis and Antibacterial Activity of *Dipterocarpus turbinatus* Leaf Extracts against *Staphylococcus aureus* and *Klebsiella pneumoniae*

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A B S T R A C T

Introduction: In this research, *Dipterocarpus turbinatus* leaf extracts in aqueous, chloroform, ethanol, hexane, and methanol were examined for their phytochemical components and antimicrobial activity.

Methods: Bacteria such as *Staphylococcus aureus* (a gram-positive bacterium) and *Klebsiella pneumoniae* (a gram-negative bacterium) were tested for antimicrobial action. The extracts from the leaves demonstrated broad-spectrum anti-bacterial action against these microbes.

Result: Acids, proteins, phenols, carbohydrates, flavonoids, alkaloids, glycosides, cardiac glycosides, steroids, tannins, starch, triterpenoids, and terpenoids were among the frequently found phytochemical components in *D. turbinatus* leaf extracts.

Conclusion: Traditional herbal medicines, which are widely used in developed countries as well as in the developing world, can benefit greatly from the antimicrobial properties of these plants, which have been used by humans for centuries without showing any signs of toxicity.

Keywords: *Dipterocarpus turbinatus*, Secondary Metabolites, Antimicrobial Activity

Introduction

Many different secondary metabolites, including flavonoids, phenols, saponins, tannins, essential oils, and alkaloids are produced by plants. While these compounds are typically not necessary for the development and reproduction of plants, they do take part in a variety of roles in the food and pharmaceutical industries.^{1,2} Plants contain a large number of biomolecules in all their different parts, hence they have historically been utilised as antibacterial substances. The secondary failure rate and serious adverse effects of synthetic drugs are both high. Plant products contain a range of molecules that can scavenge free radicals, including nitrogenous compounds, phenolics, and various other

naturally occurring compounds with strong antioxidant qualities.^{3,4}

Gurjan or *Ashwakarna*, a species of *Dipterocarpus turbinatus*, is found throughout India in places like Arunachal Pradesh, Assam, Manipur, Meghalaya, Tripura, and the Andaman and Nicobar Islands. It is used to treat gonorrhoea, leprosy, psoriasis, other skin conditions, urinary system infections, hearing problems, and abscesses. It also has hepatoprotective properties.^{5,6} Additionally, gonorrhoea, gleans, ulcers, dermatitis, and skin conditions are treated with *D. turbinatus*.⁷ Thus, the objective of the present research was focused on the qualitative phytochemical analysis as well as analysing the antimicrobial effects of *D.*

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turbinatus leaf extracts against *S. aureus* and *K pneumoniae*.

Materials and Method

Plant Material

The leaves of plant *D. turbinatus* were gathered in Assam, India's forested area during the month of May 2018. Dr Madhukar Bachulkar, Sivaji University, Maharashtra verified the plant's identity.

Preparation of Leaf Extracts

Using the cold extraction technique and the five solvents (aqueous, chloroform, ethanol, hexane, and methanol), fresh leaves were ground into a coarse powder and were allowed to air dry in the shade. 200 ml of each of the five solvents was introduced after 20 grams of powdered leaves sample had been placed in a beaker. After being left in the beaker at an ambient temperature for a period of 48 hours, the resulting solution was purified using Whatman Filter paper 1. For further research, the leaf extract was utilised.⁸

Qualitative Phytochemical Analysis

The standard methodology⁹ was used to determine the presence and absence of various phytochemicals such as alkaloids, acids, carbohydrates, proteins, flavonoids, phenols, coumarins, glycosides, quinones, anthocyanins, cardiac glycosides, saponins, terpenoids, starch, triterpenoids, tannins, and steroids in the leaf extracts of *D. turbinatus*.

Antibacterial Activity Test

The Kirby-Bauer technique was used to conduct antibacterial tests using disc diffusion.¹⁰ *S. aureus* and *K. pneumoniae* were the bacteria used in the study's bacterial cultures.

The inoculums were regulated to have 1106 CFU/ml when compared to turbidity standards (0.5 MacFarland tube). A Petri plate was filled with Muller Hinton Agar (MHA) medium. A sterile swab dampened with the bacterial suspension was used to distribute the inoculums on the MHA plates after the medium had solidified. The MHA plates were filled with sterile samples containing 20 µl of normal antibiotic (ampicillin) disc and discs of various concentrations (1000, 750, and 500 µg/ml). For 24 h, the dishes were incubated at a temperature of 37 °C. The antimicrobial activity was identified by quantifying the size of the zone of inhibition (ZOI).

Statistical Analysis

Data from the experiment were collected, and the findings were expressed as mean and SD. Version 8.0.2 of GraphPad Prism was used to conduct the statistical tests.

Results and Discussion

Qualitative Phytochemicals Analysis of *D. turbinatus* Leaf Extracts

Selecting the proper solvent system, solvent type (based on polarity), and extraction technique are crucial steps in the separation and purification of bioactive ingredients from plant materials.¹¹ The separation and characterisation of bioactive substances from the *D. turbinatus* leaves were thus carried out in the current research using five solvents of varying polarity. The existence of the tested secondary metabolites was confirmed by qualitative phytochemical analysis of *D. turbinatus* leaves extracts and the results have been shown in Table 1.

Table 1. Phytochemical Analysis of *D. turbinatus* Leaf Extracts

S. No.	Tests	Aqueous	Chloroform	Ethanol	Hexane	Methanol
1.	Acids	-	-	+	-	+
2.	Alkaloids	+	+	-	+	+
3.	Anthocyanins and betacyanins	+	+	-	-	-
4.	Carbohydrates	+	-	+	+	+
5.	Cardiac glycosides	+	-	+	+	+
6.	Coumarins	+	-	-	-	-
7.	Flavonoids	+	-	-	-	-
8.	Glycosides	-	-	+	-	-
9.	Phenols	-	-	+	+	+
10.	Proteins	+	-	-	-	+
11.	Quinones	-	-	-	-	-
12.	Saponins	+	-	+	+	+
13.	Starch	-	-	-	+	-
14.	Steroids	-	+	+	+	+
15.	Tannins	+	-	+	+	+
16.	Terpenoids	+	+	-	+	+
17.	Triterpenoids	+	+	-	+	-

+: Present, -: Absent

The secondary metabolic compounds namely alkaloids, anthocyanins, betacyanins, saponins, carbohydrates, tannins, cardiac glycosides, triterpenoids, coumarins, flavonoids, proteins, and terpenoids were present in liquid extract; anthocyanins, alkaloids, betacyanins, terpenoids, steroids, and triterpenoids were found in chloroform extract; acids, cardiac glycosides, saponins, glycosides, carbohydrates, steroids, phenols, and tannins were present in ethanol extract; alkaloids, carbohydrates, cardiac glycosides, phenols, saponins, triterpenoids, tannins, starch, steroids, and terpenoids were found in hexane extract; and acids, phenols, alkaloids, carbohydrates, cardiac glycosides, proteins, saponins, steroids, tannins and terpenoids were present in methanol extract. In the past, leaf extracts of plants *Aristolochia bracteolata*,¹² and *Cardiospermum halicacabum*, were found to contain a variety of phytonutrients, including alkaloids, steroids, phenols, glycosides, tannins, flavonoid glycosides, and saponins.

Antibacterial Activity of *D. turbinatus* Leaf Extracts

The results obtained from aqueous, chloroform, ethanol, hexane, and methanol extraction of *D. turbinatus* leaves showed substantial inhibition of the growth of both gram-positive and gram-negative bacteria by all the examined extracts. The zone of inhibition at the end of 1000 µg/ml showed the highest efficacy in ethanol extract as 17 mm against *S. aureus* and 12 mm against *K. pneumonia*, whereas in methanol extract, it was seen as 15 mm against both *S. aureus* and *K. pneumoniae*. Also, the aqueous extract

showed the ZOI size as 8 mm against *S. aureus* and 3 mm against *K. pneumoniae* followed by hexane and chloroform extracts with 7 mm against *S. aureus* (for both) and 3 mm (hexane) and 1 mm (chloroform) against *K. pneumonia*. Likewise, standard (ampicillin) showed the ZOI size as 18 mm against

S. aureus and 13 mm against *K. pneumoniae* (20 µl) (Table 2 and Figure 1). According to these findings, ethanol and methanol at higher concentrations exhibited the greatest zone of inhibition against clinical bacteria like *S. aureus* and *K. pneumonia*, indicating a wider range of efficacy.

As previously reported, ethanol and methanol extracts from various plants demonstrated more positive antibacterial action than the other solvents. Similar other experiments revealed that *P. vulgaris* (26.3 mm), *P. aeruginosa* (21.30 mm), and *S. typhi* (18 mm) bacteria were all susceptible to the antibacterial effects of hydro-alcoholic extracts at 750 µg/ml. In the leaf extracts of *Aristolochia tagala*, methanol extract successfully inhibited the multiplication of *B. subtilis* and *R. equi* with the zone of inhibition of sizes 21 and 21.87 mm, respectively (at 750 µg/ml concentration).

The development of *P. vulgaris*, *B. subtilis*, and *E. coli* was also noticeably inhibited by the hydro-alcoholic solution of *Dregea volubilis*,¹² and the methanolic extract of *Pisonia grandis* successfully restrained the multiplication of *P. aeruginosa*.¹³ Essential oils from the aerial parts and rhizome of *Aristolochia mollissima* were found to have considerable antibacterial activity against 21 bacterial strains, with gram-positive bacterial strains being more potent.¹⁴

Table 2. Antibacterial Activity of *Dipterocarpus turbinatus* Leaf Extracts

Samples	Microorganisms	Zone of Inhibition (mm)			Standard (20 µl) (Mean ± SD)
		1000 (µg/ml) (Mean ± SD)	750 (µg/ml) (Mean ± SD)	500 (µg/ml) (Mean ± SD)	
Aqueous	<i>S. aureus</i>	8 ± 0.23	7 ± 0.05	6 ± 0.1	18 ± 0.21
	<i>K. pneumoniae</i>	3 ± 0.26	1 ± 0.08	1 ± 0.08	13 ± 0.09
Chloroform	<i>S. aureus</i>	7 ± 0.23	7 ± 0.26	4 ± 0.21	18 ± 0.21
	<i>K. pneumoniae</i>	1 ± 0.18	1 ± 0.18	1 ± 0.18	13 ± 0.09
Ethanol	<i>S. aureus</i>	17 ± 0.19	15 ± 0.23	12 ± 0.11	18 ± 0.21
	<i>K. pneumoniae</i>	12 ± 0.44	11 ± 0.32	9 ± 0.20	13 ± 0.09
Hexane	<i>S. aureus</i>	7 ± 0.26	6 ± 0.15	5 ± 0.21	18 ± 0.21
	<i>K. pneumonia</i>	3 ± 0.08	2 ± 0.28	1 ± 0.17	13 ± 0.09
Methanol	<i>S. aureus</i>	15 ± 0.44	13 ± 0.23	13 ± 0.26	18 ± 0.21
	<i>K. pneumonia</i>	15 ± 0.26	15 ± 0.28	9 ± 0.11	13 ± 0.09

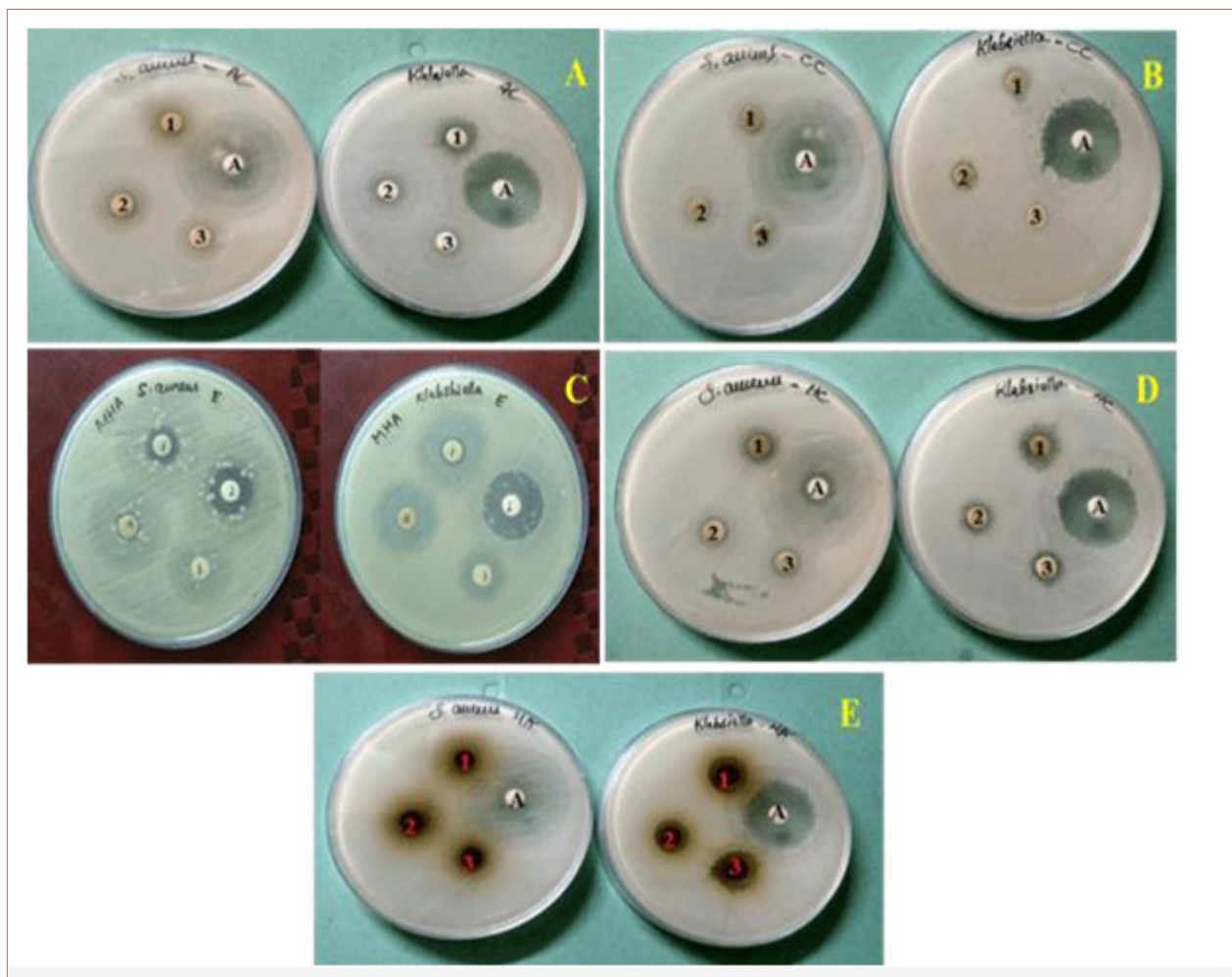


Figure 1. Antibacterial Activity of *Dipterocarpus turbinatus* Leaf Extracts against *S. aureus* (Left) and *K. pneumoniae* (Right) (A). Aqueous (B). Chloroform (C). Ethanol (D). Hexane (E). Methanol (A or a - Standard; 1 - 1000 µg/ml; 2 - 750 µg/ml; 3 - 500 µg/ml)

Conclusion

Qualitative phytochemical analysis of the *D. turbinatus* leaf extracts in ethanol and methanol showed the existence of phenols, terpenoids, proteins, saponins, carbohydrates, acids, alkaloids, carbohydrates, proteins, and cardiac glycosides. The growth of the tested bacterial strains, particularly *S. aureus* and *K. pneumoniae*, was effectively controlled by higher concentrations of ethanol and methanol extracts of *D. turbinatus* leaves, according to our observed findings of the current research. This perceived biological trait may result from a variety of components found in the plant that are separated using various chemical solvents. It is advised to conduct additional studies on the isolation of pure pharmaceutically significant compounds from the studied plants to demonstrate their therapeutic values for the creation of novel medicines.

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