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Antibacterial activity of various stem extracts of *Dalbergia Coromandeliana*

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ABSTRACT

Objective: To investigate the antibacterial activity and phytochemical screening of the hexane, chloroform, ethyl acetate, ethanol and aqueous stem extract of *Dalbergia coromandeliana* (*D. coromandeliana*). **Methods:** The antibacterial activity of stem extracts of *D. coromandeliana* were evaluated by agar well diffusion method against four selected bacterial species. **Results:** The presence of alkaloids, saponins, coumarins, tannins, steroids, flavonoids, quinones, proteins and carbohydrates in the different stem extracts was established. **Conclusions:** The results in the present study suggest that *D. coromandeliana* stems can be used in treating diseases caused by the tested organisms.

1. Introduction

Traditional plant medicines serve as a source of various types of active principle & WHO estimates 70% of the world population still relies on the herbal medicines. Out of the total 2, 25,000 species of plants, only less than 10% have been studied so far for their medicinal uses. India has rich flora of herbal plants and ancient medical system are several hundred years old[1]. The Indian Systems of medicine can be classified into traditional and classical systems. The traditional system is the local folk stream, which is prevalent in rural and tribal villages in India. The system like Ayurveda, Sidha, Unani, Yoga, and Naturopathy are expressions of classical systems. Thus the term Indian Systems of Medicine covers the system which originated in India or which originated outside but got adapted in the course of time[2]. Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives[3]. Most are secondary metabolites, of which only 12,000 have been isolated, which are estimated to be less than 10% of the total[4]. Of these, only small percentage has been investigated phyto-chemically and the fraction submitted to biological or pharmacological screening is even lower. Since plants may contain hundreds or even thousands of metabolites, there is currently a great interest in the medicinal plant research as a possible source of new lead compounds for introduction into therapeutical screening programmes. The new branch of Science, ethnobotany (or ethno-pharmacology), whose goal is to utilize the impressive array of knowledge assembled by indigenous peoples about the plant and animal products they have used to maintain health[5]. The motivation behind the study was detection of a phytoremedy for microbial infections as an alternative to chemotherapeutics and to have constructive exploitation of bio-resources of a region thereby developing special protection over rare species. The screening of plant extracts and plant products for antimicrobial activity has shown that plants represent a potential source of new anti-infective agents[6-9].

Dalbergia coromandeliana (*D. coromandeliana*) belongs to the family of Papilionaceae considered to be different from *D. spinosa* Roxb.[10]. is randomly found at the foot of Alagar hills near Madurai. The plant is a stiff shrub with white flowers and thorns arranged in a distichous manner. It has leaflets crowded on branchlets. The leaflets are 8 to 10 mm oblong, tip and base rounded. Plants of the genus *Dalbergia* are reported to be useful in the treatment of arthritis, gonorrhea and rheumatic pains[11],[12]. Detailed pharmacological studies conducted earlier on *D. sisoo*[13] and *D. lanceolaria*[14] showed that the crude and purified extracts of these plants possessed anti-inflammatory, anthelminthic, antidysentric, analgesics and antipyretic activities.

D. coromandeliana was chemically investigated for the first time in our laboratory[15] and there is no record of any pharmacological work on this plant. In view of our interest on the biological properties of the plants of

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the genus *Dalbergia* and since no such information is available on the extracts of *D. coromandeliana*, Although, the leaves and root of *D. coromandeliana* were subjected to chemical examination, its stems have not hitherto been chemically investigated. The present study was carried out to test the antibacterial efficacy of the stems extract of *D. coromandeliana* against bacterial spps.

2. Materials and methods

2.1 Plant material

The stems of *D. coromandeliana* were collected from the foot of Alagar Hills near Madurai District (Tamilnadu, India) and authenticated by the Director, Centre for Biodiversity and Forest Studies, Madurai Kamaraj University, and voucher specimens were deposited in the herbarium of Centre for Biodiversity and Forest Studies of our university (No.DC-02) and the extract was collected by using different solvents.

2.2 Extraction procedure

Shade dried pieces of stems coarsely powdered and subjected to successive solvent extraction by continuous Soxhlet extraction. The extraction was done with different solvents in their increasing order of polarity such as hexane, chloroform, ethyl acetate, ethanol and water in succession. All the extracts were concentrated by distilling the solvent in a rotary flash evaporator. The dried extracts were dissolved in dimethyl sulphoxide (DMSO) and subjected to antibacterial activity.

2.3 Preliminary phytochemical screening

For screening the phytochemical constituents standard methodologies given by Harborne[16] were adopted.

(Table 1 : Preliminary Phytochemical Screening of *D. coromandeliana* stems.)

2.4 Test Organisms

All the microbial strains of human pathogens used in the antimicrobial bioassay were procured from Institute of Microbial Technology (IMTECH), Chandigarh. These microbes include the Gram-negative bacteria such as Escherichia coli (MTCC 724) and Pseudomonas aeruginosa (MTCC 741); the Gram-positive bacteria such as Staphylococcus aureus (MTCC 96) and Streptococcus pyogenes MTCC 389. Amikacin (the antibacterial drug) were used as standards for comparison. The activity was measured as a function of zone of inhibition in mm and the results were compared with those of the reference drug by measuring their zone of inhibition (Table 2).

2.5 In vitro antibacterial activity

The extracts obtained above were screened for their antibacterial activity in comparison with standard antibiotic amikacin (10 μg / mL) by disc diffusion method[17] using *E. coli*, *P. aeruginosa*, *S. aureus* and *S. pyogenes* as test organisms. Each extract was individually loaded on the 5 mm sterile disc at the concentration of 25 μg / mL, 50 μg / mL and 100 μg / mL and subjected to antibacterial activity. The results were recorded by measuring the zone of growth inhibition surrounding the disc. The experiments were done in triplicate.

Table1

Preliminary Phytochemical analysis of *D. coromandeliana* stemsextracted with different solvents

Phytoconstituents	HE	CE	EAE	EE	AE
Alkaloids	+	+	+	+	−
Triterpenoids	+	+	+	−	−
Flavonoids	+	+	+	+	+
Carbohydrates	+	+	+	+	+
Amino acids	−	−	−	−	+
Glycosides	−	−	−	+	+
Coumarin	−	+	+	+	+
Steroids	+	+	−	−	+
Tannins	+	+	+	+	+
Quinones	−	−	+	+	+
Anthocyanins	+	+	+	+	+
Anthroquinones	−	−	−	+	+
Phenols	+	+	+	+	+

HE: Hexane extract; CE: Chloroform extract; EAE: Ethyl acetate extract; EE: Ethanol extract; AE: Aqueous extract; '+' indicate present; '–' indicate absent.

2.6. Statistical analysis

The results were expressed as mean ± SEM. Statistical analysis of the data were carried out using Student's t-test and the results considered significant when $P<0.05$. The results of antibacterial activity are presented in Table 2.

3. Results

The phytochemical analysis of the extracts of *D. coromandeliana* stems revealed the presence of flavonoids, triterpenoids, steroids, anthocyanin, phenolic compounds and carbohydrates (Table 1). Phytochemical Screening shows presence of carbohydrate in all five extracts while triterpenoids are positive in hexane, chloroform & ethylacetate extract. Steroids are present strongly in hexane and chloroform extract while marginally in aqueous extract. These are not found in ethyl acetate and ethanolic extract. Phenolic compounds, tannins, flavonoids and anthocyanins are positive in all five extracts. The concentration of secondary metabolites varies amongst the extracts evaluated. The presence of these components in this species shows that it may have some medicinal potential. This is probably due to the fact that each of the components identified has record of one therapeutic usage or another.

The antimicrobial activity (Table 2) of the hexane, chloroform, ethyl acetate, ethanol and aqueous extract of stems of *D. coromandeliana* showed concentration-dependent activity against all the tested bacteria with the zone of inhibition ranged from 10-24 mm at various

Table 2

Zone of inhibition (Mean±SEM) (mm) of various extracts of the stems of *D. coromandeliana*

Sl. No	Extract	Conc.(μg / mL)	*S. aureus*	*S. pyogenes*	*P. aeruginosa*	*E. coli*
1	Hexane	100	12.00±0.01	14.03±0.23	09.10±0.12	14.02±0.09
		500	08.20±0.12	11.05±0.17	05.04±0.05	10.03±0.12
		25	06.20±0.20	08.00±0.12	–	06.02±0.17
2	Chloroform	100	16.03±0.12	18.04±0.05	16.05±0.17	18.20±0.12
		500	10.02±0.09	12.10±0.12	11.03±0.23	16.00±0.01
		25	06.09±0.01	08.20±0.03	05.06±0.10	10.02±0.05
3	Ethyl acetate	100	15.08±0.03	16.01±0.09	14.80±0.06	18.09±0.02
		500	07.02±0.14	11.07±0.08	10.00±0.11	12.02±0.01
		25	02.08±0.06	08.00±0.01	06.02±0.01	05.08±0.12
4	Ethanol	100	10.06±0.01	12.06±0.03	09.05±0.25	12.00±0.00
		500	04.01±0.23	10.04±0.04	04.02±0.01	06.05±0.03
		25	–	04.05±0.03	–	–
5	Aqueous	100	11.70±0.23	14.05±0.03	08.00±0.01	16.20±0.15
		500	06.01±0.12	08.03±0.16	04.08±0.23	12.14±0.03
		25	–	06.01±0.08	–	07.05±0.08
6	Amikacin(10 μg / mL)		18.01±0.12	20±0.01	18.01±0.16	22.04±0.01

concentrations. Chloroform extract showed more antimicrobial activity against the gram-negative bacteria than the hexane extract. The zone of inhibition recorded was ranged from 5-18 mm against gram-negative bacteria. The solvents used for extraction were used as control and all the solvent control did not show any activity. Standard antibiotics were also used along with the extracts for comparison as given in the Table 2. Chloroform and ethyl acetate extract showed the maximum zone of inhibition ranged from 16 to 18 mm against the bacterial strains at 100 mg/ml concentration.

4. Discussion

Drug resistance in human pathogenic microorganisms has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. This condition has forced scientists to search for new antimicrobial substances from various sources. *In vitro* evaluation of plants for antimicrobial property is the first step towards achieving the goal for developing eco-friendly management of infectious diseases of humans by search for new biomolecules of plant origin. Considering these, the stems of *D. coromandeliana* was screened *in vitro* for antibacterial activity against four human pathogenic bacteria. On the basis of zone of inhibition, the result of the present investigation revealed that the plant is active against both gram-negative bacteria and gram-positive bacteria. In case of solution with low activity, a large concentration or volume is needed. In general gram-positive bacteria are considered more sensitive than gram-negative bacteria towards different antimicrobial compounds because of the difference in the structure of their cell walls[18-32] but the present result showed that the extracts are effective against both gram-positive and gram-negative bacteria. Antimicrobial properties of substances are desirable tools in the control of harmful microorganisms especially in the treatment of infectious diseases and in food spoilage. The active components usually interfere with growth and metabolism of microorganisms and prevent them from contamination[33].

The solvent extracts of stem showed presence of many phytochemicals. The presence of such phytochemicals may be correlated with the fact that solvent extracts showed maximum activity against the bacterial strains. Several phenolic compounds like tannins found in plant cells are potent inhibitors of hydrolytic enzymes used by plant pathogens. These bioactive components of the plants which are naturally occurring in most plant materials are known to be bactericidal, pesticidal and fungicidal in nature thus conferring the antimicrobial property of this plant. These phytochemicals like phenolic compounds (tannins) present in the extract of these species are potent inhibitor of microbial growth.

The presence of bioactive substances have been reported to confer resistance to plants against bacteria, fungi and pests and therefore explains the antibacterial activity of plant extracts. Many plants release phenolic compounds that are toxic to microbial pathogens[34]. Flavonoids on the other hand are potent water soluble antioxidants and free radical scavengers which prevent oxidative cell damage and have strong anticancer activity[35]. Hence the compound detected may be responsible for antimicrobial activity of the plant extracts.

From the results of antimicrobial activity, it was found that the chloroform and ethyl acetate extracts exhibited maximum antimicrobial activity against the tested human pathogens. It might be attributed to the presence of secondary metabolites such as flavonoids, phenolic groups and steroids as suggested by previous reports[36],[37],[38].

Further research is necessary to determine the identity of the therapeutic compounds within these plants and also to determine their full spectrum of efficacy. However, the present study may serve as primary platform for further phytochemical and pharmacological studies.

Conflict of interest statement

We declare that we have no conflict of interest.

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