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PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY OF *DALBERGIA PANICULATA* ROXB.

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ABSTRACT: Methanolic extracts of leaf, bark, stem and root of *D. paniculata* were screened for phytochemicals and antibacterial activity against ten different pathogens. Various phytochemicals such as terpenoids, flavonoids, coumarins, saponins and tannins were found to be present. Leaf and bark extract exhibited maximum antibacterial activity against *S. aureus*, *P. vulgaris* and *E. coli* with MIC value of 2.5 mgmL-1. Significant antibacterial activity was shown by all extracts with MIC ranging from 10 mgmL-1 to 2.5 mgmL-1. Further isolation and characterization of phytochemicals could result in development of drugs against tested bacteria.

INTRODUCTION: In recent times, more emphasis is given to herbal medicine as it has become an integral part of primary health care system [1] and has gained importance as an alternative to synthetic chemical drugs which are associated with adverse side effects. Medicinal plant, as a whole or any of its parts can be used for therapeutic purpose or as precursors for pharmaceuticals [2]. Hence secondary metabolites in the plants have been extensively investigated for their medicinal and pharmaceutical properties.

Plants have been widely screened for phytochemicals like carbohydrates, alkaloids, esters, aldehydes, oils, steroids and ketones [3].

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These secondary metabolites are responsible for various biological activities viz. antioxidant, antimicrobial, antiviral, antidiabetic, anti-inflammatory and anticancerous activities of plants. Antibiotics are the mainstay of bacterial infections' treatment [4]. Indiscriminate use of antibiotics and chemical drugs has led to increase in the incidence of multi drug resistant bacteria [5]. Therefore, lot of attention has been given to natural products derived mainly from plants, with antimicrobial potential and novel treatment efficacy. In view of this, the present study was aimed at preliminary screening of phytochemicals present and evaluation of antibacterial activity of *D. paniculata*.

Dalbergia paniculata is a tree belonging to the family Fabaceae. The genus *Dalbergia* is known for timber yielding trees. However many species are proven to have medicinal uses. *D. paniculata* possesses antimicrobial, oestrogenic and insecticidal properties [6-8]. Plant was found to have potent antioxidant and anti-inflammatory activities [9]. A qualitative analysis has been carried out to determine different chemical constituents of

D. paniculata [10]. A number of steroids and flavonoids have been isolated from plant [11-15]. Considering the various biological activities of *D. paniculata*, the present work was focused on detailed phytochemical profiling and evaluating antibacterial activity of methanolic extracts of leaf, bark, stem and root against pathogenic bacteria.

MATERIALS AND METHODS:

Plant materials:

Plant samples were collected from Devarayanadurga in Tumkur district, Karnataka, in the month of September, 2013 and were identified as *Dalbergia paniculata* Roxb. Further, the plant was authenticated by National Ayurveda Dietetics Research Institute, Bangalore with voucher specimen number RRCBI-3748 and the same was maintained in the herbarium of the institute.

Extraction:

Leaves, bark, stem and root of *D. paniculata* were shade dried and powdered using pulverizer and stored in air tight containers. Each sample was subjected to exhaustive extraction in soxhlet apparatus using methanol as solvent. The methanolic extracts were then concentrated under vacuum in rotary evaporator. The solvent free methanolic extracts were then stored at 4 ˚C for further use.

Preliminary Phytochemical Screening:

Methanolic extracts of leaf, bark, stem and root were screened for the presence of secondary metabolites such as tannins ($FeCl_3$ and lead acetate tests), saponins (foam test), flavonoids (alkaline and lead acetate tests), terpenoids (Salkowski test), alkaloids (Mayer's, Dragendroff's, Hager's and Wagner tests), coumarins (NaOH test), antharaquinones (magnesium acetate test), anthocyanin (Shinoda test), resins (acetone-water test), fixed oils (stain test), phlobatanins (HCl test), gums and mucilages (alcohol precipitation test) by using specific and standard qualitative tests [16-18].

Antibacterial study:

Test microorganisms:

The bacteria used for antibacterial studies were *Salmonella abony* ACM 5080, *Bacillus cereus* NCIM 2155, *Staphylococcus aureus* ATCC 25923, *Klebsiella pneumoniae* NCIM 2957, *Proteus vulgaris* NCIM 2027, *Escherichia coli* NCIM 2931, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus polymyxa* NCIM 2540, *Enterobacter aerogenes* NCIM 2340 and *Enterobacter cloacae* NCIM 2015.

They were obtained from various institutes such as NCIM (National Collection of Industrial Microorganisms, Pune, India), ACM (Australian Collection of Microorganism, Queensland, Australia) and ATCC (American Type Culture Collection, VA, USA). The bacterial strains were maintained on nutrient agar and subcultured at regular time intervals.

Minimum Inhibitory Concentration (MIC) assay:

Minimum inhibitory concentration (MIC) is defined as the lowest concentration that inhibits the growth of a test organism over a defined interval related to the organism's growth rate, most commonly 18-24 h [19]. The MIC was determined by broth microdilution method. Different aliquots of extracts were added aseptically to MH broth to prepare concentrations ranging from 10 to 0.31 $mgmL^{-1}$. Similarly Gentamicin was used as a positive control.

The assay was performed in 96-well micro titer plates [20] by filling wells with 150 µL of sterile MH broth having extracts of different concentrations. Two wells were used as sterility and growth control respectively with the sterility control containing only MH broth, whilst the growth control containing both MH broth as well as test organism. After adding 100 µL of the bacterial suspension (105 CFU/mL) to each row (except for the sterility control), the micro titer plate was covered and incubated overnight at 37 °C. To overnight cultured plates, 40 µL of a 0.2 $mgmL^{-1}$ solution of *p*-iodonitrotetrazolium violet (INT) dye was added to each well and the plate was returned to the incubator for at least half an hour to ensure adequate color development. The colourless tetrazolium salt acts as an electron acceptor and is reduced to a red-coloured formazan product by biologically active organisms. Hence pink colour indicates growth while no colour change infers inhibition of growth.

RESULTS:

Phytochemical Screening:

The methanolic extracts of *D. paniculata* showed presence of various secondary metabolites. Leaf and bark extracts were found to have flavonoids, whereas bark, stem and root have terpenoids which are therapeutically important group of chemical compounds. But alkaloids were absent in all the four extracts tested. The results of phytochemical screening are as shown in **Table 1**.

TABLE 1: PHYTOCHEMICAL ANALYSIS OF METHANOLIC EXTRACTS OF *D. PANICULATA*

Phytochemicals	Leaf	Bark	Stem	Root
Tannins	+	+	-	-
Saponins	-	+	-	+
Flavonoids	+	+	-	-
Terpenoids	-	+	+	+
Alkaloids	-	-	-	-
Coumarins	+	-	+	-
Anthocyanin	-	-	-	-
Anthraquinones	-	-	+	+
Resins	-	+	+	-
Phlobatains	-	+	-	-
Fixed oils	-	-	+	-
Gums and mucilages	+	-	+	-

+ present, - absent

Antibacterial activity:

Methanolic extracts of *D. paniculata* were tested against ten different bacterial strains including both Gram positive and Gram negative strains. Minimum concentration of extract required to inhibit the microbial growth, Minimum Inhibition Concentrations (MICs) were determined. The MIC value for each organism tested varied significantly ranging from 2.5 to 10 $mgmL^{-1}$ **(Table 2).**

Methanolic extract of bark was found to be more potent inhibiting *S. aureus, P. vulgaris, E. coli* and *P. aeruginosa* with MIC value 2.5 $mgmL^{-1}$. Leaf extract inhibited growth of *P. vulgaris, E. coli* along with *B. cereus* at a minimal concentration of 2.5 $mgmL^{-1}$ and *Salmonella abony* was inhibited by leaf and bark extracts.

Most of the extracts found to have MIC value of 10 $mgmL^{-1}$ as they showed potent inhibitory activity against all the test organisms except *Enterobacter cloacae*, which was not inhibited by any of the extracts.

TABLE 2: MINIMUM INHIBITORY CONCENTRATIONS OF *D. PANICULATA*.

Sample	Conc.	*S. abony*	*B. cereus*	*S. aureus*	*K. pneumoniae*	*P. vulgaris*	*E. coli*	*P. aeruginosa*	*B. polymyxa*	*E. aerogenes*	*E. cloacae*
Leaf	10	+	+	+	+	+	+	+	+	+	-
	5	+	+	+	-	+	+	+	-	+	-
	2.5	-	+	-	-	+	+	-	-	-	-
	1.25	-	-	-	-	-	-	-	-	-	-
	0.62	-	-	-	-	-	-	-	-	-	-
	0.31	-	-	-	-	-	-	-	-	-	-
Bark	10	+	+	+	+	+	+	+	+	+	-
	5	+	+	+	-	+	+	+	+	-	-
	2.5	-	-	+	-	+	+	+	-	-	-
	1.25	-	-	-	-	-	-	-	-	-	-
	0.62	-	-	-	-	-	-	-	-	-	-
	0.31	-	-	-	-	-	-	-	-	-	-
Stem	10	-	+	+	+	+	+	-	+	-	-
	5	-	-	+	-	-	-	-	-	-	-
	2.5	-	-	-	-	-	-	-	-	-	-
	1.25	-	-	-	-	-	-	-	-	-	-
	0.62	-	-	-	-	-	-	-	-	-	-
	0.31	-	-	-	-	-	-	-	-	-	-
Root	10	-	-	+	-	-	+	+	+	-	-
	5	-	-	-	-	-	-	-	-	-	-
	2.5	-	-	-	-	-	-	-	-	-	-
	1.25	-	-	-	-	-	-	-	-	-	-
	0.62	-	-	-	-	-	-	-	-	-	-
	0.31	-	-	-	-	-	-	-	-	-	-
Gent	10	+	+	+	+	+	+	+	+	+	+
	5	+	+	+	+	+	+	+	+	+	+
	2.5	+	+	+	+	+	+	+	+	+	+
	1.25	+	+	+	+	+	+	+	-	+	+
	0.62	+	+	+	+	+	+	+	-	+	+
	0.31	+	+	+	+	-	+	+	-	-	-

n=9, + inhibition, - no inhibition, Gent- Gentamicin, Conc- concentration in $mgmL^{-1}$

DISCUSSION: Phytochemicals identified from traditional medicinal plants are presenting an exciting opportunity for the development of new types of therapeutics. In view of this the present study on D. paniculata seems to be very significant as methanolic extracts of the plant showed greater potential as they proved to have different phytochemicals.

Preliminary phytochemical screening of D. paniculata revealed the presence of tannins, saponins, terpenoids, steroids, coumarins and anthraquinones. The plants belonging to genus Dalbergia are known to contain a number of flavonoids and their derivatives 15. Leaf and bark extracts showed presence of flavonoids and they contribute to antioxidant and antimicrobial activity of the plant 21, 22. Terpenoids are the most important phytochemicals currently known as they confer antimicrobial, insecticidal, wound and scar healing property to the plants 23. D. paniculata was found to have terpenoids, hence the plant has potential to be used as an antimicrobial agent.

The presence of tannins shows that the D. paniculata can be used as purgative in the treatment of cough, asthma and hay fever 24. Previous study on whole plant alcoholic extract of D. paniculata was reported to have phytosterols, triterpenoids, glycosides, flavonoids and tannins 10. The present study was carried out using different parts of the same plant and revealed the presence of terpenoids, flavonoids and tannins. But in contrary to earlier work, saponins were found to be present in bark and root extracts. However in both the studies alkaloids were found to be absent.

The antimicrobial screening of traditional medicinal plants has been the source of innumerable therapeutic agents. The reason being that large number of antimicrobial agents derived from medicinal plants are readily available for treating various diseases caused by microorganisms 25. Recent reports have shown that there is reduction in the discovery of new antimicrobial agents globally, coupled with alarming cases of resistance to available antimicrobials. Minimum inhibitory concentration (MIC) is the minimal concentration of extract required to inhibit the growth of the organism and it is used as an index for measuring the efficacy of antibacterial agents 26.

The leaf and bark extracts of D. paniculata presented a better inhibitory effect on test bacteria than stem and root extracts. This could be attributed to the presence of different phytochemicals in different parts present in varying concentrations. Though antimicrobial activity of oil from seeds of D. paniculata has been carried out 27, it has been the first instance where the crude extracts have been tested for antibacterial property. The results have been promising as leaf and bark extracts inhibited growth of pathogenic bacteria like S. aureus, E. coli, P. vulgaris and with a MIC value as low as 2.5 mgmL-1.

The results strongly recommend further analysis of extracts and isolation of specific compounds which could be used as antibacterial against the above mentioned organisms. The MIC illustrates a decreasing inhibitory effect of the extracts as the concentration decreased. This implies that the antimicrobial activity of extracts was concentration dependent.

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