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RESEARCH ARTICLE

PRELIMINARY STUDIES ON *IN VITRO* ANTIBACTERIAL ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF AQUEOUS CRUDE EXTRACT OF *Shorea robusta* FLORAL PARTS

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ABSTRACT

The aqueous extract of floral parts of *Shorea robusta* (Dipterocarpaceae) was prepared with cold water maceration. Well diffusion method was employed to determine the effect of antibacterial potential against Gram positive bacteria viz. *Staphylococcus aureus* and *Bacillus subtilis* and *G*ram negative bacteria viz. *Klebsiella pneumoniae* and *Serratia marcescens.* Aqueous extract of the plant has showed significant inhibitory activity on different bacterial species tested against penicillin as standard antibacterial agent. Furthermore, the preliminary phytochemical analysis revealed that the aqueous extract found to possess tannins, flavanoids, cardiac glycosides and steroids, which may involve in showing antibacterial activity .

INTRODUCTION

Naturally occurring antibacterial compounds can be derived from plants, animal tissues, or microorganisms (Jordon and David, 2001). Due to the side effects of the present day antimicrobial compounds and emerging antibiotic resistance, the need for developing the newer antimicrobial compounds has been gaining momentum. The ethnomedicinal plants have been screened for newer compounds with potent activity (Cordell, 1993) all over the world. The antimicrobial activity of diethyl ether extract of *Cassia auriculata* and *Emblica fischeri* showed better promising results in controlling the bacterial growth (Sekar, 2010). The antibacterial activity of aqueous and organic extracts of *Thymus capitatus* L. (Lamiaceae) leaves and stems (Haitham *et al.,* 2009). It has been reported that the ethanol and methanol extracts of *Aloe vera* gel showed higher activity while acetone extract, showed least or no activity against most of the tested pathogens (Rubina *et al.,* 2009). Organic solvent leaf extracts of *Eucalyptus* have great potential as antimicrobial agents in the treatment of infectious organisms (Mumtaz *et al.,* 2011). *Albizia lebbeck*, *Cleistanthus collinus, Emblica officinalis*, *Eucalyptus deglupta*, *Eupatorium odoratum*, *Oxalis corniculata*, *Hevea brasiliensis*, and *Lantana camara* showed profound antimicrobial activity and even active against multi-drug resistant *Escherichia coli* and *Staphylococcus aureus* (Maji *et al.,* 2010).

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Five medicinal plant species, *Abrus precatorius* (Fabaceae), *Amaranthus spinosus* (Amaranthaceae), *Argyeria nervosa* (Convolvulaceae), *Vernonia cinerea* (Asteraceae), *Zizyphus nummularia* (Rhamnaceae) were screened for potential of antibacterial activity against four medically important human pathogens (Ashok and Sujata, 2011). The petroleum ether extract of *Digera muricata* (L.) Mart. (Amaranthaceae) showed inhibition against *V. cholerae* (Pratima and Sundar, 2010). The butanolic extract of *Cyanodon dactylon* was more active against most of the organisms tested (Yogesh, 2011). Methanol extract of *Medicago sativa* showed significant inhibitory activity against all the tested bacteria followed by chloroform and ethanol extracts (Doss *et al.,* 2011). *Shorea robusta* belongs to family Dipterocarpaceae is reported to possess antimicrobial properties for its resin (Alluri *et al.,* 2005), and being used in ulcers (Narayan *et al.,* 2004) and found to show anti-aging and wound healing activity (Sharma *et al.,* 2009). However no reports are available on antibacterial activity of *Shorea robusta* floral parts. Therefore, the present study has been undertaken to investigate the antibacterial activity against Gram negative and Gram positive bacteria.

MATERIALS AND METHODS

Plant material: *Shorea robusta* flowers were collected from Kharagpur, West Bengal. The collected flowers were air-dried then separated the petals and gynoecia and the crude aqueous extract was prepared at an approximate concentration of 1g/10ml for preliminary investigations.

Microorganisms: The bacteria used in this study were Gram positive bacteria *Staphylococcus aureus* (NCIM3021), *Bacillus subtilis* (NCIM2063) and Gram negative bacteria *Klebsiella pneumoniae* (NCIM2957) *and Serratia marcescens* (NCIM2396), which were obtained from National Chemical Laboratory (NCL), Pune.

Determination of antibacterial activity by well diffusion assay: Antibacterial tests were carried out by well diffusion method (Kaladhar *et al.,* 2010). The density of the bacterial suspension was standardized by using Mac Farland standard method. The 6 mm wells on inoculated agar plates were filled with 40 µl of the aqueous extract at the concentration of 4mg/well. Penicillin was used (4mg/well) as positive reference standard. The agar plates were incubated at 37^0C for 24 hours. In the present study, the antibacterial activity aqueous extract of *Shorea robusta* floral parts was examined against Gram negative and Gram positive bacteria tested and their inhibitory activity was quantitatively assessed by the presence or absence of inhibition zones and zone diameters.

Phytochemical Analysis

(a) Test for Tannins: About 0.5gm of dried powder sample was boiled in 20ml of water in a test tube and then filtered a few drops of 0.1% ferric chloride was added and observed for brown green or blue black coloration

(b) Test for Phlobatannins: Deposition of a red precipitate when an aqueous extract of each plant sample was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of Phlobatannins.

(c) Test for Saponins: About 2g of the powdered sample was boiled in 20ml of distilled water in a water bath and filtered.10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

(d) Test for Flavonoids: 5ml of dilute ammonia solution were added to a portion of the aqueous filtrate of plant extract followed by addition of concentrated sulphuric acid. A yellow coloration was observed in the extract indicating the presence of Flavonoids. The yellow coloration disappeared on standing.

(e) Test for Steroids: 2ml of acetic anhydride was added to 0.5g ethanolic extract of sample with 2ml sulphuric acid. The colour changed from violet to blue or green colour indicating the presence of steroids.

(f) Test for Terpenoids: 5ml of each extract was mixed in 2ml of chloroform and concentrated H2SO4 (3ml) was carefully added to form a layer. A reddish brown coloration of the interface was formed to show positive results for the presence of Terpenoids.

(g) Test for Cardiac Glycosides: 5ml of extract was treated with 2ml of glacial acetic acid containing 1drop of ferric chloride solution. This was underplayed with 1ml of concentrated H_2SO_4. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

RESULTS AND DISCUSSION

Well diffusion method was employed to determine the effect of antibacterial activity against Gram positive bacteria i.e., *Staphylococcus aureus, Bacillus subtilis,* and *G*ram negative bacteria i.e., *Klebsiella pneumonia* and *Serratia marcescens.* The aqueous extract of the floral parts of *S. robusta* showed significant inhibitory activity on different bacterial species tested as against standard antibiotic i.e. Penicillin. (Table 1)**.** In the floral parts tested, it was found that the extract of gynoecia showed comparatively good inhibitory activity than that of the petal extract.

Table 1. Antimicrobial activity of aqueous extract of floral parts of the *Shorea robusta* against the Gram positive and Gram negative bacteria by well diffusion method (6mm well size)

Bacterial species	Type of bacteria	Antibiotic Zone of inhibition (in mm)	Floral parts Zone of inhibition (in mm)	
			Petals	Gynoecia
S.aureus	Gram +ve	Penicillin-13	12	14
B. subtilis		Penicillin-13	12	13
K.pneumoniae	Gram -ve	Penicillin -12	11	15
S.marcescens		Penicillin-13	12	14

Table 2. Preliminary phytochemical analysis of floral parts of *Shorea robusta* types of compound

Type of compound	Result
Tannins	Positive
Phlobatannins	Negative
Saponins	Negative
Flavonoids	Positive
Steroids	Positive
Terpenoids	Negative
Cardiac glycosides	positive

Phytochemical analysis: The phytochemical analysis of aqueous extract of floral parts of *S. robusta* was carried by biochemical tests as shown in Table 2**.** The preliminary phytochemical analysis revealed that the aqueous extract found to possess tannins, flavanoids, steroids and cardiac glycosides which may involve in showing antibacterial activity. While phlobatannins, saponins and terpenoids were found to be absent in the floral parts.

Conclusion

An aqueous extract of *S. robusta* floral parts viz. gynoecia and petals has shown good inhibitory activity in the control of both Gram positive as well as Gram negative bacteria. The activity may be due to the presence of tannins, flavanoids, steroids and cardiac glycosides. Further isolation and analysis has to be conducted on floral parts of *S. robusta* for for further characterization.

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