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## ANTIBACTERIAL ACTIVITY OF METHANOLIC SEED COAT EXTRACT OF *BORASSUS FLABELLIFER* L.

Govinda Rao Duddukuri\*, Y. Nagendra Sastry, D.S.V.G.K. Kaladhar, K. Kamalakara Rao and K. Krishna Chaitanya

Department of Biochemistry & Bioinformatics, GITAM University, Visakhapatnam-530 045, Andhra Pradesh, India

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### Correspondence to Author:

Dr. D. Govinda Rao

Associate Professor, Department of  
Biochemistry, GITAM Institute of Science,  
GITAM University, Visakhapatnam – 530  
045, Andhra Pradesh, India

### ABSTRACT

The antibacterial activity of methanol extract of *Borassus flabellifer* L. (Arecaceae) seed coat (soft outer shell) was studied by agar well diffusion method *in vitro*. The tender seed coat of *Borassus flabellifer* was extracted with methanol. The effect of antibacterial potential was examined against Gram positive bacteria i.e., *Staphylococcus aureus*, *Bacillus subtilis* and Gram negative bacteria i.e., *Klebsiella pneumoniae* and *Serratia marcescens*. The methanol extract of the seed coat has showed consistently significant inhibitory activity on different bacterial species tested. Furthermore, the minimum inhibitory concentration studies carried out by broth dilution assay and found the MIC ranged between 100µg to 1 mg/ml implying the significance of antibacterial activity of *Borassus flabellifer*.

**INTRODUCTION:** 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 provide an immense scope to explore novel antimicrobial compounds<sup>1</sup> all over the world.

The methanol extract of *Terminalia bellerica* was more effective than crude extract against most of the microbes tested except *E. coli* (enteropathogen) and *P. aeruginosa*<sup>2</sup>. 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<sup>3</sup>. The antibacterial activity is exhibited by aqueous and organic extracts of *Thymus capitatus* L. (Lamiaceae) leaves and stems<sup>4</sup>. The antimicrobial activity of diethyl ether extract of *Cassia auriculata* and *Emblica fischeri* showed better promising results in controlling the bacterial growth<sup>5</sup>.

The petroleum ether extract of *Digera muricata* (L.) Mart. (Amaranthaceae) showed inhibition against *V. cholera*<sup>6</sup>. The maximum inhibitory activity of *Cassia auriculata* flowers was observed against all organisms except *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*<sup>7</sup>. Organic solvent leaf extracts of *Eucalyptus* have great potential as antimicrobial agents in the treatment of infectious organisms<sup>8</sup>.

The butanolic extract of *Cyanodon dactylon* is reported to be more active against most of the organisms tested<sup>9</sup>. Methanol extract of *Medicago sativa* showed significant inhibitory activity against all the tested bacteria followed by chloroform and ethanol extracts<sup>10</sup>. The different parts of the *Borassus flabellifer* are being used for medicinal properties viz. Male flowers are used for anti-inflammatory activity<sup>11</sup>, the juice from flowering stalks used for diabetes<sup>12</sup>. Oral feeding of mice with palmyrah flour induced the generation of T suppressor cells which were able to suppress the DTH response to SRBC<sup>13</sup>. The plant has been used in folklore.

For example, decoction used for gonorrhoea and respiratory ailments, roots, leaves and flowering stalks for restorative, antihelmintic and diuretic properties, leaf juice used for hiccups, gastric ailments, the sap is laxative<sup>14</sup>.

Reports are not available on antimicrobial activity of the seed coat. Therefore, the present study has been undertaken to investigate the antimicrobial activity against Gram negative and Gram positive bacteria and selected fungal species.

## MATERIAL AND METHODS:

### Plant material and preparation of Plant Extract:

*Borassus flabellifer* tender seeds locally termed as 'Thati munjelu' were obtained from local market in the summer season from Visakhapatnam, Andhra Pradesh.

Tender seed coat of *Borassus flabellifer* is removed and air dried then ground into powder which was dissolved in methanol so as to make 40% methanol extract. The extract is kept in orbital shaking incubator for 3 days and then centrifuged to remove the debris. Finally clear methanol extract was collected and then the solvent is removed by using rotavapour to get the dried powder of methanol dissolved components of the seed coat of the *Borassus flabellifer*. The dried powder appropriately dissolved in methanol and tested for antibacterial activity.

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

### Antibacterial activity by Agar Well Diffusion Method:

The bacteria were grown in Muller-Hinton media (HiMedia Pvt. Ltd., Mumbai, India) at 37°C and maintained on nutrient agar slants at 4°C and stored at -20°C. Inoculum of test organisms was prepared by growing pure isolate in nutrient broth at 37°C for overnight. The overnight broth cultures was sub-cultured in fresh nutrient broth and grown for 3hrs to obtain log phase culture. The agar plates were prepared by pour plate method using 20ml M-H medium. The sterile M-H agar medium is cooled to

45°C and mixed thoroughly with 1ml of growth culture of concerned test organism ( $1 \times 10^8$  cells) and then poured into the sterile petri dishes and allowed to solidify. Wells of 6 mm size were made with sterile cork borer and test extracts were added. The agar plates were incubated at 37°C for 24hrs. The diameter of zones of inhibition was measured in mm using HiMedia zone reader<sup>15</sup>.

### Determination the MIC of the Methanol Extract by Broth Dilution Assay:

The minimum inhibitory concentration of the methanol extract was determined using broth dilution assay<sup>16</sup>. The medium containing different concentrations of methanol extract of seed coat of *Borassus flabellifer* viz., 10, 1, 0.1, 0.01, 0.001 mg/ml prepared by serial dilution. After inoculation, the tubes were incubated for 24 hours at 37°C. The MIC of each sample was determined by measuring the optical density in the spectrophotometer at 620 nm and compared the result with those of the non-inoculated broth.

**RESULTS AND DISCUSSION:** The antibacterial activity of methanol extract of *Borassus flabellifer* seed coat was determined against *B. subtilis* and *S.aureus* and *K. pneumoniae*, *S. marcescens*. The methanol extract has showed consistently significant antibacterial activity when compared to penicillin. The significance of antibacterial activity is assessed by determining minimum inhibitory concentration (MIC) required to inhibit the bacterial growth.

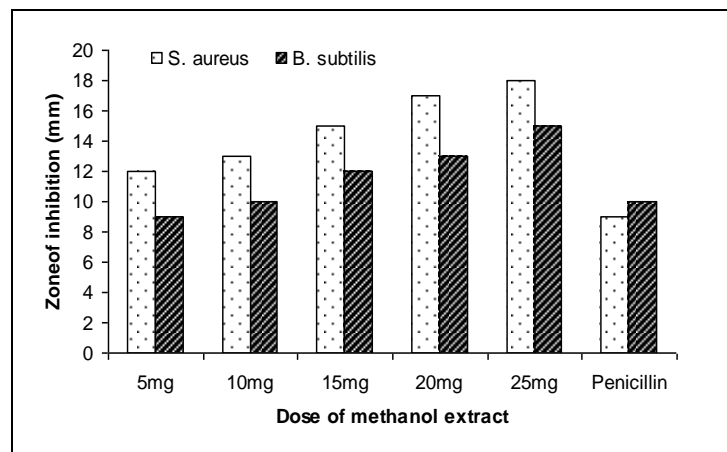
### Inhibitory effect of Methanol Extract of *Borassus flabellifer* seed coat on Gram Positive Bacteria:

As shown in Fig 1., the antibacterial activity of methanol extract was found to be very significant for *Staphylococcus aureus* as the highest zone of inhibition (18mm) was observed with 25mg dose while methanol extract exhibited significant antibacterial activity consistently on *Bacillus subtilis* as the highest zone of inhibition was found to be 15mm at the 25mg concentration of the test extract. The zone of inhibition was compared with 5mg of penicillin. The zone of inhibition is in accordance with increase in the concentration of the test extract.

### Inhibitory effect of Methanol Extract of *Borassus flabellifer* seed coat on Gram Negative Bacteria:

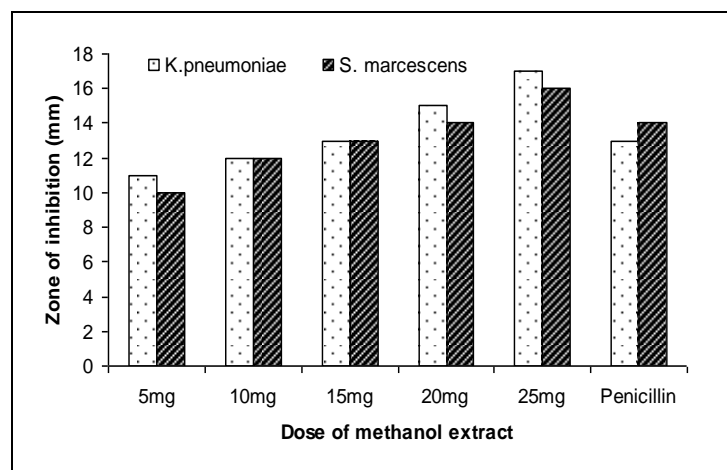
The seed coat of *Borassus flabellifer* exhibited significant

inhibitory activity against Gram negative bacteria such as *Klebsiella pneumoniae* and *Serratia marcescens*, (Fig 2.) tested. The highest zone of inhibition (17mm for *Klebsiella* and 16mm for *Serratia*) was observed with 25mg for both bacteria tested. However, the inhibitory activity is not as significant as with Gram positive bacteria.



**FIG. 1: DOSE-DEPENDENT INHIBITORY EFFECT OF METHANOL EXTRACT OF *BORASSUS FLABELLIFER* SEED COAT ON GRAM POSITIVE BACTERIA**

Indicates significant inhibitory activity of methanol extract of *Borassus flabellifer* on Gram positive bacteria viz. *S. aureus* and *B. subtilis* compared with penicillin (5 mg/ml) in a dose dependent manner



**FIG. 2: INHIBITORY EFFECT OF SOLVENT EXTRACTS OF *BORASSUS FLABELLIFER* SEED COAT ON GRAM NEGATIVE BACTERIA**

Indicates significant inhibitory activity of methanol extract of *Borassus flabellifer* on Gram negative viz. *K. pneumoniae* and *S. marcescens* compared with penicillin (5mg/ml) in a dose dependent manner

**Determination of Minimum Inhibitory Concentration (MIC):** Minimum inhibitory concentration of methanol extract of seed coat was determined against different Gram positive bacteria like *S. aureus* and *B. subtilis*

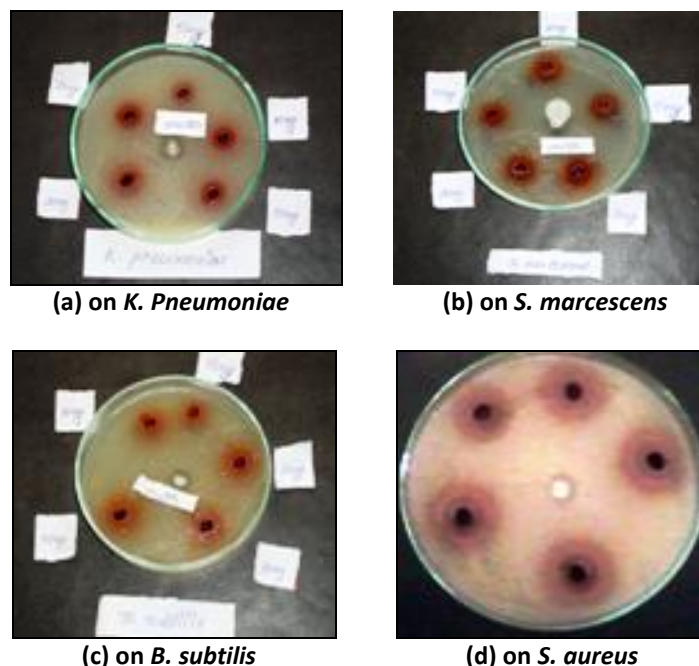
and Gram negative bacterial species like *K. pneumoniae* and *S. marcescens*.

As shown in **Table 1**, the minimum concentration of extract required to inhibit the bacterial growth varies between 0.1 and 1.0 mg methanol extract. The lowest MIC was observed to be 0.1mg/ml with *B. subtilis*, while for *S. aureus*, *K. pneumoniae*, and *S. marcescens*, the MIC was found to be 1.0 mg/ml (**Table 1 & Fig 3**). This observation implies that the presence of potent antibacterial compounds in methanol extract of *B. flabellifer*.

**TABLE 1: MINIMUM INHIBITORY CONCENTRATION (MIC) OF METHANOL EXTRACT OF TENDER SEED COAT OF *BORASSUS FLABELLIFER* FOR ANTIBACTERIAL ACTIVITY**

Bacterial species	MIC (mg/ml)
<i>B. subtilis</i>	0.1
<i>S. aureus</i>	1.0
<i>K. Pneumoniae</i>	1.0
<i>S. marcescens</i>	1.0

Indicates the significance of inhibitory activity of methanolic extract of *Borassus flabellifer* seed coat on different bacterial species, as MIC ranged between 0.1 to 1 mg/ml



**FIG. 3: INHIBITORY EFFECT OF METHANOLIC EXTRACT OF *BORASSUS FLABELLIFER* SEED COAT ON DIFFERENT BACTERIA**

Dose effect of methanolic extract of *Borassus flabellifer* on different bacterial species

**CONCLUSION:** The above results confirm that the methanol extract of seed coat of *Borassus flabellifer* exhibit significant and consistent antibacterial activity with relatively lower MIC values indicating to

undertake further fractionation analysis to isolate the antibacterial compound of therapeutic importance.

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