

***In vitro* Antibacterial Activity of Organic Solvent Seed Coat Extracts of *Borassus flabellifer* Linn**

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ABSTRACT

The *in vitro* antibacterial activity of organic solvent extracts of *Borassus flabellifer* L. (Arecaceae) seed coat (soft outer shell) was studied by agar well diffusion method. The tender seed coat of the plant *Borassus flabellifer* was extracted with different organic solvents viz. petroleum ether, acetone, ethyl acetate, ethanol and methanol. The effect of antibacterial potential was examined against Gram positive bacteria i.e., *Staphylococcus aureus*, *Bacillus subtilis* and Gram negative bacteria i.e., *Escherichia coli*, *Klebsiella pneumoniae*. Among the different organic solvent extracts of the seed coat tested, methanol extract has showed consistently significant inhibitory activity on different bacterial species. Furthermore, the minimum inhibitory concentration studies carried out by broth dilution assay and found the MIC ranged between 3.12 to 6.25 mg/ml.

Key words: *Borassus flabellifer*, Seed coat, Antibacterial assay, MIC.

INTRODUCTION

Naturally occurring antibacterial compounds can be derived from plants, animal tissues, or microorganisms¹. 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 activity² all over the world.

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 pathogens³. The antibacterial activity of aqueous and organic extracts of *Thymus capitatus* L. (Lamiaceae) leaves and stems⁴. Organic solvent leaf extracts of *Eucalyptus* have great potential as antimicrobial agents in the

treatment of infectious organisms⁵. The antimicrobial activity of diethyl ether extract of *Cassia auriculata* and *Embllica fischeri* showed better promising results in controlling the bacterial growth⁶. *Albizia lebbek*, *Cleistanthus collinus*, *Embllica 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*⁷. Five medicinal plant species, *Abrus precatorius* (Fabaceae), *Amaranthus spinosus* (Amaranthaceae), *Argyria nervosa* (Convolvulaceae), *Vernonia cinerea* (Asteraceae), *Zizyphus nummularia* (Rhamnaceae) were screened for potential of antibacterial activity against four medically important human pathogens⁸. The petroleum ether extract of *Digera muricata* (L.) Mart. (Amaranthaceae) showed inhibition against *V. cholerae*⁹. The butanolic extract of *Cyanodon dactylon* was more active against most of the

organisms tested¹⁰. Methanol extract of *Medicago sativa* showed significant inhibitory activity against all the tested bacteria followed by chloroform and ethanol extracts¹¹.

The different parts of the *Borassus flabellifer* are being used for medicinal properties viz. Male flowers are used for anti-inflammatory activity^{12,13}, the juice from flowering stalks used for diabetes¹⁴. Oral feeding of mice with palmyrah flour induced the generation of T suppressor cells which were able to suppress the DTH response to SRBC¹⁵. The plant has been used in folklore. For example, decoction used for gonorrhoea and respiratory ailments, roots, leaves and flowering stalks for restorative, antihelminthic and diuretic properties, leaf juice used for hiccups, gastric ailments, the sap is laxative¹⁶.

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 (1g/10ml) was dissolved in different organic solvents like petroleum ether, acetone, ethyl acetate, ethanol and methanol. These were placed in orbital shaking incubator for 3 days and then centrifuged to remove all the debris. Finally supernatant was collected 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 *Escherichia coli* (NCIM2066), *Klebsiella pneumonia* (NCIM2957),

Proteus vulgaris (NCIM2027) were obtained from 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 were 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 (1×10^8 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 reader¹⁷.

Determination the MIC of the methanol extract by broth dilution assay

The minimum inhibitory concentration of the methanol extract was determined using broth dilution assay¹⁸. The medium containing different concentrations of plant extract viz., 100, 50, 25, 12.5, 6.25, 3.12 1.56 and 0.78 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 comparing the result with those of the non-inoculated broth.

RESULTS AND DISCUSSION

The antibacterial activity of different solvent extracts of *Borassus flabellifer* seed coat was determined against *B. subtilis* and *S. aureus* and *E. coli*, *K. pneumonia*, , *S. marscence*. Among the solvent extracts tested, methanol extract has showed consistently significant activity. The significance of antibacterial activity is assessed by determining minimum inhibitory concentration (MIC) required to inhibit the bacterial growth.

Table 1. Minimum inhibitory concentration (MIC) of methanol extract of tender seed coat of *Borassus flabellifer* for antibacterial activity

S.No.	Bacterial species	MIC (mg/ml)
1	<i>B.subtilis</i>	6.25
2	<i>S. aureus</i>	3.12
3	<i>E.coli</i>	6.25
4	<i>K.Pneumoniae</i>	6.25
5	<i>S. marcescens</i>	6.25

Inhibitory effect of different solvent extracts of *Borassus flabellifer* seed coat on Gram positive bacteria.

As shown in Fig 1., the antibacterial activity of ethyl acetate extract was found to be very significant for *B. subtilis* as the highest zone of inhibition was observed while methanol extract exhibited significant antibacterial activity consistently on *B. subtilis* and *S. aureus*.

Inhibitory effect of different solvent extracts of *Borassus flabellifer* seed coat on Gram negative bacteria

The seed coat of *Borassus flabellifer* exhibited inhibitory activity against Gram negative bacteria such as *E. coli*, *S. marcescence*, *K. pneumonia* (Fig 2.) tested. Moreover, the significant antibacterial activity was found in methanolic extract as compared to other solvent extracts. However, the inhibitory activity is not as significant as with Gram positive bacteria.

Comparative inhibitory activity of methanol extract of tender seed coat of *Borassus flabellifer* on different bacterial species

The methanol extract of seed coat of *Borassus flabellifer* exhibited significant inhibitory activity against all the bacterial species employed. However, the highest zone of inhibition was obtained with *S. aureus* and *S. marcescens* followed by with *E. coli* and *B. subtilis*. The lowest zone of inhibition was obtained with *K. pneumoniae* (Fig 3.)

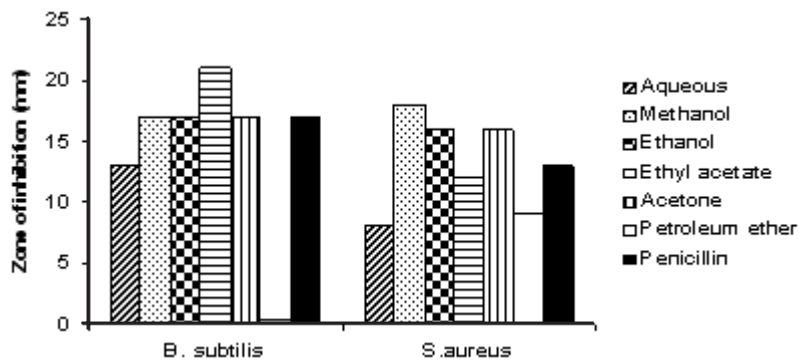


Fig. 1: Inhibitory effect of solvent extracts of *Borassus flabellifer* seed coat on Gram positive bacteria

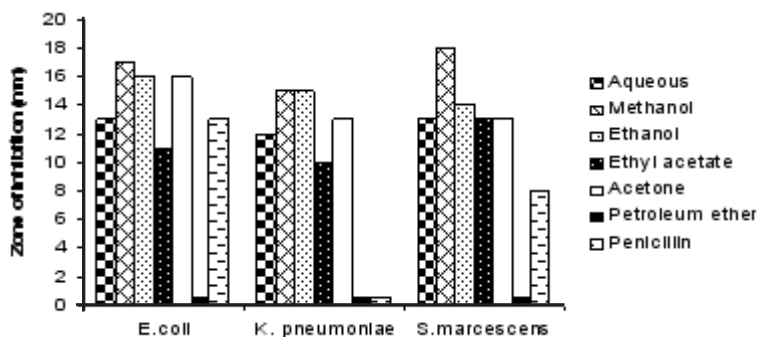


Fig. 2: Inhibitory effect of solvent extracts of *Borassus flabellifer* seed coat on Gram negative bacteria

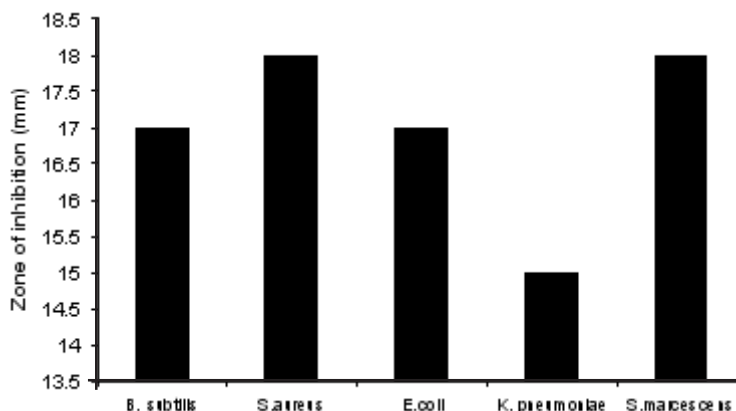


Fig. 3: Inhibitory effect of methanol extract of tender seed coat of *Borassus flabellifer* on different bacterial species

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*, *E. coli* and *S. marcescens*. As shown in Table 1., the minimum concentration of extract required to inhibit the bacterial growth varies between 3.12 and 6.25 mg crude methanol extract against. The lowest MIC was observed with 3.12 mg/ml *S. aureus*, while for *B. subtilis*, *K. pneumoniae*, *E. coli* and *S. marcescens*, the MIC was found to be 6.25mg (Table 1.). This observation implies that the presence of potent antibacterial compounds in methanol extract of *B. flabellifer*.

CONCLUSION

The above results confirm that the seed coat of *Borassus flabellifer* exhibit antibacterial activity. Among the different organic extracts tested, crude methanol extract found to exhibit 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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