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STUDY ON ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF MALE FLOWERS (INFLORESCENCES) OF *BORASSUS FLABELLIFER* LINN

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ABSTRACT

An antimicrobial and antioxidant activity of ethanol male flowers extract of *Borassus flabellifer* was evaluated by using DPPH scavenging and Minimum inhibitory concentration MIC test. For antibacterial activity, the bacterial species such as *Klebsiella pneumoniae, Vibrio cholerae, Shigella dysenteriae, Escherichia coli* and *Staphylococcus aureus* were tested in different concentrations of flower extract. The fungi species *Candida albicans, Aspergillus niger* and *Aspergillus flavus* were tested for antifungal activity. The results obtained for the present study showed that the plant has significant inhibition activity against all the tested bacteria and fungi. Among these, the bacterial species *E. coli* and fungal species *C. albicans* exhibited maximum level of inhibition such as 11mm and 13mm at 1000µg/ml of ethanol extract. From these results, it is concluded that the flower extract proved as a potential antimicrobial agent. The extract showed high antioxidant activity when compared to the standard drug vitamin E. The IC_{50} value of the extract was 213.3µg/ml. However, the extract possesses potential antimicrobial agent and a strong radical scavenger.

KEYWORDS: Borassus flabellifer, Phytochemical, Antibacterial, Antifungal, Antioxidant

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INTRODUCTION

Recent research on medicinal plants has become an intense because of its true values in disease cure and their traditional uses are supported worldwide by peoples due to less side effects and low cost. Nowadays the actual pharmacological effects of these traditional medicinal plants on disease cure and health improvement are universally accepted as an alternative form of health care. To explore the real medicinal value of our traditional knowledge on plants, the screening of medicinal plants for active compounds is very important. Traditional medicine based on plants has played a key role in the health care systems of many countries like India, China etc.[1] Herbal medicine is still the main stay of about 75-80% of the world population. Now a day most of the peoples like to use the traditional methods to cure general diseases.[2] This worldwide interest in medicinal plants is focusing on recognition of the validity of many traditional claims regarding the value of natural products in health care and the development of microbial resistance to the available antibiotics has led the authors to investigate the antimicrobial activity of medicinal plants. Antibacterial constituents of medicinal plants and their use for the treatment of microbial infections as possible alternatives to synthetic drugs to which many infectious microorganisms have become resistant seem to very much promising. Relevant literature on ethno-botanical records suggest that plants are the sleeping giants of pharmaceutical industry.[3] It provides natural source of antimicrobial drugs, novel compounds that may be employed in controlling some infections globally. *Borassus flabellifer* Linn belongs to family Arecaceae, commonly known as Palmyra palm, Asian toddy palm, Sugar palm etc. This plant is a tall tree (palm) growing in sandy soil and attaining height of 20-30m with a straight trunk ringed with leaf scars.[4] It is very fibrous and nutritious, called as Panangkizhangu in Tamil. Flowers and fruits were found during the period of December to August. The male inflorescence constitutes (borassosides and dioscin) spirostane-type steroid saponins.[5] Yielded flabelliferrins, a bitter compound of steroidal saponins. Spirosterol is a dominant aglycone in odiyal flour and palmyra inflorescence. The different parts of the *B.flabellifer* are being used for medicinal properties. Male flowers are used for anti-inflammatory activity.[6] The juice from flowering stalk used for diabetes.[7] Other than these pharmacological uses the juice of the plant is used in preparation of health drinks, jellies etc. The plant has been used traditionally as a stimulant, antileprotic, diuretic, antiphlogistic. The fruits are stomachic, sedative, laxative and aphrodisiac in nature used in hyperdipsia, dyspepsia, flatulence, skin diseases, hemorrhages, fever and general debility.[4] Ash obtained by burning the inflorescence is a good antacid, antiperiodic and heartburn, splenomegaly and in bilious fever. Literature survey revealed that *Borassus flabellifer* Linn have been used as antidiabetic, antidote, anti-inflammatory, woundhealing, anthelmintic activity, analgestic and antipyretic activity. It was also reported that the methanol extract of *Borassus flabellifer* male flower inhibits increase of serum glucose levels in sucrose loaded rats. It is due to spirostane-type steroid saponins. It possess immunosuppresant property and diuretic activity. Due to the presence of these compounds traditionally the plant has been used for treatment of several diseases and used as a remedy for urinary tract infections caused by microorganisms. The study has been carried out to reveal that the male flower consist of several active compounds which possesses antimicrobial activity.

MATERIALS AND METHODS

Plant collection

The male flowers (inflorescences) of *Borassus flabellifer* was collected from Viruthampet (Vellore). The collected flowers were dried under shade at room temperature (25 ± 1^{o}C) for few weeks and thereafter, coarsely powdered by using a blender. This powder was stored in an airtight container until it is utilized for further analysis.

Plant extraction

The plant was extracted by using cold method. About 100g of this *Borassus flabellifer* flower powder was extracted with ethanol (600 ml) in flat bottom glass container, occasional stirring and shaking was done for 3 days to obtain ethanol extract of the flowers respectively. The whole mixture was then filtered through Whatman no.1 filter paper and the obtained filtrate was then transferred to a china dish and kept in a water bath at 50^{o}C to concentrate to dryness. The obtained dried form of extract of *Borassus flabellifer* was transferred to 10ml small glass vial and stored in an air tight container at freezing temperature to avoid contamination until it was analyzed.

Preliminary phytochemical screening

The male flowers of *Borassus flabellifer* were qualitatively screened to determine the presence of active compounds which are responsible for the various activities related to health care. Phytochemical constituents such as phenolic compounds, carbohydrates, flavones, saponins, alkaloids, quinones, tannins and phlobotannins present in the extract were qualitatively analyzed. About 500mg of ethanol extract is weighed and dissolved in 25ml of ethanol respectively, and then it is subjected to various preliminary phytochemical analysis.[8]

Microorganisms used

The microorganisms used for antibacterial activity was *Klebsiella pneumoniae*, *Vibrio cholerae, Shigella dysenteriae*, *E.coli* and *Staphylococcus aureus.* Fungi used to test the antifungal activity were *Candida albicans*, *Aspergillus niger* and *Aspergillus flavus.*

Inoculum preparation

A slant of nutrient agar at 4^{o}C was maintained for the preparation of stock cultures. Test tubes with nutrient broth were taken and loop full of cells from stock cultures were transferred to the autoclaved clean test tubes at aseptic condition for the preparation of active cultures, which was used for the experiments. They were incubated for 24 hrs at 37^{o}C. The Assay was performed by agar disc diffusion method.

Antibacterial activity

Disc diffusion method was used to determine the antibacterial activity of the sample by using Muller Hinton Agar (MHA) medium. 3.8g of Muller Hinton Agar was weighed and dissolved in 100ml of distilled water. This medium was subjected for sterilization and was poured in autoclaved petriplates and allowed it to solidify for 1 hr. After solidification, inoculums were spread on the solid plates using sterile swab moistened with the bacterial suspension. In this way, five experimental discs with 20 µl of culture with different concentrations of extracts (1000µg/ml, 500µg/ml, 250µg/ml, 125µg/ml and 62.5µg/ml) and one negative control 20µl of 1% v/v DMSO and one positive control 10µg/ml streptomycin were prepared. These plates were incubated for 24 hrs at 37ºC. Finally the microbial growth was determined by measuring the diameter of zone of inhibition in all petriplates.

Antifungal activity

Antifungal activity of the sample was determined by Disc diffusion method by using Potato Dextrose Agar (PDA) medium. 3.9g of potato dextrose agar medium was weighed and dissolved in 100ml of distilled water. Then the medium is subjected for sterilization. This media was transferred to sterile petriplates after sterilization and were allowed to solidify for thirty minutes. After solidification, the inoculums were spread on the solid plates with sterile swab moistened with the fungal suspension. In this way, five experimental discs with 20 µl of fungal suspension with different concentrations of flower extract (1000 µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml and 62.5 µg/ml) and one negative control 20 µl of 1% v/v DMSO and one positive control 20 µg/ml ketacanazole were prepared. These plates were incubated for 24 hrs at 37ºC. Finally the zone of inhibition was measured to determine the antifungal activity.

Antioxidant assay

DPPH radical scavenging activity

Different concentration of the standard vitamin E and the extract (31.2µg/ml, 62.5µg/ml, 125µg/ml, 250µg/ml, 500µg/ml and 1000µg/ml) were taken in different test tubes with 3.7 ml of methanol and 200µl of DPPH reagent were added. All the test tubes were incubated in dark condition at room temperature for 30 minutes and finally absorptions were read at 517nm. The absolute methanol without extract was taken as blank. The IC_{50} value was determined. The percentage inhibition was calculated as following

$$\% \text{ Antioxidant activity} = \frac{(\text{absorbance at blank}) - (\text{absorbance at test})}{(\text{absorbance at blank})} \times 100$$

RESULTS AND DISCUSSION

Phytochemical screening

The preliminary phytochemical qualitative analysis for the ethanolic extract of *Borassus flabellifer* male flowers (inflorescences) was performed by using an appropriate methodology. It indicated the presence of phenols, carbohydrates, flavones, saponins, alkaloids, quinones, tannins and phlobotannins. Among these carbohydrates, saponins and phlobotannins were present in high amount. Whereas phenols, flavones, alkaloids, quinones and tannins were present in moderate amount and the compounds such as reducing sugars, glycosides, steroids, amino acids and proteins are absent (Table 1). The preliminary phytochemical screening on aqueous extract of fruits of *Borassus flabellifer* Linn showed the presence of phytoconstituents such as carbohydrates, amino acids, flavonoids, tannins, saponins, vitamin C and phenolic compounds.[4] The Phytochemical screening of *Borassus flabellifer* parts such as young fruit, ripe seed coat, cotyledon and palm sugar showed the presence of different constituents using ethanol extract. It revealed the presence of reducing sugar, terpenoids, tannins, flavonoids and coumarin in all parts, but palm sugar showed positive only for the presence of reducing sugar.[9] Phytochemical screening of *Borassus flabellifer* root was performed in extracts such as acetone, benzene, chloroform, ethanol and methanol. Among all the solvents, ethanol and methanol showed the presence of tannins, phenols, steroids, flavanoids and saponins.[10]

Antibacterial activity

The Antibacterial activity of ethanolic extract of *Borassus flabellifer* was effective for all the tested bacteria except *Staphylococcus aureus*. The activity of the extract varies depending on the sample concentration and the tested bacteria. Among these the extract showed maximum zone of inhibition against *E.coli* (11mm) at high sample concentration (1000µg/ml) and for 500µg/ml, inhibition was measured as (10mm), followed by that *Klebsiella pneumoniae* (8mm) for all the concentrations, similarly *Vibrio cholerae* (8mm) for 1000µg/ml and the remaining concentrations showed (7mm) of inhibition, parallel to that *Shigella dysenteriae* was also measured as (7mm) of inhibition. Streptomycin which was used as the standard compound showed significant activity against all the tested bacteria (Table 2 and Fig. 1). *Borassus flabellifer* root extract exhibited significant antibacterial activity. Methanol and ethanol extracts of Palmyra palm root extract exhibited maximum zone of inhibition against *E.coli* (14mm); *Pseudomonas aeruginosa* (13mm) and *Bacillus subtilis* (11mm). The zone of inhibition is in accordance with increase in the concentration of the tested extract[10] which is less similar to our results, in the present study *E.coli* showed (11mm) of inhibition at 1000µg/ml concentration. The male inflorescences of *Borassus aethiopum* Mart was tested against *Escherichia coli* and *Enterobacter cloacae* respectively, in which the zone of inhibition was measured as 12mm and 11mm. The extracts were found to be completely ineffective at lower concentrations which are correlated to the present study. Similarly *B. aethiopum* was totally ineffective against the *Staphylococcus aureus,* which are more parallel to the results obtained in this study. According to

the results obtained from the tested plant, it is clear that plant possesses various active principles in flower which are responsible for the antibacterial activity. The therapeutic value of medicinal plant lies in the various chemical constituents in it. The bioactivity of plant extracts is attributed to phytochemical constituents. For instance, plant rich in tannins have antibacterial potential due to their character that allows them to react with proteins to form stable water soluble compounds thereby killing the bacteria by directly damaging its cell membrane.[11] Flavonoids are a major group of phenolic compounds reported for their antiviral,[12] antimicrobial,[13] and Spasmolytic properties.[14] Alkaloids isolated from plant are commonly found to have antimicrobial properties.[15] Antimicrobial property of saponins is due to its ability to cause leakage of proteins and certain enzymes from the cell.[11] Hence *Borassus flabellifer* male flowers (inflorescences) are reported to contain the above mentioned compounds, which makes the plant to be efficient against tested bacteria.

Antifungal activity

Antifungal activity of male flower *B. flabellifer* extract showed maximum zone of inhibition against the tested fungi except *Aspergillus flavus*. Among the fungi tested the best antifungal activity was obtained against *Candida albicans* and the zone of inhibition was measured as (13mm) at 1000µg/ml which was more than the standard drug (Ketacanazole) used (Table 3 and Fig. 2). Similarly *Aspergillus niger* was measured as (11mm) for 1000µg/ml, (9mm) for 500µg/ml, (8mm) for 250µg/ml and the remaining concentrations do not showed any zone of inhibitions. However the extract not proved effective for *Aspergillus flavus. Murraya koenigii* root extract was tested for antifungal activity. For each fungal strain, controls were maintained where pure solvents were used instead of (*Murraya koengii*) extracts. The results clearly show that all the extracts have shown antifungal activity against the entire tested organisms. Ethanol, methanol, ethyl acetate, acetone, chloroform, petroleum ether, hexane and hot water extracts have shown better activity against all the five microorganisms. Among these, ethanol extract was more effective against *Aspergillus niger* (12.17±0.15), *Candida tropicalis* (12.17±0.15) and *Candida albicans* (11.07±0.12)[16] which are showing similarity with the present study results, that the male flower extract shows maximum zone of inhibition against *Candida albicans* and *Aspergillus niger* at 1000µg/ml of sample concentration and the zone of inhibition of the present study was measured as 13mm and 11mm respectively. Investigation on the potential antifungal activities of *Borassus aethiopum* male inflorescences showed that dermatophytic fungi were sensitive to the extract. The presence of saponin compounds is responsible for the antifungal activity. These compounds are well known for antifungal task. In various plants tested against fungal strains, the results were shown to possess tannin, alkaloids, saponins and phenolic compounds such as flavonoids which have been responsible for antimicrobial activities against a number of microorganisms.[17]

Antioxidant activity

The ethanolic extract of *B. flabellifer* male flower showed high radical scavenging activity. Here vitamin E is used as the standard. The flower extract at 1000µg/ml of concentration it gives 95.3% of activity, which is more than the activity of standard vitamin E. The flower has 1.6% of more antioxidant activity compared to the standard compound used. At minimum concentration of the sample at 31.2µg/ml, the flower extract showed 79.6% of antioxidant activity and the standard vitamin E showed 78.1% (Table 4 and Fig. 3). IC_{50} value determined for the sample was 213µg/ml and for the standard drug Vitamin E was 159.4µg/ml. Radical scavenging activity of methanolic seed coat extract of *B. flabellifer* was carried out using DPPH. IC_{50} value for the standard (ascorbic acid) was 2.85 µg/ml and 7.90µg/ml were the IC_{50} values of seed coat extract. The antibacterial as well as radical scavenging activities dose dependently increased with seed coat extract. The study revealed that seed coat of *B. flabellifer* has shown significant anti-bacterial and radical scavenging (anti-oxidant) activities.[18] Antioxidant activity of *Borassus flabellifer* fruits extract was tested by its ability to bleach the stable DPPH radical. DPPH gives strong absorption band at 517nm and when it is quenched by the extract, there is a decrease in absorbance. Depending on the concentration of the extract the antioxidant activity also varies. More activity was showed at maximum concentration which correlate to the present study results.[4] Many medicinal plants have been analyzed and reported for their DPPH scavenging activity. The DPPH scavenging ability of medicinal plants has been attributed to several components, including phenolics, flavonoids. Hence herbal drugs containing antiradical constituents are gaining importance in prevention and treatment of diseases and free radical scavengers like phenolics are known for their therapeutic activity. Moreover based on the above results, it is demonstrated that the *B. flabellifer* male flowers are potential radical scavengers. The radical scavenging activity of the plant is due to the presence of active compounds such as phenols, saponins and flavonoids. Phenols are compounds that have the ability to destroy radicals because they contain hydroxyl groups. These important plant components give up hydrogen atoms from their hydroxyl groups to radicals and form stable phenoxyl radicals. Hence, they play an important role in antioxidant activity.The therapeutic effects of several medicinal plants are usually attributed to their antioxidant phytochemicals. It has been suggested that there is an inverse relationship between dietary intake of antioxidant rich food and incidence of human disease.[19]

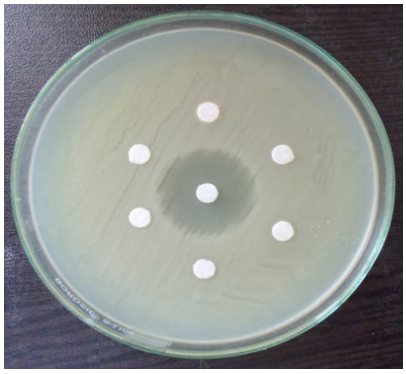
Table 1
Phytoconstituents in Borassus flabellifer ethanolic male flowers (inflorescences) extract

S. NO	COMPOUND	ETHANOL EXTRACT
1.	Phenols	++
2.	Reducing sugars	-
3.	Carbohydrates	+++
4.	Flavones	++
5.	Glycosides	-
6.	Saponins	+++
7.	Steroids	-
8.	Alkaloids	++
9.	Quinones	++
10.	Proteins	-
11.	Amino acids	-
12.	Tannins	++
13.	Phlobotannins	+++

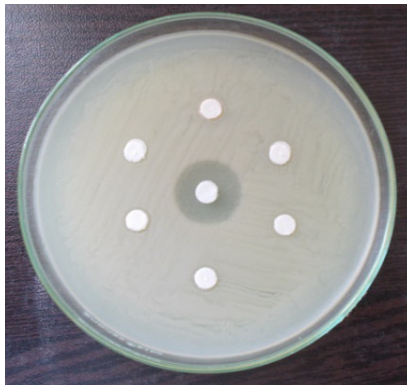
+: Presence (+ mild, ++ moderate, +++ high), -: Absence

Table 2
Antibacterial activity of ethanolic extract of Borassus flabellifer male flower

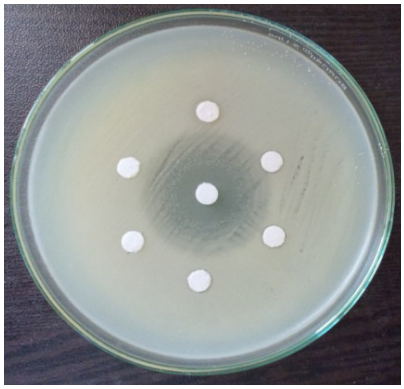
S.No	MICROORGANISMS	ZONE OF INHIBITION (mm) CONCENTRATION (µg/ml)					DMSO 1% v/v	Streptomycin	
		1000	500	250	125	62.5	20µl	(10µg/ml)	
1.	*Klebsiella pneumoniae*	8	8	8	8	8	-	2	2
2.	*Vibrio cholerae*	8	7	7	7	7	-	2	0
3.	*Shigella dysenteriae*	7	7	7	-	-	-	2	1
4.	*Escherichia coli*	1	1	1	0	-	-	-	-	2	1
5.	*Staphylococcus aureus*	-	-	-	-	-	-	1	9



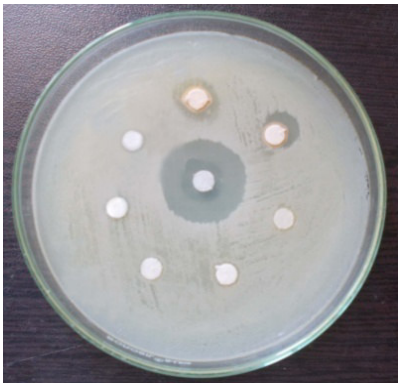
A-*Klebsiella pneumoniae*



B- *Vibrio cholerae*



C-*Shigella dysenteriae*



D-*Escherichia coli*

E-*Staphylococcus aureus*

Figure 1
Antibacterial activity of ethanolic extract of Borassus flabellifer male flower

Table 3
Antifungal activity of ethanolic extract of Borassus flabellifer male flower

S.No	MICROORGANISMS	ZONE OF INHIBITION (mm) CONCENTRATION (µg/ml)					DMSO 1% v/v	Ketacanazole
		1000	500	250	125	62.5	20µl	(20 µg/ml)
1.	*Candida albicans*	13	11	10	9	9	-	12
2.	*Aspergillus niger*	11	9	8	-	-	-	11
3.	*Aspergillus flavus*	-	-	-	-	-	-	-

A-*Candida albicans*

B-*Aspergillus niger*

C-*Aspergillus flavus*

Figure 2
Antifungal activity of ethanolic extract of Borassus flabellifer male flower

Table 4
DPPH antioxidant activity of ethanolic extract of Borassus flabellifer male flower and standard vitamin E

S.No	Concentration (µg/ml)	% of inhibition activity	
		Sample	Standard Vitamin E
1	31.2	79.6	78.1
2	62.5	82.8	81.2
3	125	82.8	85.9
4	250	89.0	87.5
5	500	92.1	90.6
6	1000	95.3	93.7

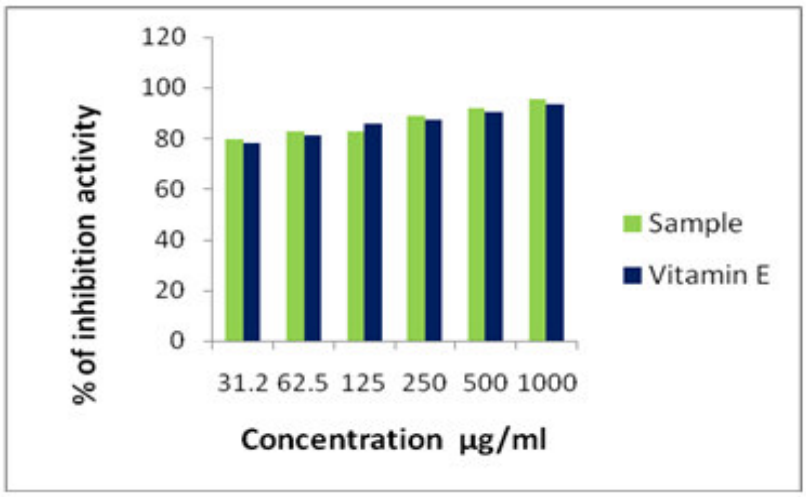


Figure 3
DPPH antioxidant activity of ethanolic extract of Borassus flabellifer male flower and standard vitamin E

CONCLUSION

From this study it is concluded that *Borassus flabellifer* possess moderate antibacterial activity and significant antifungal activity. It shows high antioxidant activity and prevents oxidative stress in humans. Hence *Borassus flabellifer* male flower can be utilized as a source of new antimicrobial compound for the tested bacteria and fungal strains, as it inhibit the growth of microorganisms and thereby prevent infectious disease. It is essential that research should continue to isolate and purify the active components and use in experimental animals.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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