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ORIGINAL ARTICLE

Biological activities of leaves of ethnomedicinal plant, *Borassus flabellifer* Linn. (Palmyra palm): An antibacterial, antifungal and antioxidant evaluation

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Abstract Plants contain a variety of phytochemicals that have the ability to exert effect on human body. Among them *Borassus flabellifer* Linn. is a medicinally important plant. In traditional medicine different parts of plants are being used for their medicinal properties. The methanol extract was obtained from powdered leaves and further fractions were prepared. Antimicrobial potential was investigated using eight pathogenic strains of bacteria and fungi by agar well diffusion method. Broth dilution method was employed to MIC and MMC of active samples and MIC index value was determined. ME was subjected to preliminary phytochemical analysis; and 1,1-diphenyl-2-picrylhydrazyl (DPPH) and hydrogen peroxide (H_2O_2) radical scavenging activity. Phytochemical screening revealed the presence of several phytochemicals. The ME showed dose dependent radical scavenging activity as evidenced by IC_{50} values for DPPH (40.19 μg/ml) and H_2O_2 (30.92 μg/ml) radicals. The inhibition zones and MIC values for bacterial strains were in the range of 10–16 mm and 50–70 μg/ml, respectively. All the samples showed an inhibitory effect on fungal strains with inhibition zone (10–17 mm) and MFC (50–70 μg/ml). Samples exhibited diverse patterns of antibacterial and antifungal effects. Among the tested samples, methanol extract and acetone fraction (AF) had potent antibacterial and antifungal activities. These results lead to the conclusion that the plant has a broad spectrum antimicrobial and antioxidant activity and could be a potential option for treating various infectious diseases. The strong antioxidant property of methanol extract might be employed in the development of natural antioxidants for agro-food and pharmaceutical industries.

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1. Introduction

The progression of improvement and dissemination of the knowledge about the plants and its traditional medicinal uses has become one of the basis for the cure of general ailments from the midst of primordial epoch. *Borassus flabellifer* Linn. is high in stature and distinctly differ as male and female one with a sturdy trunk and is unbranched. It is generally cultivated in most of the regions of India, Bangladesh, Burma, Sri Lanka, Malaysia and tropical Africa. The people residing in these regions are mostly dependent upon vegetation around them for the treatment of small ailments such as cuts and wound focusing on the medicinal importance of plant parts.[1,2] *Borassus flabellifer* Linn. is commonly known as Palmyra palm which is immensely distributed in the tropical regions of the Asian continent. Appreciable noteworthy economic value to the local population is aided by the *Borassus flabellifer* Linn.[2] It belongs to the family Palmae and the sub-family Boracidae. *Borassus aethiopum* Mart., *Borassus flabellifer* Linn. and *Borassus sundaicus* Becc. are the three most economically important species of Borassus.[3]

The plant mainly contains gums, albuminoids, fats, steroidal glycosides, and carbohydrate like sucrose. It also contains spirostane type steroids like borassosides and dioscin.[4] Seed coat extract of the *Borassus flabellifer* Linn. has been reported to possess antimicrobial activity.[5] Male inflorescence shows a significant anti-inflammatory activity.[6] Different parts of the *Borassus flabellifer* Linn. plant have been reported to comprise biological activities and pharmacological functions, including anthelmintic, diuretic[7], antioxidant[8] and antibacterial activities of the fruits, wound healing[9], immunomodulatory[10], and antimalarial.[11]

Fundamental parameter in domineering and sustaining human life is the biochemical reactions which take place within the organelle and cells of the body.[12] The chemical constituent of the plant produces free radicals that regulate biochemical processes by acting as an antioxidizing agent.[13] Many studies have shown a close relationship between a highly nutritious diet, maintenance of good health and reduction in the risk of chronic diseases. Besides nutrients such as carbohydrates, protein, and fibers another class of essential substances that has been studied in the last few years is antioxidant compounds which are present at low concentrations and can help to prevent cell damage such as cancers, inflammations, aging and atherosclerosis caused by free radicals throughout the body.[14] Many studies revealed that synthetic antioxidants produce toxic effects like carcinogenesis and liver toxicity.[13] Microbial infection is a one of the major motives responsible to evoke oxidative reactions which intern lead to cell injury.[15–17] Although many antimicrobials have been effectively used but remarkable resilience and the emergence of resistance are major problems.[18] It is known that leaves of *Borassus flabellifer* Linn. are rich in an abundant number of phytochemicals.[4] Several antimicrobial herbs like *Borassus flabellifer* Linn. have not revealed for all their facets so there is surge to reveal their medicinal properties. Therefore, the aim of this study was to investigate the antioxidant activity of leaf extract and antimicrobial efficacy of extract and fractions against most common human pathogenic strains.

2. Material and methods

2.1. Chemicals and reagents

1,1-diphenyl-2-picrylhydrazyl (DPPH), nutrient broth (NB), potato dextrose broth (PDB), bacteriological agar and antibiotic disk (amoxicillin, ciprofloxacin and griseofulvin) were supplied by Himedia (Mumbai, India). Hydrogen peroxide (H_2O_2), sodium hydroxide and potassium dihydrogen phosphate were purchased from Rankem (India). Ascorbic acid was obtained from Oxford laboratory, India. All the reagents were of analytical grade purity and obtained from Rankem (India).

2.2. Collection and authentication of plant material

The *Borassus flabellifer* Linn. leaves were collected from Kurnapalli village of Nizamabad district of Andhra Pradesh state, India and was authenticated by Botanist, Dr. Gachande B.D., Associate Professor of Botany Department, N.E.S. Science College, Nanded, India. The voucher specimens were deposited at herbarium of School of Pharmacy, S.R.T.M. University, Nanded, Maharashtra, India.

2.3. Extraction and fractionations

The collected leaves were converted into small pieces and shade dried for 7 days at room temperature, and then powdered using a grinder (coarse powder by sieve No. 10, manual). The dried powder material (250 g) was Soxhlet extracted with 1 L methanol for 8 h at 64 °C. After extraction the solvent was evaporated and concentrated by rotary evaporation (Superfit, India). The obtained methanol extract was then mixed with 150 ml of distilled water and sequentially partitioned (3×; three times) using separatory funnel with an equal volume of pet. ether, n-butanol, chloroform and acetone solvents, respectively. Solvents were selected on solubility and polarity of phytochemicals. The fractions were later concentrated under reduced pressure in a rotary evaporator, and weighed and a percentage yield of 5%, 13.5%, 12% and 12.5% for pet. ether, n-butanol, chloroform and acetone fractions, respectively was obtained. The extracts were stored at 10 °C, protected from light and used within one week.

2.4. Qualitative phytochemical investigation

The methanol extract of *Borassus flabellifer* Linn. was subjected to phytochemical screening using standard procedures.[19] Extract was primarily intended for the phytochemical analysis and detection of major chemical constituents.

2.5. Antimicrobial assay

2.5.1. Microbial strains and culture media preparation

The methanol extract (ME) and fractions n-butanol (NF), chloroform (CF) and acetone (AF) of *Borassus flabellifer* Linn. were individually tested against a set of eight strains of bacteria (four gram positive and four gram negative) and fungi. Microbial strains were provided by School of Life Science, Swami Ramanand Teerth Marathwada University, Vishnupuri, Nanded, Maharashtra, India. Bacteria strains used include

Staphylococcus aureus (MTC 96), *Staphylococcus epidermidis* (MTCC 1228), *Proteus vulgaris* (ATCC 33420), *Bacillus subtilis* (B 28), *Escherichia coli* (MTCC 170), *Pseudomonas aeruginosa* (CC 488), *Klebsiella pneumoniae* (ATCC 15380), and *Salmonella typhi* (ATCCB 23564). Antifungal activity was tested against *Aspergillus niger* (MTCC A), *Aspergillus flavus* (MTTC 873), *Aspergillus fumigates* (MTCC 2551), *Vestilago myditis* (MCIM 983), *Microsporum canis* (MTCC 2520), *Candida albicans* (MTCC 3018), *Saccharomyces cerevisiae* (MCIM 170), and *Candida blanki* (MTCC 1442).

Culture media for antibacterial study was prepared by adding 2.6 g agar to 5 g of nutrient broth (NB) and volume was adjusted to 200 ml using distilled water. Later it was sterilized by autoclave at 121 °C for 15 min. The same procedure was performed to prepare culture media for antifungal screening using 5 g agar and 4.8 g potato dextrose broth (PDB). The bacterial strain along with nutrient agar was cultured overnight at 37 °C and fungi were cultured overnight at 30 °C in potato dextrose agar.

2.5.2. Agar well diffusion assay

Determination of antimicrobial activities of ME, NF, CF and AF were accomplished by agar well diffusion method.[20,21] The molten and cooled media was poured in sterilized petri dishes (20 ml/dish). The plates were kept overnight at room temperature to ensure contamination. Briefly, wells of 10 mm diameter were prepared in the agar plates with the help of sterilized stainless steel cork borer. Lawns were prepared on agar plates by the spreader employing 100 µl NB culture of each organism. All the samples (100 µg/ml) were prepared in DMSO and from that 100 µl was used for activity. The wells on each plate were loaded with samples (ME, NF, CF and AF) and the same procedure was carried out for standard antibiotics. Amoxicillin and ciprofloxacin were considered as standard for antibacterial whereas griseofulvin was used for antifungal screening. The plates were aerobically incubated at 37 °C for 24 h for bacteria and at 28 °C for 48 h for fungi. The diameters of inhibition zones were used as a measure of antimicrobial activity and compared with standard antimicrobials.

2.5.3. Determination of minimum inhibitory concentration (MIC)

Bacterial and fungal strains sensitive to samples in agar well diffusion assay were studied for their minimal inhibition concentration (MIC) values using broth dilution method.[20,22] The 0.5 ml of active sample (90, 80, 70, 60, 50, 40, 30, 20, 10, 5, 1 µg/ml) was mixed with 2 ml of NB and a loopful of the test organism. The same procedure was repeated on the test organisms using the standard antibiotics. A tube containing NB was seeded with the test organisms and served as control. Then, tubes were incubated at 37 °C for 24 h for bacteria and at 28 °C for 48 h for the fungi. Microbial growth was determined by the presence of turbidity. MIC was defined as the lowest concentration of compounds at which the microorganism tested did not demonstrate visible growth.

2.5.4. Determination of minimum microbicidal concentration (MMC)

The minimum microbicidal concentration (MMC) includes minimum bactericidal (MBC) and minimum fungicidal concentrations (MFC) of the active samples were determined according to the MIC values. The samples showing no increases in turbidity were streaked on nutrient agar medium and inoculated on sterile nutrient agar for 37 °C for 24 h. Similar procedure was repeated for MFC determination using potato dextrose agar and plates were incubated at 28 °C for 48 h. The lowest concentration at which no visible growth was noted was considered as the MMC.[21,22]

2.5.5. Determination of MIC index

MIC index values were determined for active samples using their MMC and MIC values calculated against the test strains of bacteria and fungi.[23,24] MIC index value for bacteria and fungi was calculated using the following formula:

$$\text{MIC index value for bacteria} = \text{MBC}/\text{MIC}$$

$$\text{MIC index value for fungi} = \text{MFC}/\text{MIC}$$

2.6. In vitro antioxidant assay

2.6.1. DPPH radical scavenging activity

The scavenging activity of ME was estimated using 1, 1-diphenyl-2-pycrylhydrazyl (DPPH) as a free radical model and a method adapted from Ye et al.[25] 0.1 mM DPPH solution was prepared by dissolving 1.9 mg of DPPH in methanol and the volume was made up to 100 ml using methanol. The solution was kept in darkness for 30 min to complete the reaction. An aliquot of 1 ml of ME at different concentrations (20, 40, 60, 80 and 100 µg/ml) was mixed with 1 ml of 0.1 mM DPPH-methanol solution. The mixture was shaken vigorously and left to stand at room temperature for 30 min in the dark. Then the mixture was measured spectrophotometrically (Shimadzu Kyoto 1800) at 517 nm against methanol as blank. The free radical scavenging activity was calculated as follows:

$$\text{Scavenging effect}\ (\%) = [(\text{Absorbance of blank} - \text{Absorbance of sample})] \times 100$$

A standard of ascorbic acid was run at the concentrations 20, 40, 60, 80 and 100 µg/ml. Antioxidant activity of the ME was expressed as IC_{50}, the concentration (in µg/mL) that inhibits the formation of DPPH radicals by 50%. The IC_{50} was calculated by plotting the graph of inhibition percentage versus the ME concentration.

2.6.2. Hydrogen peroxide radical scavenging activity

The abilities of the ME to scavenge H_2O_2 were determined according to the method of Muruhan et al. and Alam et al.[26,27] The ME (1 ml) of different concentrations (20, 40, 60, 80 and 100 µg/ml) was added to a 40 mM hydrogen peroxide solution (0.6 ml) prepared in 0.1 M phosphate buffer saline (pH 7.4). Absorbance of mixture at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide using UV–Visible spectrophotometer (Shimadzu Kyoto 1800) against a blank solution containing phosphate buffer solution without H_2O_2. Standard known antioxidant such as ascorbic acid was used as positive control. The IC_{50} values were calculated by linear regression of plots, where the abscissa represented concentration of ME and the ordinate represented average percent of scavenging capacity

from three replicates. Percentage scavenging of hydrogen peroxide of ME and ascorbic acid at different concentrations was calculated using the formula:

$$\%\text{Scavenging } [H_2O_2] = [1 - (\text{Absorbance of extract}/\text{Absorbance of control})] \times 100$$

2.7. Statistical analysis

All the determinations in antioxidant activity were carried out in triplicate. The results were expressed as mean ± SEM. Statistical analysis was carried out by a one way ANOVA followed by Post hoc Tukey test using GraphPad InStat version 3 USA. The level of statistical significance was set at $p < 0.05$.

3. Results

3.1. Phytochemical screening

The methanol extract of leaves of *Borassus flabellifer* Linn. was analyzed for the presence of major chemical constituents using qualitative phytochemical tests. Extract showed the presence of flavonoids, glycosides, tannins, proteins, steroids, triterpenoids, carbohydrates, fats and fixed oils.

3.2. Antimicrobial activity

Antimicrobial activity of ME, NF, CF and AF from the leaves of *Borassus flabellifer* Linn. against the tested bacteria and fungi strains was assessed qualitatively and quantitatively by the presence and absence of inhibition zones using agar well diffusion method. Results of anti-bacterial activity are presented in Table 1. The tested samples exhibited erratic effect on bacterial strains being ME and AF most sensitive. The zone of inhibition produced by ME on different bacterial strains was between 10 mm to 16 ± 0.57 mm. Among the bacterial strains tested, the diameters (mm) of the inhibition zone for ME were 12.33 ± 0.33, 16 ± 0.57, 14.33 ± 0.33, 10.33 ± 0.33, 10.66 ± 0.66 and 10 for *B. subtilis*, *S. aureus*, *P. vulgaris*, *E. coli*, *K. Pneumonia* and *P. aeruginosa*, respectively. *S. aureus*, *P. vulgaris* and *B. subtilis* were more sensitive to the ME and showed prominent inhibitory activity. The AF was found to have inhibitory effect on all the bacterial strains with a maximum zone of inhibition against *S. aureus* (14.33 ± 0.33), and *S. typhi* (12) and minimum zone of inhibition against *K. pneumonia* (10). The AF exhibited moderate antibacterial activity against *B. subtilis* (11.33 ± 0.33), *S. epidermidis* (11 ± 0.57), *P. vulgaris* (11.66 ± 0.33), *E .coli* (10.33 ± 0.33), *K. Pneumonia* (10) and *P. aeruginosa* (10.33 ± 0.33). The CF and NF were not effective against most bacterial strains but showed inhibitory effect only against *S. aureus* (11 ± 0.57) by CF and *P. aeruginosa* (10) by NF. The zone of inhibition produced by standard antibiotics was larger than those produced by samples.

Antifungal activity of samples was determined against eight different fungal strains and recorded as inhibition zone. The results are presented in Table 2. ME showed inhibitory effects against all the tested fungal strains except *A. niger* with highest inhibition zone diameter of *A. flavus* (11.66 ± 0.33). However, ME of the plant showed moderate (10– 10.66 ± 0.33) inhibitory activity against *M. canis*, *A. fumigates*, *S. cerevisiae*, *V. myditis*, *C. albicans* and *C. blanki*. The AF exhibited prominent inhibition zone against *A. flavus* (13.33 ± 0.33) whereas no inhibition was observed against *C. blanki* and *A. niger*. The *M. canis* (9.33 ± 0.33), *S. cerevisiae* (10.33 ± 0.33) and *C. blanki* (11.00 ± 0.57) showed moderate susceptibility toward CF. However, CF was found ineffective against *A. flavus*, *A. fumigates*, *V. myditis*, *C. albicans* and *A. niger*. NF exerted a potent inhibitory effect against *C. blanki* (12.00 ± 0.57) and moderate against *M. canis* (10) while it was found to be ineffective against other tested fungal strains.

3.3. Minimum inhibitory concentrations (MIC), minimum microbicidal concentration (MMC) and MIC index value

The ME and AF depicted its versatile potential against most of the tested pathogenic bacterial and fungal strains so they were selected for MIC and MMC. MIC, MBC and MIC index values against *B. subtilis, S. aureus, E. coli* and *P. aeruginosa* were shown in Table 3. These results demonstrated that ME and AF displayed a potential antibacterial property. In general, the MIC values of the ME against the tested bacteria ranged from 50 μg/ml to 70 μg/ml and MBC from 60 μg/ml to 80 μg/ml, respectively. MIC index values of ME for *B. subtilis, S. aureus, E. coli* and *P. aeruginosa* were 1.16, 1.20, 1.14 and 1.16, respectively. The MIC and MBC values of AF were in the range of 60 μg/ml to 70 μg/ml and from 60 μg/ml to 80 μg/ml,

Table 1 Inhibitory zone diameter of extract and fractions of *Borassus flabellifer* Linn. leaves against pathogenic bacteria and reference standard strains.

Bacterial strain	Diameter of inhibition zone (mm)					
	ME	AF	CF	NF	Amoxicillin	Ciprofloxacin
B. subtilis	12.33 ± 0.33	11.33 ± 0.33	–	–	20.33 ± 0.33	23.33 ± 0.33
S. epidermidis	–	11 ± 0.57	–	–	21.33 ± 0.33	15.33 ± 0.33
S. aureus	16 ± 0.57	14.33 ± 0.33	11 ± 0.57	–	14.33 ± 0.33	21.33 ± 0.33
P. vulgaris	14.33 ± 0.33	11.66 ± 0.33	–	–	18.33 ± 0.333	19.33 ± 0.33
S. typhi	–	12 ± 0.00	–	–	18 ± 0.57	12 ± 0.57
E. coli	10.33 ± 0.33	10.33 ± 0.33	–	–	21 ± 0.57	21.66 ± 0.33
K. pneumonia	10.66 ± 0.66	10.00 ± 0.00	–	–	24.00 ± 0.57	20.66 ± 0.66
P. aeruginosa	10.00 ± 0.00	10.33 ± 0.33	–	10.00 ± 0.00	16.66 ± 0.33	16.66 ± 0.66

ME: methanol extract; NF: n-butanol fraction; CF: chloroform fraction; AF: acetone fraction.
Values are mean ± SEM of triplicate measurements.

Table 2 Inhibitory zone diameter of extract and fractions of *Borassus flabellifer* Linn. leaves against pathogenic fungi and reference standard strains.

Fungal strain	Diameter of inhibition zone (mm)				
	ME	AF	CF	NF	Ciprofloxacin
A. flavus	11.66 ± 0.33	13.33 ± 0.33	–	–	23.66 ± 0.33
M. canis	10.33 ± 0.33	10.66 ± 0.33	9.33 ± 0.33	10.00 ± 0.00	21.66 ± 0.33
A. fumigates	10.66 ± 0.33	10.00 ± 0.00	–	–	23.33 ± 0.00
S. cerevisiae	10.33 ± 0.33	10.66 ± 0.66	10.33 ± 0.33	–	14.33 ± 0.33
V. myditis	10.66 ± 0.33	10.33 ± 0.33	–	–	26.33 ± 0.33
C. albicans	10.00 ± 0.00	10.66 ± 0.33	–	–	15.66 ± 0.33
C. blanki	10.33 ± 0.33	–	11.00 ± 0.57	12.00 ± 0.57	13.66 ± 0.33
A. niger	–	–	–	–	23.66 ± 0.33

ME: methanol extract; NF: n-butanol fraction; CF: chloroform fraction; AF: acetone fraction.
Values are mean ± SEM of triplicate measurements.

Table 3 The MIC, MBC and MIC index values of ME and AF of *Borassus flabellifer* Linn. leaves for antibacterial activity.

Bacterial strain	MIC (μg/ml)		MBC (μg/ml)		MIC index values	
	ME	AF	ME	AF	ME	AF
B. subtilis	60	60	70	80	1.16	1.33
S. aureus	50	60	60	60	1.20	1
E. coli	70	70	80	80	1.14	1.14
P. aeruginosa	60	60	70	70	1.16	1.16

ME: methanol extract; AF: acetone fraction.

respectively. AF showed 1.33, 1, 1.14 and 1.16 MIC index values for *B. subtilis, S. aureus, E. coli* and *P. aeruginosa*, respectively.

The MIC and MFC for ME, AC fractions were investigated against *A. flavus, M. canis*, *S. cerevisiae,* and *V. myditis* and are listed in Table 4. MIC values of ME for fungal strains were in the range of 50 μg/ml to 60 μg/ml and MFC value range was 50 μg/ml to 70 μg/ml. The MIC and MFC values for AC against the tested microorganisms ranged from 50 μg/ml to 70 μg/ml and from 60 μg/ml to 70 μg/ml, respectively.

3.4. Antioxidant activity

3.4.1. DPPH radical scavenging assay

DPPH radical scavenging assay is a standard method in antioxidant activity studies and offers a rapid technique for screening the radical scavenging ability of phytochemicals. The antioxidant activity of ME was measured by the ability to scavenge DPPH free radicals and it was compared with the standard antioxidant, ascorbic acid. Compounds possessing antioxidant property have the ability to scavenge DPPH radicals by providing hydrogen atom or electron donation and decreases characteristic absorption of radical DPPH at 517 nm. A lower absorbance at 517 nm indicates a higher radical scavenging activity of ME. The scavenging effects of the ME and ascorbic acid on the DPPH radical were expressed as half maximal inhibitory concentration (IC_{50}) values. The IC_{50} values were recorded as 40.19 μg/ml for ME and 21.80 μg/ml for ascorbic acid. ME represents a concentration dependent decrease in absorbance which is represented in Table 5.

Table 4 The MIC, MFC and MIC index values for ME and AF of *Borassus flabellifer* Linn. leaves for antifungal activity.

Fungal strain	MIC (μg/ml)		MFC (μg/ml)		MIC index values	
	ME	AF	ME	AF	ME	AF
A. flavus	50	50	50	60	1	1.2
M. canis	50	60	70	70	1.4	1.16
S. cereviceae	60	70	60	70	1	1
V. myditis	50	60	60	60	1.2	1

ME: methanol extract; AF: acetone fraction.

3.4.2. H_2O_2 radical scavenging assay

The radical scavenging property of a compound, which may serve as a significant indicator of its potential antioxidant activity and it was determined using H_2O_2 radical scavenging assay. The scavenging ability of the ME on H_2O_2 is shown in Table 5 and compared with ascorbic acid as control standard. ME and ascorbic acid demonstrated hydrogen peroxide radical scavenging activity in a dose dependent manner. The H_2O_2 radical scavenging increased from 26.19 ± 0.01% to 59.52 ± 0.01% when the concentration of the ME increased from 20 to 100 μg/mL. The IC_{50} value of the ME and ascorbic acid were 30.92 μg/ml and 18.85 μg/ml, respectively.

4. Discussion

Many pharmaceutical and health allied communities are focusing toward medicinal properties of plants, as the herbal formulations prepared from parts or whole plant are generally safe with fewer side effects if used in the proper therapeutic dosages.[28] Unremitting development of resistance to existing and newer antibiotics is also responsible to spotlight over traditional claims of medicinal plants.[18,28] The plant, *Borassus flabellifer* Linn. is one of the most popular traditional medicinal plants.[8,9] Several previous studies showed that extracts and fractions obtained from different plant parts have the ability to resist microbial growth.[29] The antimicrobial activity of extract and fractions against pathogenic strains of bacteria and fungi supported the scientific validity of the plant being used traditionally as a medicine.[30] The inhibition of almost all the bacterial strains by the methanol extract may be attributed to the presence of antibacterial phytochemicals such as phenolic and polyphenolic compounds.[31] Same results have been

Table 5 Antioxidant activity of ME of *Borassus flabellifer* Linn. leaves and ascorbic acid.

Conc. (μg/ml)	Radical scavenging assay			
	DPPH		H_2O_2	
	% inhibition			
	ME	Ascorbic acid	ME	Ascorbic acid
20	42.01 ± 0.58	48.57 ± 0.01	26.19 ± 0.01	50 ± 0.57
40	47.92 ± 0.00	57.76 ± 0.00	38.09 ± 0.05	61.90 ± 0.57
60	59.76 ± 0.00	65.94 ± 0.05	42.85 ± 0.02	72.61 ± 0.57
80	68.04 ± 0.02	72.53 ± 0.00	52.38 ± 0.01	84.52 ± 0.00
100	72.18 ± 0.57	80.63 ± 0.01	59.52 ± 0.01	91.66 ± 0.00
IC_{50} (μg/ml)	40.19	21.80	30.92	18.85

ME: methanol extract.
Each value is represented as mean ± SEM (n = 3).

published by Nain et al. and Kuete et al. in which it was shown that the methanol extract exhibits potent inhibitory effect, suggesting that the antibacterial activity of the methanol extract may be related to triterpenoids, phenolics and other phytochemicals.[32,33] In the present study, we found that AF exhibited antibacterial activity against most of the tested bacterial strains. Lack of antibacterial potential in some of the fractions is not surprising as the number of plants has been found to be ineffective against pathogenic strains which might be due to the absence or fewer numbers of antibacterial phytochemicals.[23,34] Polyphenolic compounds are soluble in acetone and these could be responsible for antibacterial efficacy of acetone fraction.[35]

The methanol extract exhibited broad antimycotic activity against the tested fungal strains which was similar to its antibacterial activity. The results obtained are in consonance with several previous studies showing that the antifungal activity of methanol extract was due to the presence of glycosides, steroids, tannins and polyphenolics.[36,37] The AF also showed an inhibitory effect on most fungi which may be attributed to the presence of antifungal compounds as shown by Aderogba et al.[36,38] The antifungal effects of the extracts could be due to the disruption of proteins in bacterial membranes.[31,39]

The MIC index values for ME and AF values supported the results obtained in the antibacterial study, showing clearly that both samples encompass prominent inhibitory effect on bacteria as the MIC values were lower than the MBC values, similar to the results of Humeera et al. This difference in MIC values might be due to the presence of inert materials in crude plant extract and fractions which are not present in synthetic compounds as they are in a pure form.[23] The MIC index value obtained for ME and AF was less than four indicating bactericidal effect on the growth of bacteria.[23,24] In case of antifungal activity, same MIC and MFC values were exhibited by ME for *A. flavus* and *S. cerevisiae*; and AF for *S. cerevisiae* and *V. myditis*. The ME and AF was fungicidal in effect as the MIC index value was less than four.

Different assays have been employed to recognize the property of plant extracts to scavenge the free radicals.[27] To evaluate complex reactive properties of the phytochemicals at least two antioxidant test systems have been recommended as the antioxidants act by several mechanisms such as direct inhibition of reactive oxygen species or scavenging of free radicals.[40] For this reason the antioxidant activity was evaluated using DPPH and H_2O_2 radical scavenging assay in the present study.

Neutralization of DPPH free radicals by the plant extract either by transfer of hydrogen or of an electron is the basis of DPPH free radical scavenging assay.[41] Ascorbic acid is a standard antioxidant and it has a strong DPPH scavenging property.[42,43] The results revealed that methanol extract has a potent scavenging activity which may be attributed to the numerous phenolics.[44] The methanol extracts of *Borassus flabellifer* Linn. leaves scavenged free radicals in a dose-dependent manner corresponding with the results of Chandran et al. and Guntupalli et al. demonstrating that secondary metabolites of plants possess a strong antioxidative property.[44,45] Flavonoids, tannins, catechins and other phenolics are the examples of common plant metabolites having prominent antioxidant activity.[46]

Hydrogen peroxide is a weak oxidizing agent and restrains enzymes by the oxidation of essential thiol (–SH) groups.[47] H_2O_2 itself is not very reactive but it has the ability to cross cell membrane rapidly and react with Fe^{2+} and Cu^{2+} ions to form hydroxyl radical which further leads to cell toxicity.[48] The methanol extract scavenges H_2O_2 in a dose-dependent manner as that of standard, ascorbic acid. This activity may be attributed to phytochemicals of *Borassus flabellifer* Linn. leaves such as flavonoids, tannins and phenolics which neutralize H_2O_2 to water by donating electrons. Oyedemi et al. demonstrated that phenolics compounds have the capacity to neutralize H_2O_2 to water by donating electrons.[49]

5. Conclusion

Because of life threatening undesirable problems and side effects of synthetic antimicrobials, the phytochemicals of plant obtained in the form of extract or fraction, especially traditionally used edible ethnomedicinal plant have gained considerable interest of researchers working in the field of pharmaceuticals and health sciences. The present study indicated that methanol extract and acetone fraction shows a prominent antimicrobial activity against most referenced bacterial and fungal strains. Methanol extract possesses an interesting antioxidant activity as that of ascorbic acid. The antioxidant activity might be attributed to the presence of natural antioxidants such as

phenolic compounds. Thus considering together, these outcomes show that the *Borassus flabellifer* Linn. leaves could be considered as good sources of natural antioxidants and antimicrobials and may find several applications in agro-food and pharmaceutical industries.

Conflict of interest

The authors declare that there are no conflicts of interest.

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