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A Study of Antioxidant and Anti-bacterial activities of *Borassus flabellifer*

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Authors' contributions

This work was carried out in collaboration among all authors. Author PN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors DSS and AH managed the analyses of the study. Authors SP, PK, AK managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The present study was designed to estimate the antimicrobial and antioxidant activities of ethanolic and aqueous extract of *Borassus flabellifer* fruit, juice, leaves and sap. The antioxidant evaluation was carried out by various radical scavenging assays and antimicrobial activity by the disk diffusion method. For antibacterial activity, bacterial species such as *Escherichia coli and Staphylococcus aureus* were tested for all extracts. The extract showed moderate antioxidant activity when compared to the standard vitamin C. In both DPPH and nitric oxide oxide assay when related IC_{50} value, ethanolic fruit extract showed promising results of 132 and 119 µg/mL respectively with ascorbic acid as standard which showed 25 and 35 µg/mL respectively. In Ferric Thiocyanate (FTC) Method and Thiobarbituric Acid (TBA) Method the radical scavenging activity of ethanolic fruit extract showed satisfactory results of 160 and 148 µg/mL ascorbic acid being the standard which showed 94 and 97 µg/mL respectively. The total flavonoid contents were 32.7 /100g for ethanolic sap extracts and 53.9/100g in aqueous extract of sap. The fruit juice extract showed

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maximum inhibition zone with 31.4 and 32.1 mm against *E. coli* and *S.aureus* correlated other extracts .Ethanolic and aqueous sap extracts zone of inhibition was 25.2 and 26.8 against *E. coli* and zone of inhibition was 28.3 against *S.aureus* in both ethanolic and aqueous sap. Ethanolic and aqueous leaves extracts zone of inhibition was 28.5 and 24.1 against *E. coli.* Ethanolic and aqueous leaves extracts zone of inhibition was 30.4 and 25.8 against *S.aureus.*
Herein, the results suggest that the *Borassus flabellifer* plant extracts have potential antioxidant and antimicrobial properties.

Keywords: Borassus flabellifer; phytochemical; antibacterial; antioxidant; IC50.

1. INTRODUCTION

The use of herbal plants for infection prevention and treatment is a common remedy. It ranges from ancient and general medicines of each nation to consistent and titrated herbal extracts. Even though traditional medicine has become a popular treatment of health care, the main drawback is the lack of final and complete data about the composition of extracts and their pharmacological activities. For this purpose plant-based obtained medications require a thorough evaluation of their pharmacological activities. To establish their herbal pharmacological activities, conventional testing methods in many plant extracts have been effective for specific ailments [1].

Antioxidants or oxidation inhibitors are compounds that delay or prevent oxidation, generally prolonging cells' lives. Overproduction of free radicals causes oxidative stress which results in the form of diseases or disorders. Free radicals are agents with a very short half-life, high reactivity and are responsible for harmful activity towards macromolecules such as proteins, DNA and lipids [2]. In general, the reactive oxygen species have higher reactivity with some other molecules than that of ground-state oxygen, due to the additional electron they carry, Which affects various enzyme systems and cause harm that can further lead to conditions such as cancer, ischemia, ageing, adult syndromes of respiratory distress, rheumatoid arthritis, etc [3].A diet mainly composed of medicinal herbs protects against chronic illnesses linked to oxidative stress. Variable chemical families and quantities of antioxidants include dietary plants. Animal antioxidants suggest leading to the positive health effects of nutritional plants [4].

Borassus flabellifer belongs to the Arecaceae family, generally referred to as Palmyra palm, which is native to tropical Africa but is grown in India (Fig. 1) [5].Customarily the various parts of the plant, for example, root, leaves, organic product, and seeds are utilized for different human health issues. Blossoms of *B. flabellifer* were examined for antipyretic impacts and pain relieving, mitigating movement, hematological, biochemical boundaries, and immunosuppressant property [6]. The various portions of the herb are used for healing properties like antihelminthic and diuretic [7].

B. flabellifer fruit pulps are typical in traditional, and sap in diabetic patients as a sweetener. Phytochemical investigations of the plant uncovered spirostane-type steroid saponins; steroidal glycoside also contains an unpleasant compound called flabelliferrins. However, more studies are necessary for the comprehension and use of these compounds [8].

Fig. 1. *B flabellifer* palm tree (a) and fruits of *B. flabellifer* (b)

2. MATERIALS AND METHODS

1, 1-diphenyl-2-picryl-hydrazyl (DPPH), Ascorbic acid were procured from Sigma-Aldrich Bangalore.

Sodium nitroprusside, Sulfanillic acid reagent, Naphthyl ethylenediamine dihydrocholoride, Hydrogen peroxide were purchased from Himedia labs, Mumbai.

Iron(III) chloride($FeCl_3$), Ethylene diamine tetraacetic acid (EDTA), Tetracholoro acetic acid, Thiobarbituric acid, Potassium ferricyanide, Ammonium molybdate, Folin-Ciocalteu, Sodium carbonate, Nitro blue tetrazolium (NBT) and Dimethyl sulfoxide (DMSO) were procured from Lab India, Thane Mumbai.

2.1 Collection of Plant Materials and Preparation of Extracts

For this purpose, we collected fruits, leaves, sap, and dried parts of *B. flabellifer* from Mangalore and Puttur district of Dakshina Kannada, India. The unrefined homegrown medication was cleaned, spread under the shade at room temperature, and dried under sunlight. For powdered material, submitted the dried plant material to a mechanical grinder and the extraction in a Soxhlet extractor with 500mL of ethanol or water. The extracts were concentrated under vacuum evaporator and stored in a desiccator for further use [6].We used an aqueous and ethanolic extract from leaves, fruits, and sap to measure the antioxidant and antibacterial assays.

FE= fruit juice extract of ethanol
FW= fruit juice extract of water
LE= leaves extract of ethanol
LW= leaves extract of water
SE = sap extract of ethanol
SW = sap extract of water

2.2 Antioxidant Activity Evaluation by Different Methods

2.2.1 DPPH radical scavenging activity

The samples were evaluated for the DPPH radical scavenging behavior according to the method proposed by Barros et al. (2007) method with some modification. In 5 mL of a 100 µM solution of DPPH (in methanol), 50 µL of the various plant extracts concentrations in ethanol incorporated. The ascorbic acid was used as a standard and prepared as mentioned above without the extracts. For 30 minutes, the samples were incubated at room temperature, and later absorbance of all the samples, including blank, were measured at 517 nm using shimadzu uv-spectrophotometer 1300 series .Determined the percentage of inhibition activity from the following equation:

$$\% \text{ inhibition} = [(A_o - A_1) / Ao] \times 100$$

Where A_o is the control absorbance, and $A_1$is the extract/ standard absorbance [9,10]. Here ascorbic acid was used as a standard. The experiments were performed in triplicate.

2.2.2 Nitric oxide radical inhibition assay (NO°)

The use of the Griess Illosvoy reaction is possible to estimate NO° inhibition. The assay was carried out by a modified GriessIl losvoy reagent using 0.1 % N-(1-Naphthyl) ethylene diamine dihydrochloride and ascorbic acid as standard [11,12].

The samples were measured at 540 nmusing shimadzu uv- spectrophotometer 1300 series wavelength were compared to the blank using the following formula:

Scavenging activity of Nitric oxide radical = ($A_o$blank - A_1sample) × 100 / $A_o$blank

Where Ao is the blank absorbance, and A_1 is the sample absorbance
Ascorbic acid was used as standard.

2.2.3 Ferric thiocyanate (FTC) method

The assay was developed by Kikuzaki method, to determine ferric thiocyanate content. For this, 4 mg of extract liquefied in 4 mL of 99.5% ethanol, 4.1 mL of 2.51% linoleic acid in 99.5 ethanol, 8.0 ml of 0.02 M phosphate buffer (pH 7.0), add 3.9 mL of distilled water in test tubes keep it in the over at 40^{o}C. To a test tube, 0.1 mL of the reaction mixture was transferred from the above solution to a test tube, later added 9.7 mL of 75% hydroethanolic solution, followed by 0.1 mL of 30 % aqueous mL ammonium thiocyanate and 0.1 mL of 0.02 M ferrous chloride in 3.5 % hydrochloric acid. Once the red color is found in the samples indicating ferric thiocyanate presence, the samples were measured spectrometrically to get the absorbance at 500 nm using shimadzu uv- spectrophotometer 1300 series [13].

The ascorbic acid value was used as a positive control, while the reactant without the herbal drug was used as a negative control. The FTC assay was done using the following formula.

% Inhibition= 100- (A_1/A_0) ×100)

A_0 is the absorbance of the control and A_1 is the absorbance of the sample extracts/standard.

2.2.4 Thiobarbituric Acid (TBA) Method

This experiment, added 2 mL of 20% of TCA and 2 mL of 0.67% of TBA was added to 1 ml of the FTC method for sample plant extract solution. As a standard, we used ascorbic acid. The resultant was kept in the water bath for 10 minutes then cooled and certrifuged at 3000 rpm. The supernatant's absorbance was measured at 552 ηm and using shimadzu uv- spectrophotometer 1300 series [14].

2.2.5 Estimation of total phenolic content

The Folin-Ciocalteu method is used to evaluate total phenolic content. In an ultrasonic water bath, extracted 200 mg of finely ground plant material for 20 minutes with 10 mL of 50 %methanol. Under cold condition, the content was centrifuged for 10 minutes and the supernatant was collected. The remaining residue again extracted for 20 minutes with 5 mLof 50% methanol and finally both supernatants were resting for 20 minutes at room temperature [15].

2.2.5.1 (A) Analysis of total phenolics (TP)

Tannic acid standard (0.02-0.10mg/mL) and extract / fraction (0.005 mL) were prepared with triple distilled water (TDW) up to 0.5 mL volume. It was vigorously mixed and kept at room temperature for 40 minutes. Measured the absorption of all tested samples at 725 nm using shimadzu uv- spectrophotometer 1300 series and the total amount of tannic acid equivalent total phenols were determined from the standard tannic acid curve.

2.2.5.2 Analysis of simple phenolics (SP) –

Weigh100 mg polyvinyl pyrrolidone into a test tube. Add to it 1.0 ml distilled water and then 1.0 ml of herbal extract. It was vigorously mixed and kept at room temperature for 40 min. The absorption of all tested samples were measured at 725 nm using shimadzu uv-spectrophotometer 1300 series and the total amount of tannic acid equivalent simple phenols were determined from the standard tannic acid curve.

Total tannins were calculated as follows:

Total tannin content = Total phenols –Simple phenols

2.2.6 Estimation of total flavonoids

The aluminum chloride colorimetric assay estimated total flavonoids. For the experiment 250 µL of each sample with 1.25mL of deionized water and 0.075mL of 5% sodium nitrite were incubated for 6 minutes. Posteriorly, we added, 0.15mL of 10% aluminum chloride waiting for 6 minutes, and then included 0.5 mL of 1M sodium hydroxide and 2.5mL of deionized water in the solution. After 6 minutes, read the samples at 510 nm and the total flavonoids were estimated using shimadzu uv- spectrophotometer 1300 series [16].

Total flavonoids = ($A \times M_o$. /A_0 × M)

A is the absorbance of the plant extract solution, A_o is the absorbance of the standard ascorbic acid solution, M is the weight of plant extract, and M_o is the weight of ascorbic acid solution.

2.3 Antibacterial Activity

Several extracts of plants were performed on antibacterial activity, mainly to *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923.The method of disk diffusion was able to assess the antibacterial activity of *B. flabellifer*. We prepared the culture plates by pouring 20mL (nutrient media containing Müller Hinton Agar, MHA) molten media into sterile Petri plates, with ciprofloxacin (5 mg) standard or control. The experiment included extracts of *B. flabellifer* in the holes of inoculated plates at 37^{o}C for 24 hours, posteriorly inhibition zones around the area of holes were measured in millimeters [17].

3. RESULTS AND DISCUSSION

Table 1 shows IC_{50} values of different extracts of *Borassus flabellifer* for all the 4 methods of antioxidant activity comparing with ascorbic acid as standard**.**

In both DPPH and nitric oxide oxide assay when IC_{50} value were compared, ethanolic fruit extract showed results of 132 and 119 µg/ml respectively as compared to standard ascorbic acid which showed 25 and 35 µg/ml as shown in

Fig 2 . As compared to the other tested extracts, the fruit extracts of *Borassus flabellifer* results revealed good antioxidant properties.

The IC_{50} results of FTC and TBA assay for all the extracts along with ascorbic acid are represented in Fig.3**.** Radical scavenging activity of Ethanol fruit extract showed good results of 160 and 148 µg/mL as compared to standard ascorbic acid 94 and 97 µg/mL. Fair results were observed which can be interpreted as good antioxidant agent.

The antioxidant activity of herbal drugs was reported that phenolic compounds play an important role in stabilizing lipid peroxidation. Table 2 shows the estimated values of the assay. The total phenolic contents of ethanol extracts of leaves are highest (80.23 ± 3.19) and total tannins were also highest (59.77 ± 1.45) as compared to fruit and sap extracts. The aqueous leaf extract showed good results (16.27 ± 4.91) and total tannins in sap extract of water were found to be good with 11.69 ± 5.3.

TFC activity is the process which tells the amount of flavanoid content in the samples. Phenolic compounds that contained in the plants have redox properties, and the properties allow them acting as antioxidants.

As quantified and analysed in Table 3 the ethanol and aqueous extracts of plant, the total flavonoid contents were found to be 42.8µg/100g of aqueous fruit extracts and 53.9 µg/100g of aqueous sap extracts and 21.5µg/100g of ethanol fruit extracts and 32.7 µg/100g of ethanol sap extracts respectively.

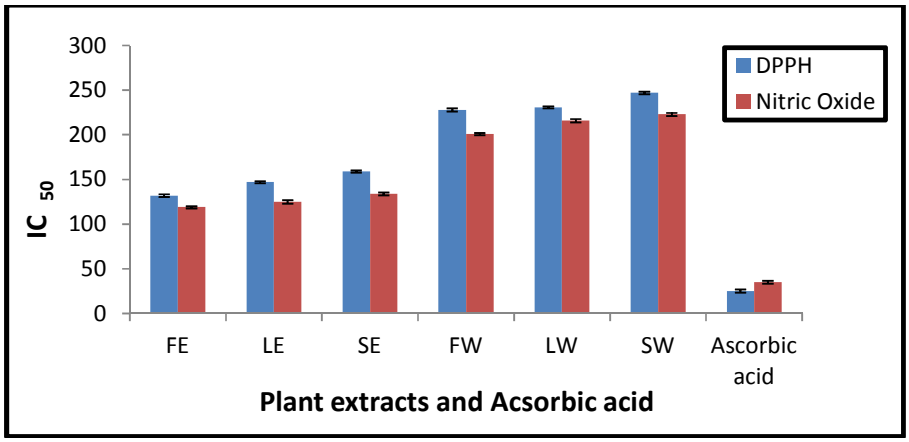

Fig. 2. Comparison of DPPH and nitric oxide assay

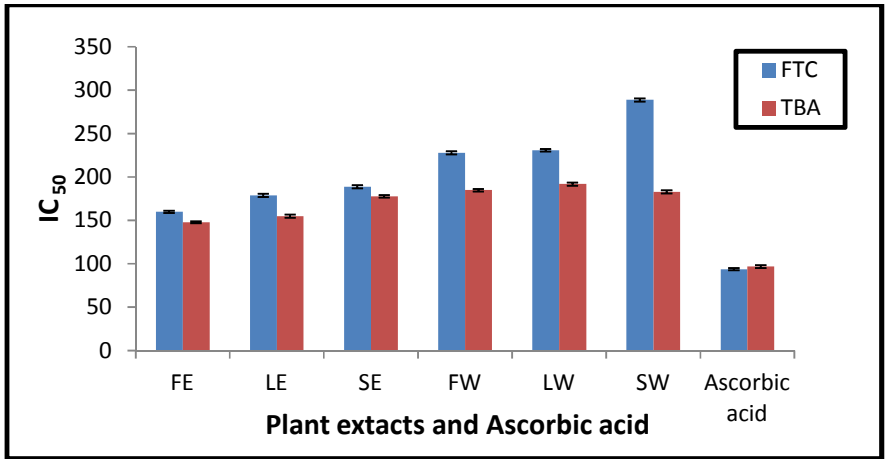

Fig. 3. Comparison of FTC and TBA assay

Table 1. IC_{50}values of *Borassus flabellifer* different extracts by different methods

Name of the compound	Vegetal organ	IC_{50} values			
		DPPH (µg/mL)	Nitric oxide radical inhibition assay (µg/mL)	FTC method	TBA Method
Borassus flabellifer	FE	132 ± 1.58	119 ± 1.28	160 ± 1.23	148 ± 1.16
	LE	147 ± 1.23	125 ± 1.88	179 ± 1.83	155 ± 1.87
	SE	159 ± 1.26	134 ± 1.58	189 ± 1.78	178 ± 1.46
	FW	228 ± 1.88	201 ± 1.34	228 ± 1.91	185 ± 1.46
	LW	231 ± 1.18	216 ± 1.81	231 ± 1.38	192 ± 1.83
	SW	247 ± 1.35	223 ± 1.64	289 ± 1.85	183 ± 1.82
Ascorbic acid		25 ± 1.82	35 ± 1.57	94 ± 1.38	97 ± 1.73

Table 2. Estimation of total phenolic content for different extracts

Phytoconstituent class	Successive extracts (Mean ± SEM)					
	FE	LE	SE	FW	LW	SW
Total phenolics (mg/g TAE)	11.86±4.58	80.23 ± 3.19	50.93 ± 5.11	5.34 ± 2.56	16.27 ± 4.91	14.7 ± 3.5
Total Simple phenolics (mg/g TAE)	2.09 ± 2.3	20.46 ± 1.74	21.16 ± 1.17	1.16 ± 1.78	6.51 ± 3.89	33.94 ± 4.3
Total Tannins (mg/g TAE)	9.77± 2.28	59.77 ± 1.45	29.77 ± 3.33	4.18 ± 0.78	9.76 ± 1.02	11.69 ± 5.3

Table 3. Estimation of total flavonoids content

Sample	Total flavonoid content in µg/100g of extracts
FE	21.5 ± 0.41
LE	25.2 ± 0.52
SE	32.7 ± 0.47
FW	42.8 ± 0.35
LW	48.4 ± 0.36
SW	53.9 ± 0.28

Table 4. Antibacterial activity

Sample tested	Zone of inhibition (mm) for *E. coli*	Zone of inhibition (mm) for *S.aureus*
FE	31.4± 0.58	32.1± 0.34
LE	28.5± 0.23	30.4± 0.68
SE	25.2± 0.82	28.3± 0.74
FW	22.3± 0.49	26.6± 0.82
LW	24.1± 0.49	25.8± 0.79
SW	26.8± 0.28	28.3± 0.49
Ciprofloxacin	32 .4± 0.76	34.8± 0.38

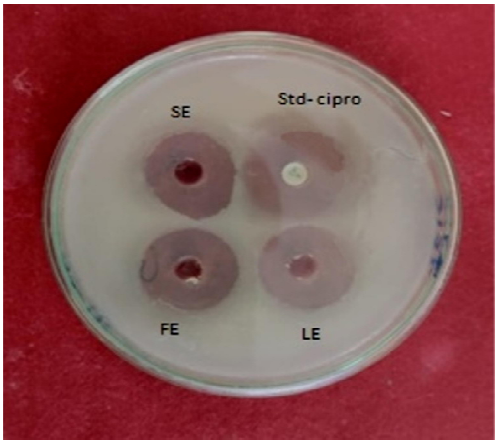

Fig. 4. Zone of inhibition of ethanolic fruit extracts

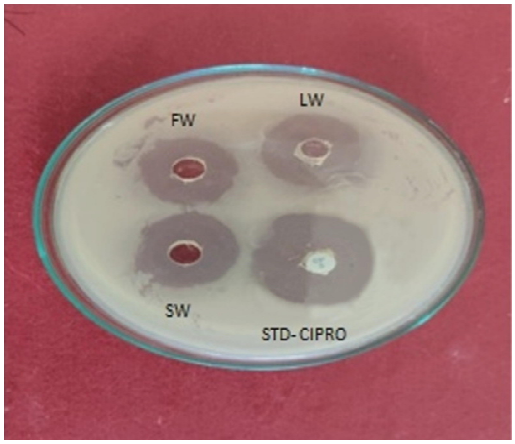

Fig. 5. Zone of inhibition of and aqueous fruit extracts

These properties of plant are because of the phytochemical compounds are responsible for the antioxidant properties of *Borassus flabellifer.*

The extracts prepared were investigated to evaluate their antibacterial property against two strains of organisms, *E. coli* (Gram negative) and *S. aureus* (Gram positive) by cup plate method. Evaluation of antibacterial activity of these plant extracts was recorded in Table 4 and illustrated in Fig.4 and Fig.5.

The results showed the suppression of the growth of bacteria by the plant extracts. The growth inhibition property was found to be 31.4 mm in ethanolic fruit extract which was compared with ciprofloxacin as standard which showed the zone of inhibition of 32.4 mm against *e-coli*. In case of *s-aureus,* ciprofloxacin showed 34.8 mm of zone of inhibition and zone of inhibition for ethanol fruit extract was found to be 32.1 mm. Thus it was observed, that both ethanolic and aqueous fruit extracts more potent results as compared to all other plant extracts analyzed.

4. CONCLUSION

In the present study, *Borassus flabellifer* fruit, juice, sap and leaves were tested for antioxidant and antibacterial activities. Extracts were prepared by Soxhlet extractor. Ethanol and water extracts of *Borassus flabellifer* fruit have been shown to promote antioxidant function in conventional pharmaceutical products for the treatment of inflammatory conditions. This study also verified that extracts of *Borassus flabellifer* fruit are possible source of antioxidants and could be used to prevent free radical-related illnesses. However, the isolation and characterization of the active constituents responsible for the above activities require further work. Antimicrobial studies of both ethanolic and aqueous fruit extract also showed a potent zone of inhibition when compared to the standard. The fruit extract results revealed promising antimicrobial and antioxidant properties.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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