EVALUATION OF INVITRO ANTIOXIDANT POTENTIAL OF BORASSUS FLABELLIFER

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Abstract

The present study was carried out on the plant of Borassus Fabilifer having several pharmacological actions including analgesic and antipyretic effects, anti- inflammatory activity, haematological, biochemical parameters and immunosuppressant property. In the present study in-vitro antioxidant activities of aqueous, ethanol & hydro alcoholic extracts of Palm shoot of Borassus fabilifer was evaluated by DPPH, Hydroxyl, Nitric oxide, H_2O_2 methods respectively. In antioxidant studies ethanol extract shows the significant activity in DPPH method and hydroxyl radical scavenging activity, aqueous extract shows significant activity in nitric oxide method and hydro alcoholic extract had better activity in H_2O_2 scavenging method than the standard (Ascorbic acid).

Key words: - Borassus fabilifer, DPPH, Hydroxyl, Nitric oxide, H_2O_2, Ascorbic acid.

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INTRODUCTION[1-3]

Borassus flabellifer (Arecaceae) is a tall tree (palm) growing in sandy soil and attaining a height of about 20-30 meters with a straight trunk. The fruits are large and fibrous, containing usually three nuts like portions each of which encloses a seed. Flowers and fruits during December to August. The plant has been used traditionally as a stimulant, anti-leprotic, diuretic and antiphlogistic. The fruits are stomachic, sedative, laxative and aphrodisiac in nature useful in hyperdipsia, dyspepsia, flatulence, skin diseases, haemorrhages, fever and general debility. The roots and juice of the plant are useful in inflammatory reactions. The ash obtained by burning the inflorescence is a good antacid antiperiodic, and is useful in heart burn, splenomegaly. Survey of literature revealed that the medicinal plant Borassus flabellifer Linn. have been used as antidiabetic, antidote, anti-inflammatory, wound healing, anthelmintic activity, analgesic and antipyretic. It has been reported that the methanolic extract from the male flowers of Borassus flabellifer Linn. was found to inhibit the increase of serum glucose levels in sucrose-loaded rats which may be due to presence of spirostane-type steroid saponins. It also has been documented to possess immunosuppressant property.

MATERIALS AND METHODS

Collection of Plant material

The shoot of Borassus fabilifer were collected from Chinthareddypalem village, Nellore district, Andhra Pradesh, India. The shoots were authenticated by botanist Dr. K. Madhav chetty assistant professor in Department of botany, Sri Venkateswara University, Tirupathi-517 502, A.P., India. The voucher specimen was preserved in the Department of Pharmacognosy, Narayana pharmacy college, Nellore, Andhra Pradesh, India.

Extraction Procedure[4]

The shoots were shade dried and powdered mechanically. The powder was extracted individually by using water, ethanol and hydro alcohol (2:3) The ethanol, aqueous and hydro alcoholic extracts were screened for phytochemical constituents and evaluated for in-vitro antioxidant activity.

Phytochemical screening[5,6]

Extracts were screened for the presence of active principles such as flavonoids, phenols, steroids and triterpenoids, using following standard procedures.

Determination of total phenolic content[7]

The total phenolic content was determined by Folin- Ciocalteau reagent method. The content of the phenols was determined as gallic acid equivalent. Stock solution (1mg/ml) of the ethanol aqueous and hydro alcoholic extracts were prepared in respective solvents. From the stock solutions 1ml of the extract was taken into a 25 ml volumetric flask. To this 10 ml of water and 1.5 ml of Folin-Ciocalteau reagent were added. The mixture was kept aside for 5 min, followed by addition of 4 ml of 20% Sodium Carbonate solution and volume was made up to 25ml with distilled water. The mixture was kept aside for 30 min and the absorbance was recorded at 765nm using UV-Vis spectrophotometer. The total phenolic content was determined from extrapolation of calibration curve which was made by preparing gallic acid solution (50-150 µg/ml) in distilled water.

Determination of total Flavonoid content[8]

The total flavonoid content was determined by aluminium chloride colorimetric method. The content of the flavonoids was determined as gallic acid equivalent. 1 ml of sample (1 mg/ml) was mixed with 3 ml of methanol, 0.2 ml of 10% aluminium chloride, 0.2 ml of 1 M potassium acetate and 5.6 ml of distilled water and remains at room temperature for 30 min. The absorbance of the reaction mixture was measured at 420nm with UV- Visible spectrophotometer. The total flavonoid content was determined from extrapolation of calibration curve which was made by preparing gallic acid solution (50-150 µg/ml) in distilled water.

In-vitro antioxidant and radical scavenging activity

DPPH radical scavenging activity [9,10]

Ascorbic acid and dried extracts were weighed (10 mg each) and dissolved in 10 ml of methanol to get 1 mg/ml (1000 µg/ml) stock solutions separately. Lower concentrations of ascorbic acid and extracts (20, 40, 60, 80, 100 µg/ml respectively) were prepared by serially diluting stock solutions. The stable DPPH radical was used for determination of free radical-scavenging activity of the extracts. The 0.1 mM solution of DPPH in methanol (2.22 mg in 100 ml) was freshly prepared. Different concentrations of 3 ml of each extracts were added to 1 ml of methanolic solution of DPPH. After 30 min at room temperature, the absorbance was recorded at 517 nm. IC_{50} values denote the concentration of sample, which is requiring scavenging 50% of DPPH free radicals.

Radical scavenging activity was calculated by the following formula.

% of $radical\ scavenging\ activity$

$$= [(\frac{A_C - A_S}{A_C})] \times 100$$

Where Ac: Absorbance of control, As: absorbance of standard/extract.

Hydroxyl radical scavenging activity[11,12]

The stable Hydroxyl radical was used for determination of free radical-scavenging activity of the extracts. 2 ml of various (20, 40, 60, 80, 100 µg/ml) concentrations of extracts or standard were added to deoxy ribose (3 mM, 0.4 ml), ferric chloride (0.1 mM, 0.4 ml), EDTA (0.1 mM, 0.4 ml), ascorbic acid (0.1 mM, 0.4 ml) and hydrogen peroxide (2 mM, 0.4 ml) in phosphate buffer (pH, 7.4, 20 mM) and the reaction mixture was incubated at 37ºC for 30 min after incubation, the reaction was stopped by adding ice cold trichloroacetic acid (0.2 ml, 15% w/v) and thiobarbituric acid (0.2 ml, 1% w/v), in 0.25 N HCl. The mixture was kept in a boiling water bath for 30 min, cooled and absorbance was measured at 532 nm. IC_{50} values denote the concentration of sample, which is requiring scavenging 50% of hydroxyl free radicals. Radical scavenging activity was calculated by the following formula.

% of $radical\ scavenging\ activity$

$$= [(\frac{A_C - A_S}{A_C})] \times 100$$

Where A_C**:** Absorbance of control,
A_S**:** Absorbanceof standard/extract.

Nitric oxide radical scavenging activity[13-15]

The stable nitric oxide radical was used for determination of free radical-scavenging activity of the extracts. At different (20, 40, 60, 80, 100 µg/ml) concentrations 4 ml of extracts were added to 1 ml of sodium nitroprusside (25 mM), and incubated at 37ºC for 2 hr. an aliquot (0.5 ml) of the incubation solution was removed and diluted with 0.3 ml Griess reagent (1% sulfanilamide in 5% H_3PO_4 and 0.1% naphthylethylenediamine dihydrochloride equal amount), the absorbance was recorded at 517 nm. IC_{50} values denote the concentration of sample, which is requiring scavenging 50% of nitric oxide free radicals. Radical scavenging activity was calculated by the following formula.

% of $radical\ scavenging\ activity$

$$= [(\frac{A_C - A_S}{Ac})] \times 100$$

Where A_C**:** Absorbance of control,
A_S**:** Absorbanceof standard/extract.

Hydrogen peroxide scavenging activity[16,17]

The stable nitric oxide radical was used for determination of free radical-scavenging activity of the extracts. At different (20, 40, 60, 80, 100 µg/ml) concentrations 4 ml of extracts were added to 0.6 ml of 4 mM H_2O_2 solution was prepared in phosphate buffer (0.1 M pH 7.4) and incubated for 10 min and the absorbance of the solution was recorded at 230 nm. IC_{50} values denote the concentration of sample, which is requiring scavenging 50% of hydrogen peroxide free radicals. Radical scavenging activity was calculated by the following formula.

% of $radical\ scavenging\ activity$

$$= [(\frac{A_C - A_S}{A_C})] \times 100$$

Where A_C**:** Absorbance of control, A_S**:** Absorbance ofstandard/extract.

RESULTS & DISCUSSION

Phytochemical Screening

The ethanol, aqueous & hydro alcoholic extracts of *Borassus fabillifer* was screened for its various phytoconstituents by standard chemical tests. Aqueous extracts were found to contain flavonoids, phenols, and steroids, ethanolic extracts was found to contain flavonoids, anthocyanin, phenols and hydro alcoholic extract contains phenols, flavonoids and triterpenoids. The results were represented in Table No.1.

Determination of total phenolic and total flavonoid Content of extracts

In the three extracts ethanol extract contains high total phenolic and total flavonoid content and aqueous, hydro alcoholic extracts having less phenolic and flavonoid contents compared to ethanol extract. The results were represented Table No.2,Figure No.1, 2.

In-vitro **antioxidant studies DPPH radical scavenging activity**

This assay showed the abilities of the extract and standard ascorbic acid to scavenge DPPH radical at concentration range of 20-100µg/ml in a concentration dependent manner. Decrease in absorbance with increase in concentration indicates a concentration response relationship in DPPH scavenging activity of extracts. The ethanol extract of *Borassus fabilifer*. Shown significant $DPPH^{\cdot}$ scavenging effect with an IC_{50}value of 14µg/ml and the percentage inhibition is 82.72% compared to ascorbic acid. The results were represented in Table No.3,

Figure No. 3.

Hydroxyl radical scavenging activity of extracts

The degradation of hydroxyl radical scavenging wassignificantly decreased by *Borassus fabilifer*. and ascorbic acid at concentration range of 20-100 µg/ml in concentration dependent manner, proving the significant hydroxyl radical scavenging activity of extracts. The ethanol extract showed significant antioxidant activity (IC_{50}=18 µg/ml and percentageinhibition value is 78.73%) compared to standard (IC_{50}=14.5 µg/ml and percentage inhibition value is 79.33%). The results were represented in Table No.4, Figure. No.4.

Nitric oxide radical scavenging activity of extracts

The extracts showed a significant nitric oxide scavenging activity between concentration range of 20 to 100 μg/ml in a concentration dependent manner. Aqueous extract of *Borassus fabilifer*. showed significant nitric oxide scavenging activity, the (IC_{50}) value of the acetone extract was found to be 44 µg/ml and percentage inhibition value is 78.21% and standard ascorbic acid (IC_{50}) value was 40 µg/ml and percentage inhibition value is 83.56%. The results were represented in Table No. 5, Figure No. 5.

Hydrogen peroxide scavenging activity of extracts

The extracts was able to neutralize H_2O_2in a concentration dependent manner at a concentration range of 20-100 µg/ml. Hydro alcoholic (IC_{50}=15 µg/ml and the percentage inhibition value is 82.72%) extract showed better antioxidant activity than the standard ascorbic acid (IC_{50}=41 µg/ml and the percentage inhibition value is 81.86%). The results were represented in Table No.6, Figure No. 6.

Table No.1: Phytochemical screening of the methanol, acetone and ethyl acetate extracts of Borassus fabilifer

S.No.	Plant constituents	Inference		
		Ethanol extract	Aqueous extract	Hydro alcoholic extract
1	Alkaloids	+	+	+
2	Carbohydrates	+	+	+
3	Flavonoids	+	+	+
4	Phenols	+	+	+
5	Steroids	-	+	+
6	Triterpenoids	-	-	-

Table No.2: Total phenolic and total flavonoid contents in ethanol, aqueous and hydro alcoholic extracts of *Borassus fabilifer*

S. No	Extract	Total phenolic content (Mean±SEM) (GAE µg/g of dry material)	Total flavonoid content (Mean±SEM) (GAE µg/g of drymaterial)
1	Ethanol extract	100.5±0.402	116.25±0.465
2	Aqueous extract	89.5±0.358	58.2±0.233
3	Hydroalcoholic extract	73±0.292	43.5±0.174

Table No.3: DPPH radical scavenging activity of ethanol, aqueous and hydroalcoholic extracts of *Borassus fabilifer*

S. No	sample	Concentration	Absorbance (Mean±SEM)	Percentageinhibition (Mean±SEM)	IC_{50}
1	**Methanol extract**	20µg/ml	0.058±0.003	64.41±1.623	14µg/ml
		40µg/ml	0.047±0.001	71.16±0.710	
		60µg/ml	0.051±0.002	74.84±7.110	
		80µg/ml	0.037±0.003	77.09±1.781	
		100µg/ml	0.020±0.001	82.72±0.707	
2	**Acetone extract**	20µg/ml	0.077±0.004	52.75±0.707	19µg/ml
		40µg/ml	0.059±0.002	63.80±1.275	
		60µg/ml	0.055±0.002	66.25±1.062	
		80µg/ml	0.047±0.002	70.95±1.082	
		100µg/ml	0.044±0.006	73.00±3.418	
		20µg/ml	0.057±0.002	65.02±0.938	

3	**Ethyl acetate extract**	40µg/ml	0.054±0.002	66.87±1.062	15µg/ml
		60µg/ml	0.054±0.002	67.07±1.139	
		80µg/ml	0.041±0.001	74.84±1.062	
		100µg/ml	0.040±0.002	75.72±1.242	
4	**Ascorbic acid**	20µg/ml	0.056±0.003	65.84±1.816	12µg/ml
		40µg/ml	0.059±0.005	63.59±2.861	
		60µg/ml	0.048±0.002	70.01±0.775	
		80µg/ml	0.039±0.002	76.07±1.062	
		100µg/ml	0.057±0.002	89.63±1.005	

Table No.4: Hydroxyl radical scavenging activity of ethanol, aqueous and hydroalcoholic extracts of Borassus fabilifer

S. No	Sample	Concentration	Absorbance (Mean±SEM)	Percentage inhibition (Mean±SEM)	IC_{50}
1	**Ethanolextract**	20 µg/ml	0.187±0.001	52.29±0.294	18 µg/ml
		40 µg/ml	0.176±0.003	55.10±0.779	
		60 µg/ml	0.162±0.001	58.58±0.372	
		80 µg/ml	0.122±0.006	68.95±1.532	
		100 µg/ml	0.092±0.005	78.73±3.351	
2	**Aqueous extract**	20 µg/ml	0.171±0.002	56.29±0.450	18.5 µg/ml
		40 µg/ml	0.154±0.002	60.71±0.589	
		60 µg/ml	0.142±0.002	63.77±0.442	
		80 µg/ml	0.118±0.001	69.97±0.372	
		100 µg/ml	0.095±0.003	75.76±0.739	
3	**Hydro alcoholic extract**	20 µg/ml	0.174±0.007	55.52±1.863	19 µg/ml
		40 µg/ml	0.175±0.005	55.35±1.170	
		60 µg/ml	0.150±0.002	61.64±0.615	
		80 µg/ml	0.143±0.002	63.60±0.456	
		100 µg/ml	0.135±0.002	65.55±0.532	
4	**Ascorbic acid**	20 µg/ml	0.142±0.002	64.93±0.450	14.5 µg/ml
		40 µg/ml	0.117±0.001	70.23±0.226	
		60 µg/ml	0.106±0.003	72.86±0.756	
		80 µg/ml	0.090±0.004	76.95±1.001	
		100 µg/ml	0.081±0.002	79.33±0.388	

Table No.5: Nitric oxide radical scavenging activity of ethanol, aqueous and hydroalcoholic extracts of Borassus fabilifer

S. No	Sample	Concentration	Absorbance (Mean±SEM)	Percentageinhibition (Mean±SEM)	IC_{50}
1	**Ethanol extract**	20µg/ml	0.120±0.001	44.16±0.55	44µg/ml
		40µg/ml	0.113±0.003	50.07±1.351	
		60µg/ml	0.087±0.001	59.22±0.677	
		80µg/ml	0.070±0.003	63.87±0.674	
		100µg/ml	0.057±0.001	73.33±0.674	
2	**Aqueous extract**	20µg/ml	0.114±0.001	46.66±0.674	38µg/ml
		40µg/ml	0.111±0.001	48.36±0.805	
		60µg/ml	0.091±0.001	57.51±0.677	
		80µg/ml	0.074±0.002	65.26±1.085	
		100µg/ml	0.057±0.002	78.21±1.351	
3	**Hydroalcoholic extract**	20µg/ml	0.118±0.003	44.95±1.480	48µg/ml
		40µg/ml	0.114±0.001	46.81±0.558	
		60µg/ml	0.093±0.002	56.89±0.820	
		80µg/ml	0.078±0.001	63.87±0.674	
		100µg/ml	0.059±0.003	72.55±1.493	
		20µg/ml	0.120±0.002	44.18±0.97	
		40µg/ml	0.090±0.005	56.42±1.528	

4	**Ascorbic acid**	60µg/ml	0.067±0.001	68.74±0.544	40µg/ml
		80µg/ml	0.058±0.002	73.01±0.805	
		100µg/ml	0.035±0.001	83.56±0.677	

Table No.6: H_2O_2 radical scavenging activity of ethanol, aqueous and hydroalcoholic extracts of *Borassus fabilifer*

S. No	Sample	Concentration	Absorbance (Mean ±SEM)	Percentageinhibition (Mean±SEM)	IC_{50}
1	**Ethanol extract**	20µg/ml	0.128±0.001	37.41±0.712	80µg/ml
		40µg/ml	0.121±0.006	40.68±2.786	
		60µg/ml	0.119±0.003	41.49±1.276	
		80µg/ml	0.103±0.002	49.67±0.867	
		100µg/ml	0.074±0.003	63.72±1.497	
2	**Aqueous extract**	20µg/ml	0.121±0.002	40.51±0.909	53µg/ml
		40µg/ml	0.112±0.001	44.92±0.712	
		60µg/ml	0.095±0.003	53.59±1.558	
		80µg/ml	0.083±0.002	59.31±0.748	
		100µg/ml	0.056±0.002	72.70±1.143	
3	**Hydro alcoholic extract**	20µg/ml	0.115±0.002	64.41±1.623	15µg/ml
		40µg/ml	0.105±0.002	71.16±0.710	
		60µg/ml	0.097±0.003	74.84±7.110	
		80µg/ml	0.080±0.001	77.09±1.781	
		100µg/ml	0.064±0.003	82.72±0.707	
4	**Ascorbic acid**	20µg/ml	0.113±0.001	44.60±0.566	41 µg/ml
		40µg/ml	0.096±0.001	52.94±0.566	
		60µg/ml	0.056±0.004	72.38±1.928	
		80µg/ml	0.049±0.003	76.15±1.278	
		100µg/ml	0.037±0.002	81.86±1.020	

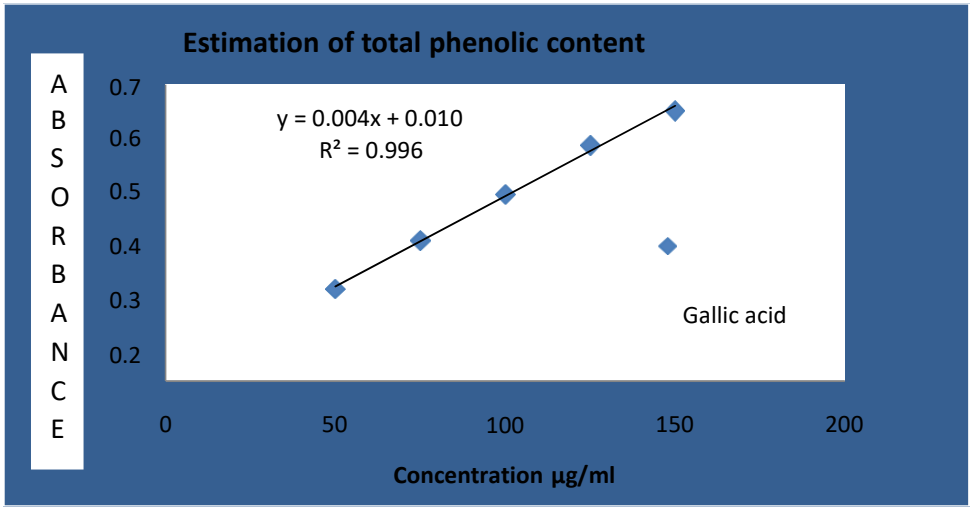

Figure No.1: Standard calibration curve of gallic acid for estimation of total phenolic content

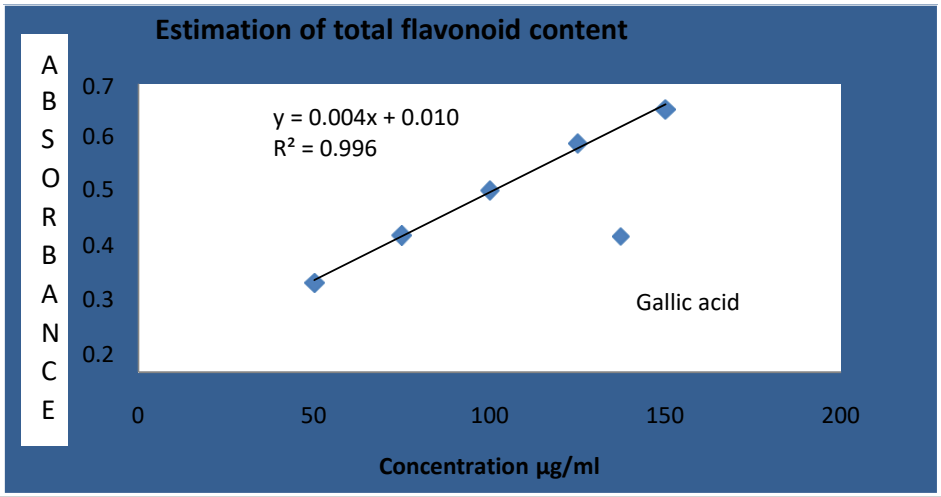

Figure No.2: Standard calibration curve of gallic acid for estimation of total flavonoid content

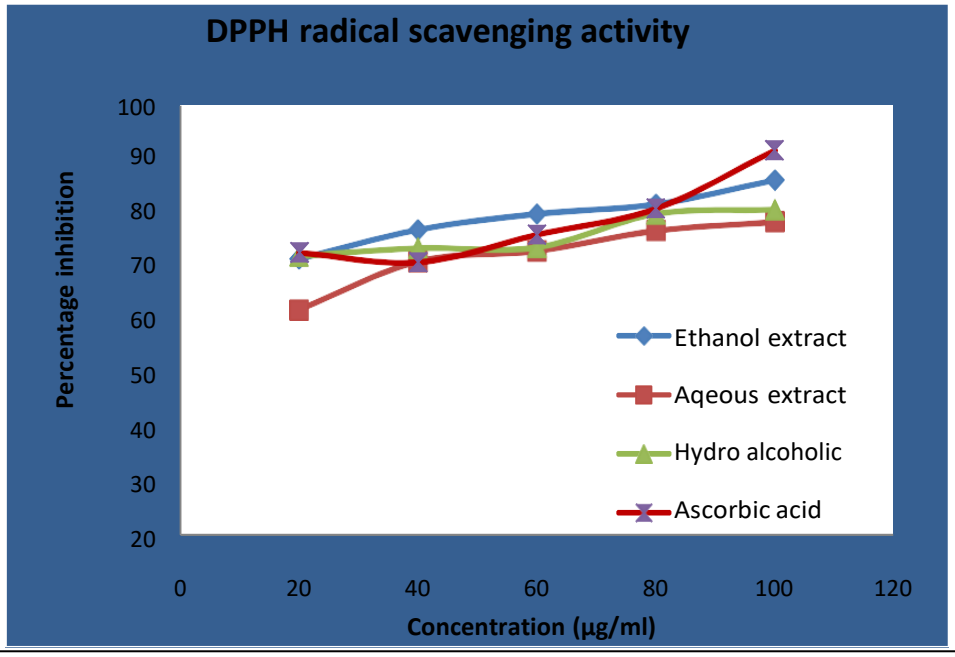

Figure No.3 DPPH radical scavenging activity of Ethanol, Aqeous extract, hydro alcoholic and ascorbic extracts of *Borassus fabilifer* Values are expressed as the Mean±SEM, (n=3)

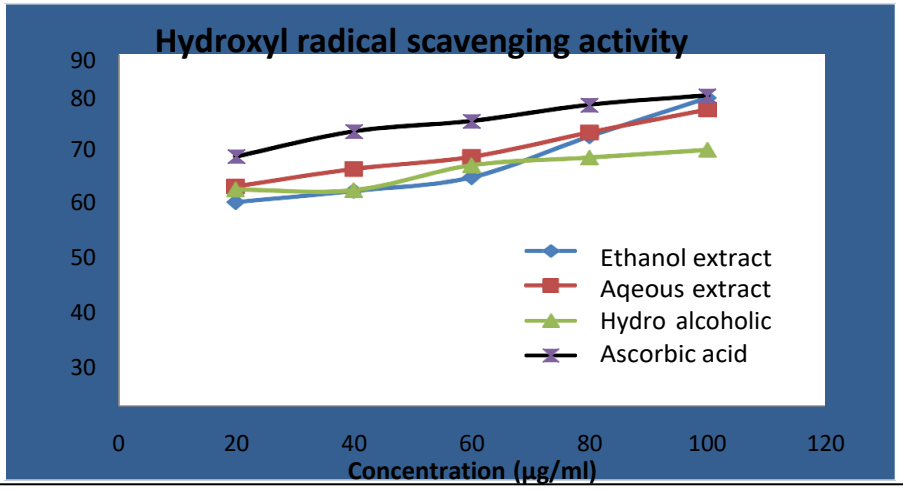

Figure No.4 Hydroxyl radical scavenging activity of Ethanol, Aqeous extract, hydro alcoholic and ascorbic extracts of *Borassus fabilifer* Values are expressed as the Mean±SEM, (n=3)

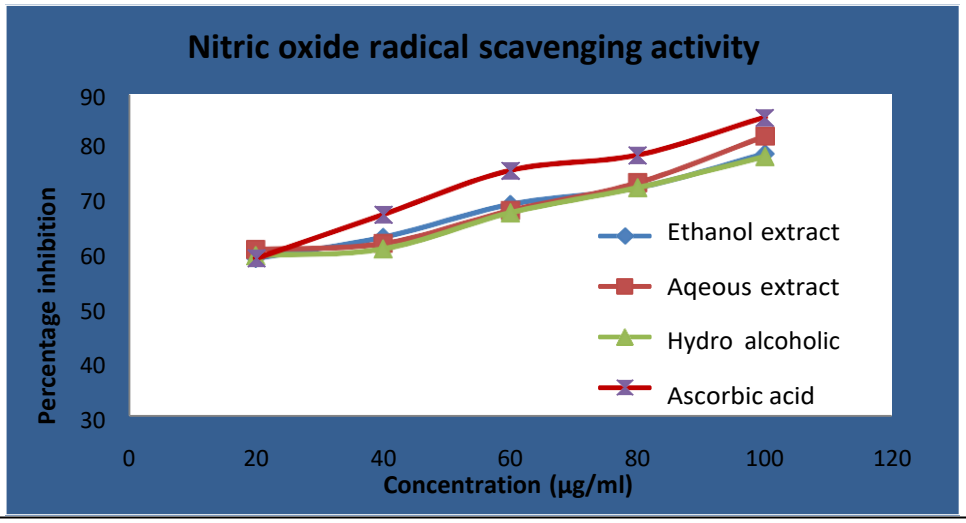

Figure No 5 Nitric oxide radical scavenging activity of Ethanol, Aqeous extract, hydro alcoholic and ascorbic extracts of *Borassus fabilifer* Values are expressed as the Mean±SEM, (n=3)

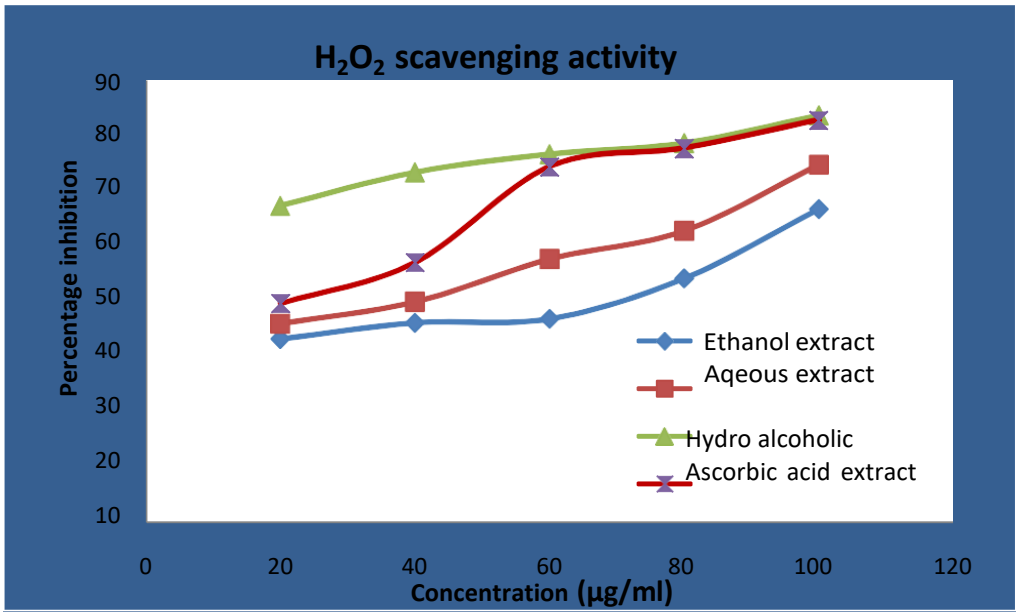

Figure no: 6 Nitric oxide radical scavenging activity of ethanol, Aqeous extract, Hydro alcoholic and ascorbic Barassus fabilifer Values are expressed as the Mean±SEM, (n=3)

CONCLUSION

Shoots were collected and authenticated, shade dried, powdered and extracted by using Ethanol, aqeous, hydro alcoholic solvents individually in Soxhlet apparatus. The Ethanol, Aqeous, hydro alcoholic shoot extracts of *Borassus fabilifer*. was evaluated for *in-vitro* antioxidant studies by using DPPH, Nitric oxide, Hydroxyl and H_2O_2 radical scavenging method. In antioxidant studies Ethanol extract showed significant antioxidant activity in DPPH and Hydroxyl radical scavenging method in nitric oxide method aqueous extract possess significant activity compared to standard and in hydro alcoholic extract shows excellent activity than the standard in H_2O_2 scavenging activity. The plant can be viewed as the potential source of natural antioxidant can afford precious functional components.

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BIBLIOGRAPHY

1. MohdAslam, ShaikhImtiyaz, Kamal Ahmad, Heenakauser S, NajeebJahan, Abdulah. Rosa damascene: a flower with medicinal properties, Universal Journal of Pharmacy, 02(04), 2013, 15-19.

2. Gholamhoseinian A, Fallah H, Sharifi far F. Inhibitory effect of methanol extract of Rosa damascena Mill.flowers on α-glucosidase activity and postprandial hyperglycemia in normal and diabetic rats, Phytomedicine, 16, 2009, 935-941.
3. Sadraei H, Asghari G, Emami S. Inhibitory effect of Rosa damascena Mill.flower essential oil, geraniol and citronellol on rat ileum contraction, Research in Pharmaceutical Sciences, 8(1), 2013, 17-23.
4. Kurhade BB, Vite MH, Nangude SL. Antibacterial activity of Rosa damascena Mill. International Journal of Research in Pharmaceutical and Biomedical Sciences, 2(3), 2011, 1015-1019.
5. Kokate CK, Purohit AP, Ghokale SP. Pharmacognosy, 2nd edition, NiraliPrakashan, 2006, 593-597.
6. Khandelwal KR. Practical Pharmacognosy, 15th edition, NiraliPrakashan, 2006, 149-153.
7. Singleton VL and Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagent, American Journal of Ecology and Viticulture, 16, 1965, 144-148.
8. Olayinka A Aiyegoro and Anthony Okoh. Preliminary phytochemical screening and In-vitro antioxidant activities of the aqueous extract of Helichrysumlongifolium DC, Bio Med Central Complementary and Alternative Medicine, 10, 2010, 21-29.
9. Harlalka VG, Patil CR, Patil MR, Protective effect of Kalanchoepinnatapers on gentamycin induced nephrotoxicity in rats, Indian Journal of Pharmacology, 39, 2007, 201-205.
10. Bushra S, Farooq A, Muhammad A (2009). Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. Molecules, 14: 2167-2180
11. Ozsoy N, Can A, Yanardag R, Akev N. Antioxidant activity of Smilax excels L. leaf extracts, Food Chemistry, 110, 2008, 571-583.
12. Chen C, Pearson AM, Gray JI (1992). Effects of synthetic antioxidants (BHA, BHT and PG) on the mutagenicity of IQ-like compounds. Food Chem., 43: 177-183.
13. Yen GC, Lai HH, Chou HY. Nitric oxide scavenging and antioxidant effects of urariacrinita root, Food Chemistry, 74, 2001, 471-478.
14. Davis W, Lamson MS, Matthew S, Brignall ND (2000). Antioxidants and cancer III: quercetin. Alter. Med. Rev., 5:196-208.
15. Antolovich M, Prenzler PD, Patsalides E, McDonald S, Robards K. Methods for testing antioxidant activity. Analyst. 2002; 127 (1): 183-98.
16. Bozin B, Mimica-Duki N, Samojlik I, Goran A, Igic R. Phenolics as antioxidants in garlic (Allium sativam L., Alliaceae), Food Chemistry, 111, 2008, 925-929.
17. Santos-Sánchez NF, Salas-Coronado R, Villanueva-Cañongo C, Hernández-Carlos B. Antioxidant compounds and their antioxidant mechanism. Antioxidants. 2019 Mar 22; 10:1-29.