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Research Article

ANTIOXIDANT ACTIVITY OF *BORASSUS FLABELLIFER*

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ABSTRACT

The present study was aimed to evaluate the antioxidant activity traditional medicinal plant, *Borassus flabellifer.* The root/rhizome part of plant was used for preparation of extracts using maceration process using different solvents. The extracts of plants were studied for free radical scavenging activity on 2, 2-diphenyl-1-picrylhydrazyl (DPPH), hydroxyl and superoxide free radicals and antioxidant capacity was studied using ferric thiocyanate (FTC) method. The selected plant extracts showed the concentration dependent percentage inhibition on tested free radicals. The extracts showed the good scavenging activity on DPPH free radical and less on hydroxyl free radical. The methanol extract of the *Borassus flabellifer* showed the good percentage inhibition on tested free radicals compared to other extracts and is comparable with the ascorbic acid. The results of the FTC method also showed that the selected plant extracts have antioxidant capacity. The extracts of selected plants showed the lower absorbance compared to the control. The methanol extract reduced the amounts of peroxides from the linolenic acid oxidation with the percentage of 53.17 on 7th day of FTC method.

Keywords: *Borassus flabellifer,* roots, free radicals and Antioxidant activity.

INTRODUCTION

Reactive oxygen species (ROS) are the unbalanced molecules generate through different cellular metabolisms in the body. These molecules search out for stable molecules to get them balance, in this process they damage the cellular components[1,2]. The over production of ROS or decline in antioxidants in body, leads to cause oxidative stress[3]. The oxidative stress leads to different acute and chronic diseases to people around the world. The balance in the oxidants and antioxidants will produce the well biological metabolisms and this provides healthy life to people[4]. In recent years many researchers showing their interest in finding the new antioxidants from natural sources, because of present lifestyle of worlds' population increased formation of more ROS in the body and they are causing aging of people and different diseases. Many researchers were reported different antioxidant from natural resources like medicinal plants, marine sources and etc[5, 6]. But, there were many medicinal plants were remained on planet without identification their medicinal use scientifically. In this point of view, we selected the one of such medicinal plant *Borassus flabellifer* in the present study. *B. flabellifer* is a dumb palm tree resident to Indian sub-continent and Africa belongs to the family Arecaceae. Different parts of *B. flabellifer* have been using as medicine for diverse illnesses in traditional medicine[7]. Different phytochemical constituents like steroids, glycosides, vitamins etc were reported from *B. flabellifer*[8]. Different biological activities like cytotoxicity, antiarthritic activity, antibacterial activity, analgesic activity, antipyretic activity and hypoglycemic activity were reported by many researchers through their studies[9-12]. But, there were no scientific reports on antioxidant activity of rhizome parts of *B. flabellifer.* In this regards, the present work carried out to evaluate the antioxidant activity of *B. flabellifer* rhizome (roots) part.

MATERIALS AND METHODS

Chemicals and Drugs

The chemicals and solvents used in the current study were analytical grade. 2,2-diphenyl-1-picrylhydrazyl (DPPH), Linolenic acid, Ammonium thiocyanate, Ferrous chloride, Butylated hydroxytoluene (BHT) were purchased from Sigma chemicals, USA, Nitroblue tetrozolium, was purchased from Sisco Research Laboratories Pvt Ltd., Mumbai. Riboflavin was purchased from Loba Chemie Pvt Ltd., Mumbai.

Collection of plant materials and preparation of extracts

The plant material *Borassus flabellifer* was collected at near Bheemili region, Visakhapatnam, Andhra Pradesh and authenticated by the taxonomist Rtd. Prof. M. Venkaiah, Depart of Botany, Andhra University. Freshly collected plant root materials were dried under shade and the dried material was milled to obtain a powder. The powdered material was separately extracted with different solvents like with ethyl acetate, chloroform and methanol successively using Soxhlet apparatus. Finally the collected solutions were concentrated to dryness under vacuum by using Rota-vapor to get the dry extract and stored in desiccators.

Antioxidant activity

Free radicals scavenging activity was studied for prepared extracts of selected plants using Dimethyl sulphoxide (DMSO) as vehicle on superoxide, hydroxyl and DPPH free radicals[13, 14]. The percentage inhibition and IC_{50} values were calculated.

Superoxide radical scavenging activity

Superoxide scavenging activity of the selected plant extracts were evaluated as per standard methods. It is by absorption of light at 560 nm induction of superoxide free radical generation by riboflavin and corresponding reduction by nitroblue tetrazolium[15].

Hydroxyl radical scavenging activity

The scavenging activity of selected plants extracts on hydroxyl radical was measured as per established method. It was studied by the competition between deoxyribose and the extract's antioxidant molecules for hydroxyl radicals generated from the Fe+2/ EDTA/H_2O_2 system[16].

DPPH radical scavenging activity

The DPPH radical scavenging activity was measured as per methods. This method is based on measure of color absorbance of alcoholic DPPH solution (Blue color) after addition of antioxidant solution (Extract/Compound). If antioxidants present in the test compound blue color yellow color due to DPPH[17, 18].

Calculation of Percentage Inhibition

The percentage inhibition of superoxide production by the extract was calculated using the formula:

Inhibitory ratio = $(Ao-A_1) \times 100/ A_0$

Ao: Absorbance of control; A_1: Absorbance of plant extract or/and Ascorbic acid.

IC_{50} calculation form percentage inhibition

The optical density obtained with each concentration of the extract/ascorbic acid was plotted taking concentration on X-axis and percentage inhibition on Y-axis. The graph was extrapolated to find the 50% inhibition concentration of extract/ ascorbic acid.

Ferric Thiocynate (FTC) method

Antioxidant activity of selected plants was evaluated using standard method[19-21]. In this method, different extracts and standard drug (4mg) were dissolved in 4mL ethanol, then mixed with 4.1mL of 2.52% linolenic acid in ethanol, 8mL of 0.02 M phosphate buffer (pH 7.0) and 3.9mL of distilled water. The mixture was placed at 40°C and then 0.1mL was mixed with 9.7mL of 75% (v/v) ethanol and 0.1mL 30% ammonium thiocyanate. Three minutes after adding the ferrous chloride (0.1mL of 2×10^{-2} M ferrous chloride in 3.5% hydrochloric acid), the absorbance was measured at 500nm in a spectrophotometer. This step was repeated every 24 h until the control (ethanol, the mixture without added sample) reached its maximal absorbance value. The percentage (%) inhibition of lipid peroxidation was estimated by the following formula:

% inhibition= $100-((A_1/A_0) \times 100)$

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

Statistical analysis

The results of the present study expressed in mean ± SEM.

Table 1: IC 50 values of *Borassus flabellifer* rhizome extracts on different free radicals

Name of the plant/ compound	Name of the extract	IC 50 value in µg on different free radicals		
		DPPH	Hydroxy l	Superoxide
Borassus flabellifer	Ethyl acetate	347	ND	292
	Chloroform	221	357	ND
	Methanol	186	175	196
Ascorbic acid	-	95	83	130

ND: Not detected

Table 2: Percentage inhibition of *B. flabellifer* on peroxidation in linolenic acid system in FTC method on seventh day

Name of the plant/ compound	Name of the extract	Percentage inhibition
B. flabellifer	Ethyl acetate	45.27±0.67
	Chloroform	49.84±0.33
	Methanol	53.17±0.34
BHT	-	84.94±0.08

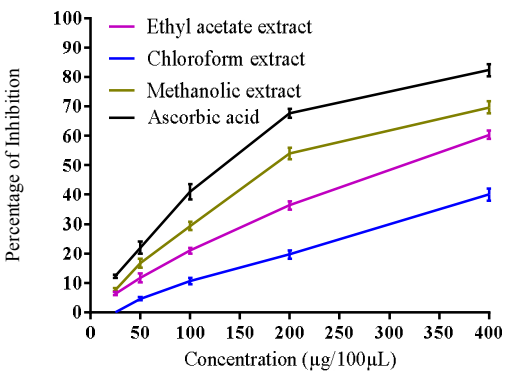

Figure 1: Concentration dependent superoxide free radical scavenging activity of *B. flabellifer* extracts

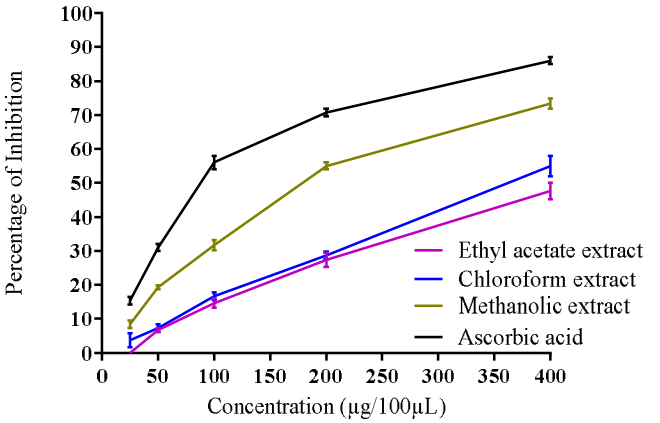

Figure 2: Concentration dependent hydroxyl free radical scavenging activity of *B. flabellifer* extracts

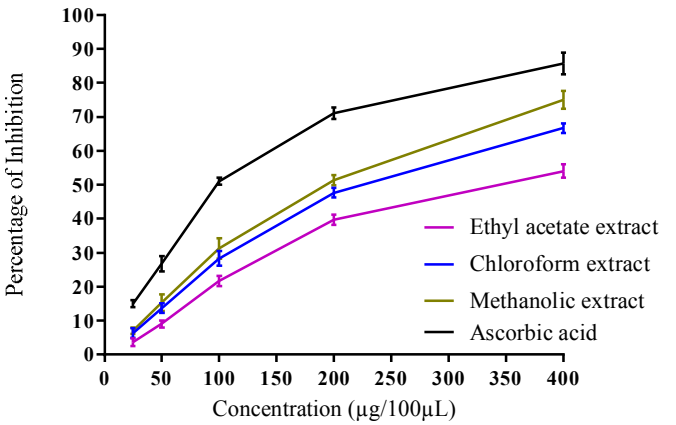

Figure 3: Concentration dependent DPPH free radical scavenging activity of *B. flabellifer* extracts

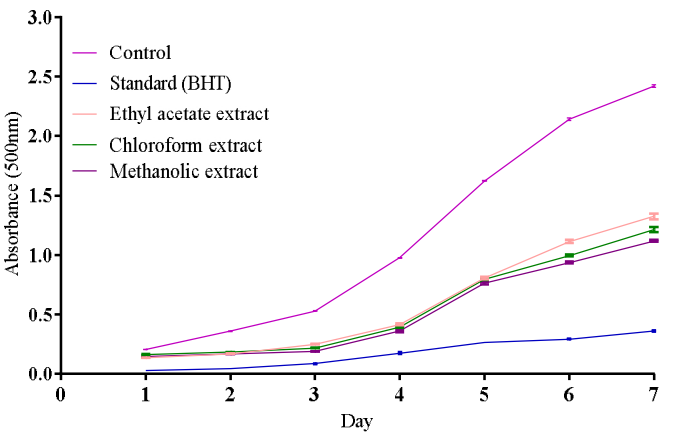

Figure 4: Antioxidant properties of different extracts of *B. flabellifer* in FTC method

RESULTS

The extracts of *B. flabellifer* showed concentration dependent inhibition on different free radicals in the present study and showed the antioxidant property in FTC method. The results of the free radical's inhibition and inhibition of linolenic acid peroxidation in FTC method were comparable with standard drugs ascorbic acid and BHT.

The chloroform, ethyl acetate and methanol extracts of *B. flabellifer* showed the percentage of inhibition on superoxide free radical was ranges from 6.33±0.33% for ethyl acetate extract at lowest concentration (25µg) to 69.67±1.20% for methanol extract at tested high concentration (400 µg) (Figure 1). The IC 50 values on superoxide radical of ethyl acetate and methanol extracts of *B. flabellifer* were found to be 292µg, and 196µg respectively. The mean IC 50 value of ascorbic acid was found to be 130µg (Table 1). The IC50 value for chloroform extract was not detectable at tested doses because the highest percentage inhibition on superoxide free radical was 40.0±1.15 at highest tested dose (400 µg).

The chloroform, ethyl acetate and methanol extracts of *B. flabellifer* showed the percentage of inhibition on hydroxyl free radical was ranges from 3.67±1.20% for chloroform extract at lowest concentration (25µg) to 73.33±0.88% for methanol extract at tested high concentration (400 µg) (Figure 2). The IC 50 values on hydroxyl radical for chloroform and methanol extracts of *B. flabellifer* were found to be 337µg, and 175µg respectively. The mean IC 50 value of ascorbic acid was found to be 83µg (Table 1). The IC50 value for ethyl acetate extract was not detectable at tested doses because the highest percentage inhibition on superoxide free radical was 47.67±1.45 at highest tested dose (400 µg).

The chloroform, ethyl acetate and methanol extracts of *B. flabellifer* showed the percentage of inhibition on DPPH free radical was ranges from 3.67±0.67% for ethyl acetate extract at lowest concentration (25µg) to 75.0±1.53% for methanol extract at tested high concentration (400 µg) (Figure 3). The IC 50 values on hydroxyl radical for ethyl acetate, chloroform and methanol extracts of *B. flabellifer* were found to be 347µg, 221µg and 186µg respectively. The mean IC 50 value of ascorbic acid was found to be 95µg (Table 1).

The results of the FTC method showed that selected plan *B. flabellifer* extracts have antioxidant capacity and their capacity was comparable with standard drug BHT. The antioxidant activities of the extracts were constant during the seven days of FTC study; the absorbance of the control was high. But, the absorbance of tested extracts in FTC method were decreased compared to control (Figure 4) and the percentage of inhibition on linolenic acid peroxidation on seventh day for chloroform, ethyl acetate and methanol extracts were 45.27±0.67, 45.27±0.67 and 53.17±0.34 respectively (Table 2).

DISCUSSION

ROS are produce during metabolisms of the body. If they were produced in the normal level, the body produced antioxidants were sufficient for neutralize them without effecting the body[22]. As earlier said, the present day lifestyle generating the more oxidants in the body and the naturally available antioxidants in the body are not sufficient for neutralize excess amount[1, 22]. The excess production of the oxidants in the body leading to unbalance physiological functions can causing different diseases like lipid peroxidation, DNA damage, atherosclorosis (oxidated LDL is more atherogenic), cancers, neurodegenerative and inflammatory bowel diseasesand accelerated aging[4,23]. So, it is important to identify antioxidants without side effects and easily available and consumable[24]. In this point of view, the present work carried out and we succeeded in identification of the antioxidant capacity of *B. flabellifer* rhizome part. The results of the current study provide scientific evidence for its traditional medicinal uses. The concentration dependent percentage inhibition of *B. flabellifer* rhizome extracts varies from one free radical to other on tested free radicals. The ethyl acetate extract showed more activity on superoxide free radical, chloroform extract showed more activity on DPPH free radical and methanol extract showed almost equal activity on three free radicals. Among three extracts, methanol extract showed the more percentage inhibition on oxidants (Free radicals/ROS). The selected plants extracts showed the appreciable antioxidant capacity along with BHT during the seven day study of FTC method supports the free radical scavenging activity. The FTC method was measures the amount of peroxide in lipid (linolenic acid) peroxidation. The peroxide reacts with the ferrous chloride forms the ferric ion, it combines with the ammonium thiocyanate forms the red color ferric thiocyanate[25]. The extracts of selected plants showed the lower absorbance compared to the control (Figure 4), the lower absorbance may be because the extracts reduced the amounts of peroxides/oxidants from the linolenic acid oxidation[26, 27] (Table 2). The *B. flabellifer* was an important palm tree and more available in Indian sub-continent; different parts of this plant are used for eaten[7]. The present study confirms the medicinal value of *B. flabellifer* rhizome part and this can be use as edible part for gaining the antioxidants.

CONCLUSION

The oxidants produced in the body can damage most cell structures through their chain reactions. This may cause many human diseases like cancer, Alzheimer's disease, cardiac reperfusion abnormalities, kidney disease and fibrosis etc. Antioxidants are the molecules can slow down the chain reaction of the oxidants by stabilizing them and able to reduce the damaging effects of them. The present study confirms the antioxidant activity *B. flabellifer* and the different extracts have different chemical compounds in them may be responsible for their activity in reduction of the oxidants.

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