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# *In Vitro* free radical scavenging potential of sap of *Borassus flabellifer*

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**Abstract**
The present investigation was intended to assess the *in vitro* free radical scavenging potential of fresh sap of *Borassus flabellifer*. The different parts of the plant are broadly utilized for their restorative properties. In the present study, the *in vitro* antioxidant activity of sap of *Borassus flabellifer* was estimated with different concentrations (10, 25, 50, 75 and 100 µg/ml) dissolved in methanol by reducing power method and total antioxidant capacity assay. The results obtained were good compared with the standard drug ascorbic acid. The outcome of the study demonstrated that sap of *Borassus flabellifer* displayed significant antioxidant activity.

**Keywords:** *borassus flabellifer*, antioxidant, phosphomolybdenum, radical scavenging activity

## Introduction
An antioxidant is any substance that at low concentration delays the oxidation of proteins, carbohydrates, lipids and DNA. Oxidative stress is a result of an imbalance between reactive oxygen species (ROS) and antioxidant defences [1]. ROS are unstable chemicals containing oxygen as free radical which reacts with biomolecules to yield various sorts of secondary radicals like lipid, sugar, nitrogenous base, amino acid and thiyl radicals. These radicals in presence of oxygen are changed over to peroxyl radicals that always induce chain reactions.
Cell membranes are prone to the oxidation by ROS because of the presence of high content of unsaturated fats in their lipid segments. ROS reaction with membrane lipids cause lipid peroxidation, bringing about development of lipid hydroperoxide (LOOH) which further disintegrate to

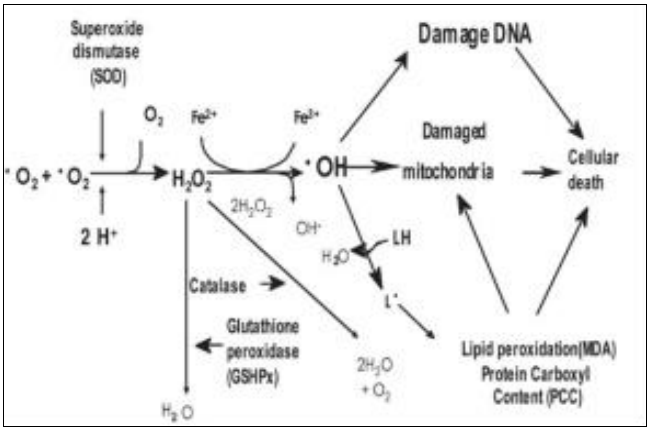


**Fig 1:** Free Radical Generation

Cyclic endoperoxide, isoprotans and hydrocarbons. The results of lipid peroxidation are cross linking of membrane proteins, modification in membrane thinness and formation of lipid-protein, lipid-DNA adduct which might be adverse to the functioning of cell, resulting in toxicities [2, 3].

The oxidative stress prompts diverse intense illnesses to individuals around the globe. The balance within the oxidants and antioxidants can produce the biological metabolisms and this provides healthy life to individuals. In recent years, numerous researchers demonstrating their enthusiasm for finding the new antioxidants from natural sources, to reduce generation of ROS in the body, aging of individuals and various ailments. Several researchers were reported different antioxidants from natural resources majorly from plants, animal and marine sources. But, there have been numerous therapeutic plants available without their medicinal uses scientifically.
In this perspective, we selected historic medicinal plant *Borassus flabellifer* in the present antioxidant examination [4, 5]. *Borassus flabellifer*, belongs to family Arecaceae, commonly known as Palmyra palm, is a native of tropical Africa but cultivated and naturalized throughout India. Presence of various phytochemical constituents in plant sap like steroids, glycosides, nutrients and organic acids were accounted for its traditional use [6, 7]. The sap of *B. flabellifer* trees exudates from the phloem tissue and it is extracted manually from the immature inflorescence of palmyra tree. Various biological activities like anti-anemic [8], anti helmintic [9], antibacterial [10], antifungal activity [11] were accounted by many researchers through their studies.
Sap of *B. flabellifer* has a crucial role in ayurvedic medication. It is a nutritious health drink and a good source of sugars, minerals and vitamins. It contains significant amount of iron, phosphorus and ascorbic acid [12]. Several organic acids such as citric acid, tartaric acid, malic acid, lactic acid, fumalic acid, pyrogalic acid and high content of succinic acid had been reported which have antioxidant activities [13]. Currently, evidence is growing that antioxidants may provide some benefit when combined with many types of diseases. In this regards, the current work was aimed to evaluate the *in vitro* inhibitory activity of *B. flabellifer* sap against reactive oxygen species.

## Materials and Methods

### Collection of Sap

Sap of *B. flabellifer* was collected early in the morning before sun rise from Ranga Reddy District, Telangana, India. It was preserved in chilled condition (0˚ C) to prevent degradation of chemical constituents due to fermentation. The sap was mixed with methanol and different concentrations were made (10, 25, 50, 75 and 100µg/ml) for testing antioxidant activity.

### Antioxidant Activity

Free radicals scavenging activity was studied for the sap of selected plants using reducing power method with potassium ferricyanide and total antioxidant capacity assay with phosphomolybdenum. The sap was gently heated to evaporate the moisture and used for measuring radical scavenging activity.

### A. Reducing Power Method [14, 15]

This method is based on the principle of increase in the absorbance of the reaction mixtures which indicates an increase in the antioxidant activity. In this method, antioxidant compound forms a colored complex with potassium ferricyanide, trichloro acetic acid and ferric chloride, which is measured at 700 nm. 2.5 ml of 0.2 M phosphate buffer and 2.5 ml of potassium ferricyanide (1% w/v) were added to different concentration of standard and test samples (10, 25, 50, 75 and 100 µg/ml). The mixtures were incubated at 50˚C for 20 min, followed by the addition of 2.5 ml of trichloroacetic acid.

The mixtures were centrifuged at 3000 rpm for 10 min to collect the upper layer of the solution (2.5 ml), mixed with 2.5 ml distilled water and 0.5 ml of ferric chloride (0.1%, w/v). A blank solution was prepared without adding extract. The absorbance was then measured at 700 nm against blank sample.

### B. Total antioxidant capacity assay [16, 17]

Total antioxidant capacity assay is a spectroscopic method for the quantitative determination of antioxidant capacity, through the formation of phosphomolybdenum complex. The assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and subsequent formation of a green phosphate Mo (V) complex at acidic pH. Different concentrations of standard and test sample solution were combined with 1 ml of reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 95˚C for 90 min. and allowed to cool to room temperature. A typical blank solution contained 1 ml of reagent solution and the 0.1 ml of the distilled water was used. The absorbance of the aqueous solutions was measured at 695 nm against blank in UV spectrophotometer.

## Results

The antioxidant activity of sap of *B. flabellifer* was studied with five different concentrations (10, 25, 50, 75 and 100 µg/ml), was determined by using reducing power method and total antioxidant capacity assay. Data in Table 1 & 2 clearly indicates that compound exhibit specific Antioxidant activity.

**Table 1:** Antioxidant activity by Reducing Power Method

| Standard and Test Samples | Absorbance (700 nm) | | | | |
|---|---|---|---|---|---|
| | 10 µg/ml | 25 µg/ml | 50 µg/ml | 75 µg/ml | 100 µg/ml |
| Standard Ascorbic Acid with Water | 1.270 | 1.944 | 2.00 | 2.172 | 2.371 |
| Sap | 0.945 | 0.115 | 0.434 | 0.173 | 1.849 |

**Table 2:** Antioxidant activity by Total Antioxidant Capacity Assay

| Strandard and Test Samples | Absorbance (695 nm) | | | | |
|---|---|---|---|---|---|
| | 10 µg/ml | 25 µg/ml | 50 µg/ml | 75 µg/ml | 100 µg/ml |
| Standard Ascorbic Acid with Water | 0.014 | 0.048 | 0.074 | 0.118 | 0.192 |
| Sap | 0.005 | 0.012 | 0.045 | 0.087 | 0.115 |

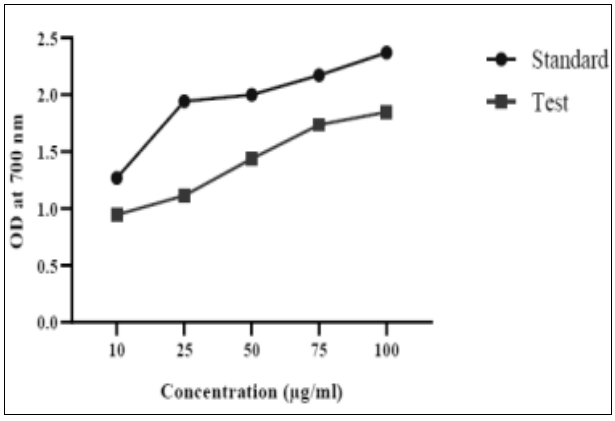


**Fig 2:** Antioxidant activity by Reducing Power Method

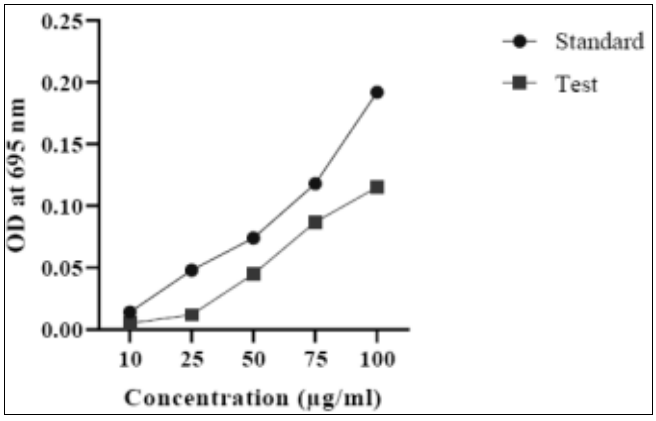


**Fig 3:** Antioxidant activity by Total Antioxidant Capacity Assay

## Discussion

In the present study, free radical scavenging activity of *B. flabellifer* sap was performed *in vitro* by reducing power method and total antioxidant capacity assay. In reducing power method, antioxidant compounds of *B. flabellifer* sap formed a green colored complex with potassium ferricyanide and ferric chloride.

The *B. flabellifer* sap exhibited a significant free-radical scavenging effect by phosphomolybdenum method. The antioxidant compounds of *B. flabellifer* sap reduced Mo

(VI) to Mo (V) and formed green phosphate Mo (V) complex at acidic pH. The scavenging activity was increased in dose dependent manner in both the methods. The maximum scavenging activity of sap was found at 100µg/ml, which is almost nearest to the standard drug. The *B. flabellifer* sap scavenging was concentration-dependent.

## Conclusion

From the results of present study, *B. flabellifer* sap at 3.6 ml/kg showed significant *in vitro* antioxidant activity. Hence, the sap can be use as an antioxidant source which can target in treating many adverse events due to free radicals.

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