Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Der Pharmacia Sinica, 2011, 2 (3): 193-199

Der Pharmacia Sinica

ISSN: 0976-8688
CODEN (USA): PSHIBD

Anti oxidant activity of methanolic extracts of female *Borassus Flabellifer* leaves and roots

Sudhakar Kommu*[a], Vijaya laxmi Chiluka[a], Gowri Shankar[a] N. L, Matsyagiri[a] L, Shankar. M[a] and Sandhya[b]. S

Swami Vivekananda Institute of Pharmaceutical Sciences, Vangapally, Yadagirigutta, Nalgonda, A.P
Nalanda College of Pharmacy, Charlapally, Nalgonda, A.P

ABSTRACT

Borassus flabellifer is a tall erect palm which can be recognized by its large and fan shaped leaves. The plant is used for the various ailments like secondary syphilis, antiperiodic, heart burns, liver and spleen enlargement etc. the various parts of the plant are a rich source of phyto constituents like gums, saponins, glycosides, carbohydrates, albuminoids, fats, vitamins A, B and C. Oxidation process is one of the most important routes for producing free radicals in food, drugs and even living systems. Lipid peroxidation in fats and fatty foods not only deteriorates their quality and brings about chemical spoilage, but also generates free radicals and reactive oxygen species. Free radicals cause many human diseases like cancer, Alzheimer's disease, cardiac reperfusion abnormalities, kidney and liver disease, fibrosis, atherosclerosis, arthritis, neuro-degenerative disorders, inflammation and aging. The present study evaluated the anti oxidant effect of methanolic extracts of Borassus flabellifer leaves and roots.

Key words: Oxidation, *Borassus flabellifer*, FRAP.

INTRODUCTION

Herbal medicine is the major stay of about 75–80% of the world population, mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and few side effects. India is sitting on a gold mine of well-recorded and well practiced knowledge of traditional herbal medicine. In spite of tremendous development in the field of synthetic drugs during these days, they are found to have some side effects. Whereas herbal medicine still hold their own unique place, by showing no side effects

[1]. World Health Organization (WHO) encourages, recommends and promotes traditional/herbal remedies in national health care programmes because these drugs are easily available at low cost and these are safe and people have faith in them [2].

Oxidation process is one of the most important routes for producing free radicals in food, drugs and even living systems. Lipid peroxidation in fats and fatty foods not only deteriorates their quality and brings about chemical spoilage, but also generates free radicals and reactive oxygen species. Free radicals cause many human diseases like cancer, Alzheimer's disease, cardiac reperfusion abnormalities, kidney and liver disease, fibrosis, atherosclerosis, arthritis, neuro-degenerative disorders, inflammation and aging [3]. The use of synthetic antioxidants like butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), and *ter*-butyl hydro quinone (TBHQ) in foods is discouraged because of their toxicity and carcinogenicity and they may cause liver swelling and influence liver enzyme activities [4]. Due to their natural origin, the antioxidants obtained from plants are of greater benefit in comparison to synthetic ones [5].

The *Borassus flabellifer* is a tall and erect palm, with large, fan-shaped leaves. *Borassus* is derived from a Greek word describing the leathery covering of the fruit and *flabellifer* means "fan-bearer". Synonyms of the plant include jaggery palm, Palmyra palm, toddy palm, wine palm. The different parts of the plant is used for the various ailments like secondary syphilis, antiperiodic, heart burns, liver and spleen enlargement etc. Other than these pharmacological uses the juice of the plant is used in preparation of health drinks, jellies etc. The leaves are used to make baskets, hats and many other useful items [6]. The midrib of the leaves and the fibers from the stalks are used in making brushes and base of young leaf stalks is used for straining the Toddy and for making torches [7]. The jelly like pulp of the fruit is pleasant to eat, while the germinated nuts with enlarged fleshy embryos are cooked and eaten as vegetables.

Borassus flabellifer contains gums, albuminoids, fats and the fresh pulp is reportedly rich in vitamins A and C [8]. The fresh sap is a good source f vitamin B-complex [9]. Male inflorescence constitutes spirostane-type steroid saponins like borassosides and dioscin. It also contains 20 known steroidal glycosides [10] and carbohydrates like sucrose [11]. It also contains bitter compound called flabelliferrins, these are steroidal saponins [6].

Recent epidemiological studies have indicated that diets rich in fruits and vegetables and those of selected natural antioxidants such as plant poly-phenols, vitamin C and flavonoids are correlated with reduced incidence of cardiovascular and chronic diseases and of certain cancer [4].

As the need for widely functional and safer natural antioxidants continues to exist, it is, therefore, imperative to measure the antioxidant activity of various plant extracts. There fore we have selected *Borassus flabellifer* which is the rich source for various phyto-chemicals.

MATERIALS AND METHODS

Chemicals

2, 4, 6-tripyridyl-s-triazine (TPTZ) was purchased from Rolex chemical industries, Mumbai. Ferric chloride was purchased from Sd fine chem. Limited, Mumbai. Potassium ferricyanide was purchased from Qualigens fine chemicals, Navimumbai. Tri chloro acetic acid (TCA) was

purchased from Qualikem fine chemicals Pvt. Limited, New Delhi. Ascorbic acid was purchased from Qualikem fine chemicals Pvt. Limited, Vadodara.

Instruments used:
U.V Spectrophotometer, centrifuge, cyclo-mixer and incubator were used in the study.

Plant material
Collection and authentication of *Borassus flabellifer* leaves and roots:
The plant species *Borassus flabellifer* were collected in regions of Nalgonda district. The plant material is collected in the months of November to December. The plant material is authenticated by Mr. A. Lakshma Reddy, Retired Professor, Dept. of Botany, Nagarjuna Govt. College (Autonomous) Nalgonda. The plant was identified as Borassus flabellifer and was certified under Voucher No: NCOP-NLG/ph'cog/2009-10/004. The collected leaves and roots were dried in shade and made into powder.

Preparation of methanolic extract of leaves of *Borassus flabellifer*:
80gm of powder of *Borassus flabellifer* leaves was extracted with 500ml of methanol in a soxhelt apparatus for 72hrs. The extract was concentrated by recovery of methanol. The concentrated product was used as methanolic extract of *Borassus flabellifer* leaves (MEBFL).

Preparation of methanolic extract of roots of *Borassus flabellifer*:
80gm of powder of *Borassus flabellifer* roots was extracted with 500ml of methanol in a soxhelt apparatus for 72hrs. The extract was concentrated by recovery of methanol. The concentrated product was used as methanolic extract of *Borassus flabellifer* roots (MEBFR).

Preliminary phytochemical studies:
Phytochemical screening was performed for the methanolic extracts of leaves and roots of Borassus flabellifer by using standard methods [khandelwal]. Tests were performed to detect the presence of various phytochemicals like flavonoids, saponins, tannins and Phenolic compounds and tannins in the methanolic extracts of leaves and roots of *Borassus flabellifer*.

Estimation of total antioxidant activity of methanolic extracts of leaves and roots of *Borassus flabellifer* by Ferric Reducing Anti oxidant Potential (FRAP) method:

FRAP reagent was prepared by mixing acetate buffer, TPTZ and ferric chloride in the ratio of 10:1:1 and 100µl of extract was mixed with 3ml of working FRAP reagent and absorbance was measured at 593nm at 0 minute after vortexing. There after the samples were incubated at 37^0C it for 4 minutes and the absorbance was measured after 4 minutes and the difference of two absorbances were calculated and plotted on the standard graph of ascorbic acid [12].

Table 1: Procedure for estimation of anti-oxidant activity of methanolic extract of *Borassus flabellifer* leaves by FRAP reagent

S.NO	REAGENTS	BLANK	TEST	STANDARD
1.	FRAP reagent	3ml	3ml	3ml
2.	MEBFL	__	100µl	__
3.	Distilled water	100µl	__	__

Table 2: Procedure for estimation of anti-oxidant activity of methanolic extract of *Borassus flabellifer* roots by FRAP reagent

S.NO	REAGENTS	BLANK	TEST	STANDARD
1.	FRAP reagent	3ml	3ml	3ml
2.	MEBFR	__	100µl	__
3.	Distilled water	100µl	__	__

Estimation of anti-oxidant activity of methanolic extracts of leaves and roots of Borassus flabellifer by reducing power assay method:

Different concentrations (20-100µg/ml) of three extracts were prepared and 1ml of each solution was mixed with phosphate buffer and potassium ferri cyanide. The mixture was incubated at 50^0C for 20 minutes. To this mixture, 2.5ml of 10% trichloro acetic acid (TCA) was added and the centrifuged at 3000 rpm for 10min. The upper layer of the solution was mixed with distilled water and ferric chloride was added and the absorbance was measured at 700nm [13].

The percentage scavenging activity was calculated by using the formula:

$$\text{Scavenging activity (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where,

$A_{control}$ is the absorbance of the solution with out extract and

A_{sample} is the absorbance of the solution with extract

Table 3: Procedure for estimation of antioxidant activity of methanolic extract of Borassus flabellifer leaves by Reducing Power Assay method

S.NO	REAGENT	BLANK	TEST	STANDARD
1.	Phosphate buffer	2.5ml	2.5ml	2.5ml
2.	Potassium ferricyanide	2.5ml	2.5ml	2.5ml
3.	Trichloro acetic acid	2.5ml	2.5ml	2.5ml
4.	Distilled water	2.5ml	2.5ml	2.5ml
5.	Ferric chloride	0.5ml	0.5ml	0.5ml
6.	MEBFL	__	1ml	__
7.	Ascorbic acid	__	__	1ml

Table 4: Procedure for estimation of antioxidant activity of methanolic extract of Borassus flabellifer roots by Reducing Power Assay method

S.NO	REAGENT	BLANK	TEST	STANDARD
1.	Phosphate buffer	2.5ml	2.5ml	2.5ml
2.	Potassium ferricyanide	2.5ml	2.5ml	2.5ml
3.	Trichloro acetic acid	2.5ml	2.5ml	2.5ml
4.	Distilled water	2.5ml	2.5ml	2.5ml
5.	Ferric chloride	0.5ml	0.5ml	0.5ml
6.	MEBFR	__	1ml	__
7.	Ascorbic acid	__	__	1ml

RESULTS AND DISCUSSION

Phytochemicals:
From the phytochemical tests performed for the methanolic extracts of *Borassus flabellifer*, chemical constituents like flavonoids, saponins, tannins and phenolic compounds were found to be present.

Table 5: Phyto-chemicals present in methanolic extracts of leaves and roots of *Borassus flabellfer*:

S.NO	PHYTOCONSTITUENTS	MEBFL	MEBFR
1.	Flavonoids	+	+
2.	Tannins and Phenolic compounds	+	+
3.	Saponins	+	+

Anti oxidant activity of methanolic extract of leaves of *Borassus flabellifer* by FRAP method:

The anti oxidant activity of 1mg/ml of methanolic extract of *Borassus flabellifer* leaves was found to be equivalent to 200µmol/L of ascorbic acid. The anti oxidant activity of 2mg/ml of methanolic extract of *Borassus flabellifer* leaves was found to be equivalent to 600µmol/L of ascorbic acid. The anti oxidant activity of 3mg/ml of methanolic extract of *Borassus flabellifer* leaves was found to be equivalent to 700µmol/L of ascorbic acid. The anti oxidant activity of 4mg/ml of methanolic extract of *Borassus flabellifer* leaves was found to be equivalent to 800µmol/L of ascorbic acid. The anti oxidant activity of 5mg/ml of methanolic extract of *Borassus flabellifer* leaves was found to be equivalent to 1000µmol/L of ascorbic acid.

Anti oxidant activity of methanolic extract of roots of *Borassus flabellifer* by FRAP method:

The anti oxidant activity of 1mg/ml of methanolic extract of *Borassus flabellifer* roots was found to be equivalent to 100µmol/L of ascorbic acid. The anti oxidant activity of 2mg/ml of methanolic extract of *Borassus flabellifer* roots was found to be equivalent to 300µmol/L of ascorbic acid. The anti oxidant activity of 3mg/ml of methanolic extract of *Borassus flabellifer* roots was found to be equivalent to 500µmol/L of ascorbic acid. The anti oxidant activity of 4mg/ml of methanolic extract of *Borassus flabellifer* roots was found to be equivalent to 600µmol/L of ascorbic acid. The anti oxidant activity of 5mg/ml of methanolic extract of *Borassus flabellifer* roots was found to be equivalent to 800µmol/L of ascorbic acid.

Anti oxidant activity of methanolic extracts of leaves of *Borassus flabellifer* by Reducing Power Assay method:
The percentage scavenging activity of 1mg/ml of methanolic extract of leaves of *Borassus flabellifer* was found to be 80.5. The percentage scavenging activity of 2mg/ml of methanolic extract of leaves of *Borassus flabellifer* was found to be 59.4. The percentage scavenging activity of 3mg/ml of methanolic extract of leaves of *Borassus flabellifer* was found to be 43.8. The percentage scavenging activity of 4mg/ml of methanolic extract of leaves of *Borassus flabellifer* was found to be 33.6. The percentage scavenging activity of 5mg/ml of methanolic extract of leaves of *Borassus flabellifer* was found to be 17.69.

Anti oxidant activity of methanolic extract of roots of *Borassus flabellifer* by reducing power assay method:

The percentage scavenging activity of 1mg/ml of methanolic extract of roots of *Borassus flabellifer* was found to be 84.6. The percentage scavenging activity of 2mg/ml of methanolic extract of roots of *Borassus flabellifer* was found to be 76.7. The percentage scavenging activity of 3mg/ml of methanolic extract of roots of *Borassus flabellifer* was found to be 61.9. The percentage scavenging activity of 4mg/ml of methanolic extract of roots of *Borassus flabellifer* was found to be 52.8. The percentage scavenging activity of 5mg/ml of methanolic extract of roots of *Borassus flabellifer* was found to be 32.6.

The present study evaluated the effect of methanolic extracts of leaves and roots of *Borassus flabellifer* in vitro FRAP and Reducing Power Assay Methods. Oxidation is defined as the interaction between oxygen molecules and all the different substances they may contact. Oxidation is a multi-factorial process occurs because of several factors like stress, pollution, toxins and drugs, genetic and dietary factors. Oxidation occurs because of the presence of free radicals which are highly reactive. Free radicals cause many human diseases like cancer, Alzheimer's disease, cardiac reperfusion abnormalities, kidney and liver disease, fibrosis, atherosclerosis, arthritis, neuro-degenerative disorders, inflammation and aging [3]. The antioxidants obtained from plants are of greater benefit in comparison to synthetic ones.

Borassus flabellifer is a tall and erect palm, with large, fan-shaped leaves. The different parts of the plant is used for treatment of various diseases like secondary syphilis, anti-periodic, heart burns, liver and spleen enlargement etc. The juice of the plant is used in preparation of health drinks, jellies etc. [6]. *Borassus flabellifer* contains gums, albuminoids, fats, saponins, carbohydrates like sucrose and vitamins A, C [7, 8]. The methanolic extracts of leaves and roots of *Borassus flabellifer* were prepared and phytochemical screening was performed. From the phyto chemical screening it was revealed that the plant is a rich source of various phyto chemicals like flavonoids, saponins, tannins and phenolic compounds which play and important role in the prevention of oxidation.

The methanolic extracts of *Borassus flabellifer* leaves and roots were evaluated for the anti oxidant activity by FRAP and Reducing Power Assay methods. The studies revealed that the methanolic extracts of *Borassus flabellifer* shown antioxidant activity and the anti-oxidant activity was to be due to the presence of the phyto chemicals like flavonoids, saponins, tannins and phenolic compounds.

From the previous studies we know that these phyto constituents shows antioxidant activity or prevents the oxidation mainly by reducing the intracellular reactive oxygen species and malondialdehyde production; increasing glutathione content; and enhancing the antioxidant enzymatic activities of catalase, superoxide dismutase and glutathione peroxidase.

The anti oxidant potential of the leaves of *Borassus flabellifer* was found to be more when compared to the anti oxidant potential of the roots of *Borassus flabellifer*. The anti oxidant potential of the methanolic extracts of the leaves of *Borassus flabellifer* was more when compared to the methanolic extract of the roots of *Borassus flabellifer* due to the presence of

more quantities of the phyto constituents like flavonoids, saponins, tannins and phenolic compounds in the leaf when compared to the roots of the *Borassus flabellefer*.

CONCLUSION

From the results of the present study, it is known that the leaves and roots of *Borassus flabellifer* possess anti oxidant activity. From the study of anti oxidant activity by different methods like FRAP and Reducing Power Assay methods it can be asserted that the investigated plant materials like leaves and roots of *Borassus flabellifer* are a viable source of natural antioxidants. The anti oxidant activity of the methanolic extracts of leaves and roots of *Borassus flabellifer* might be due to the presence of phyto constituents like flavonoids, saponins, tannins and phenolic compounds. From this study we can conclude that the leaves and roots of *Borassus flabellifer* can be used as an anti oxidant and might have potential as "nutraceuticals" for the preparation of functional foods.

REFERENCES

[1] S. Sandhya, K. Sudhakar, K.R. Vinod D. Banji., *International Journal of Pharmacology and Technology*, **2010**, 2, 67-74.
[2] N.R. Ekka. K.P. Namdeo, P.K. Samal, *Research Journal of Pharmaceutics and Technology*, **2008**, 1, 310-312.
[3] M.S. Stankovic, N. Niciforovic, M. Topuzovic et al., *Biotechnol. & Biotechnol Eq*. **2011**, 25, 2222-2227.
[4] F. Anwar, A. Jamil, S. Iqbal et al., *Grasas Y Aceites*, **2006**, 57, 189-197.
[5] R.A. Nadhala, M. Moyo, V.J. Staden, *Molecules*, **2010**, 15, 6905-6930.
[6] S. Sandhya, K. Sudhakar, D. Banji, *Annals of Biological Research*, **2010**, 1, 85-94.
[7] K.M. Nadkarni, Indian materia medica, *Popular prakashan*, Mumbai, **2002**, 209-210.
[8] J.F. Morton, *Economic botany*, **1988**, 42, 420-421.
[9] M. Yoshikawa, F. Xu, *Chem Pharma Bulletin*, **2007**, 55, 308-316.
[10] L.D. Kapoor, Hand book of Ayurvedic medicinal plants, CRC press, New york, **2005**, 118-120.
[11] K.R. Khandelwal, Practical pharmacognosy Techniques and Experiments, Nirali Prakashan, Pune, **2002**, 146-160.
[12] I.F.F. Benzie, J.J. Strain, *Analytical biochemistry*, **1996**, 239, 70-76.
[13] K.P. Ajay, A.M. Verma, D. Kavitha et al., *International Journal of Pharmaceutical Review and Research*, **2010**, 5, 181-184.