IJBPAS, May, 2022, 11(5): 2268-2278

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

ANTIOXIDANT ACTIVITY OF ETHANOL AND AQUEOUS ROOT EXTRACTS OF PALMYRA PALM (*Borassus flabellifer* L.): A COMPARATIVE STUDY

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Received 9th July 2021; Revised 30th July 2021; Accepted 18th Aug. 2021; Available online 1st May 2022

https://doi.org/10.31032/IJBPAS/2022/11.5.6070

ABSTRACT

Numerous diseases are caused by free radical damage to the human body. There is growing demand for foods with healing properties, especially foods containing phytochemicals which have higher antioxidant activity. One such food is Palmyra Palm root known to have therapeutic properties. Not many studies have been conducted on Palmyra Palmroot *Borassus flabellifer* L. which is very popular in South India, especially Southern Tamil Nadu. Therefore this study was undertaken to evaluate the antioxidant activity of ethanol and aqueous extracts of Palmyra Palm root using assays of DPPH (1, 1- diphenyl-2-picrylhydrazyl) radical scavenging activity, Superoxide radical scavenging activity, Ferric reducing power activity and Phosphomolybdenum reduction activity which were compared with Standard ascorbic acid. Results strongly suggest that both the extracts possess a good antioxidant potential, moreover ethanol extract shows a better effect when compared to aqueous extract.

Keywords: Palmyra Palm root, *Borassus flabellifer* L., antioxidant, free radical scavenging, functional food, DPPH

INTRODUCTION

Palmyra Palm root (*Borassus flabellifer* L.) is commonly grown in Tamil Nadu and its products although being economical are not widely used in the daily diet. Studies on Palmyra palm have shown numerous biological activities like cytotoxicity, anti-bacterial property, anti-arthritic, analgesic and hypoglycaemic potential **[1]**. *Borassus flabellifer* L., commonly known as Palmyra palm belongs to the family Arecaceae. Traditionally the different parts of the Palmyra plant namely root, fruit, seeds and leaves are used for various human disorders and are also known for their diuretic and anti-helminthic property. The fruit pulp of *Borassus flabellifer* has been used in traditional dishes and for diabetic patients, while the sap has been used as a sweetener **[2]**.

Functional foods are foods and food components which provide health benefits beyond the requirement of basic nutrition, maintaining health and reducing the risk of disease **[3]**. Free radicals ROS and RNS (Reactive Oxygen Species and Reactive Nitrogen Species) are the important compounds having both deleterious and beneficial effects in human health **[4]**. Although there are several enzymes systems within the body that scavenge free radicals, the principal antioxidants are vitamin E (α-tocopherol), vitamin C (ascorbic acid), and Beta carotene found naturally in the diet. The body cannot itself manufacture these micronutrients, so they must be supplied in the diet **[5]**. Oxidative stress leads to various forms of acute and chronic diseases due to the reduction in levels of antioxidants. The balance in the oxidants and antioxidants will help in producing well balanced biological metabolisms and thus directly or indirectly support a healthy life **[6]**.

Many researchers have shown interest in finding new antioxidants derived from natural sources, because of present lifestyle which influences increased production of ROS in the human body **[7, 8]**. With this view, we selected Palmyra Palm Root (*Borassus flabellifer L.)* for the present study. Different parts of *Borassus flabellifer* have been using as traditional medicine for diverse illnesses **[9]**. Different phytochemical constituents like vitamins, steroids, glycosides, etc. were reported from *Borassus flabellifer* **[10]**.

With increasing focus on sourcing locally grown seasonal foods to derive maximum nutritional benefit, this study explores the antioxidant potential of the Palmyra Palm root which is a traditional plant of Tamil Nadu. Phytochemical compounds like saponins and flavonoids were identified in Palmyra palm root samples collected from Andhra Pradesh **[11]**. The present study was undertaken to

confirm the presence of antioxidant properties of Palmyra Palm root and to examine which extract exhibits better antioxidant potential. Palmyra Palm root is generally consumed in Tamil Nadu as a snack after, boiling and removing the central shoot. It is also preserved by sun drying.

MATERIALS AND METHODS

a. Collection of Palmyra Palm Roots

The sample (Palmyra Palm Roots) for the study was collected from Nagercoil, Kanyakumari District, Tamil Nadu **(Figure 1)**. The samples were purchased from the same source to ensure homogeneity of the sample used in the study. The Palmyra Palm roots were washed, boiled in water for 30 minutes and then the central shoot was removed using a sterile-knife.

Figure 1: Palmyra Palm Roots

b. Identification and authentication of the Palmyra Palm Roots

The Palmyra Palm roots used in the study were identified and authenticated at the Central Council for Research in Siddha Chennai, Ministry of AYUSH, Government of India, Anna Govt. Hospital Campus, Chennai.

c. Lyophilisation of Palmyra palm Root

In the present study 300 grams of Palmyra Palm Root was weighed and subjected to Lyophilisation /freeze drying. A total yield of 125 grams of lyophilized Palmyra Palm Root powder was obtained. Freeze-drying was done at the Centralized Instrumentation Lab, Madras Veterinary College, Vepery, Chennai.

Preparation of aqueous extract

About 10 g of the lyophilized Palmyra Palm Root Powder was dissolved in 100 ml of distilled water and was boiled for 10 minutes. Then, the supernatant liquid was filtered using filter paper and then stored at 4°C for further analysis.

Preparation of ethanol extract

About 10 g of the lyophilized Palmyra Palm Root Powder was steeped in 100 ml of ethanol for 72 hours. Then, the supernatant liquid was filtered using filter paper and then stored at 4°C for further analysis.

Assessment of antioxidant potential of Palmyra Palm Root Powder extracts

The antioxidant potential of aqueous and ethanol extracts of Palmyra Palm Root Powder was assessed by four assays comprising of two radical scavenging assays and two reducing power assays **(Figure 2)**.

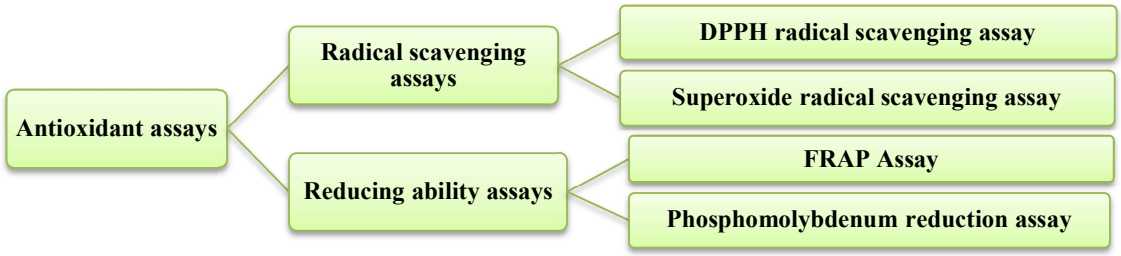

Figure 2: Antioxidant assays for Palmyra palm root powder extract

Radical scavenging assays

(i) DPPH (1, 1- diphenyl-2-picrylhydrazyl) free radical scavenging assay

The antioxidant activity of aqueous and ethanol root extracts was measured on the basis of free radical scavenging method. One mL of 0.1 mM DPPH solution in methanol was mixed with 1 mL of various concentrations (50-300 µg/mL) of aqueous and ethanol extracts. The mixtures were then allowed to stand for 30 minutes incubation in the dark. The decrease in absorbance was measured at 517 nm. Ascorbic acid was used as the standard reference **[12]**. One mL of methanol and 1 mL of DPPH solution was used as the control. The percentage of DPPH radical inhibition was calculated as:

$$\% \text{ of DPPH radical inhibition} = \left[\frac{\text{Control} - \text{Sample}}{\text{Control}}\right] \times 100$$

(ii) Superoxide ($O_2^{\cdot-}$) radical scavenging activity

Superoxide ($O_2^{\cdot-}$) radical scavenging activity was measured by riboflavin-UV light-NBT system **[13]**.The reaction mixture contained different concentrations (20-120 µg/mL) of aqueous and ethanol root extracts, 1.5 mM of riboflavin, 12 mM of EDTA and 50 mM of NBT, added in that sequence. All the reagents were prepared in 50 mM of phosphate buffer (pH 7.8). The reaction was started by illuminating the reaction mixture in short ultra violet (UV) light for 90 seconds. Immediately after illumination, the absorbance of the reaction mixture was measured at 590 nm. Ascorbic acid was used as standard reference. Superoxide ($O_2^{\cdot-}$) radical scavenging activity was calculated using the formula:

$$\% \text{ of superoxide } (O_2^{\cdot-}) \text{ radical inhibition} = \left[\frac{\text{Control} - \text{Sample}}{\text{Control}}\right] \times 100$$

Reducing power assays

(i) Ferric (Fe^{3+}) reducing power assay

The reducing power of aqueous and ethanol root extracts was determined by Ferric (Fe^{3+}) reducing power assay **[14]**. One mL of different concentrations (50-300 µg/mL) of aqueous and ethanol Palm root extracts was mixed with 1 mL of phosphate

buffer (0.2 M, pH 6.6) and 1 mL of potassium ferricyanide (1% w/v) solution. The mixture was then incubated at 50°C in the water bath for 30 minutes, after which 500 µL of trichloroacetic acid (10% w/v) was added to all the test tubes and mixed well. Then 100 µL of freshly prepared $FeCl_3$ (0.1% w/v) solution was added, mixed well and the absorbance was measured at 700 nm. Ascorbic acid was used as the standard reference. The percentage of Fe^{3+} reduction was calculated as:

$$\textbf{\% of } Fe^{3+} \textbf{ reduction} = \left[\frac{\textbf{Sample} - \textbf{Control}}{\textbf{Sample}}\right] \textbf{x 100}$$

(ii) Phosphomolybdenum reduction assay

The antioxidant capacity of aqueous and ethanol root extracts was assessed by molybdenum reduction assay method **[15]**. The aqueous and ethanol root extracts with different concentrations (50-300 µg/mL) was combined with 1 mL of reagent solution which is a combination of ammonium molybdate (4 mM), sodium phosphate (28 mM) and sulphuric acid (600 mM). The mixture was then incubated in a water bath at 95°C for 90 min. The absorbance of the coloured complex was then measured at 695 nm. Ascorbic acid was used as standard reference. The percentage of phosphomolybdenum reduction was calculated as:

$$\textbf{\% of phosphomolybdenum reduction} = \left[\frac{\textbf{Sample} - \textbf{Control}}{\textbf{Sample}}\right] \textbf{x 100}$$

Statistical analysis

The experimental data was analysed statistically using IBM SPSS Statistics version 20 software. The experimental results were expressed as a mean±standard deviation of the mean of three replicates. One way analysis of variance (ANOVA) was computed to compare the free radical scavenging activity and reducing ability of the extracts at different concentrations.

RESULTS

DPPH (1,1- diphenyl 2-picrylhydrazyl) Radical Scavenging Assay

DPPH radical scavenging assay is widely used method to measure the ability of radical scavenging activity of root extracts. The odd electron of nitrogen atom in DPPH is reduced by the antioxidants which are hydrogen donors or electron donors present in the root extracts. The antioxidants in the palm root extracts can donate hydrogen

atoms, which convert 1,1-diphenyl-2-picrylhydrazyl (free radical) to its reduced form 1,1-diphenyl-2-picrylhydrazine (non-radical) resulting in the colour change from purple to pale yellow colour **[16]**.

The results **(Table 1)** showed that ethanol and aqueous root extracts of Palmyra Palm exhibited good antioxidant activity. The maximum DPPH radical scavenging activity of ethanol root extract was 85.71±0.21% and aqueous extract was 50.87±0.12% at 300 µg/mL concentration. The IC_{50} (Inhibitory $Concentration_{50}$) of DPPH radical scavenging activity of ethanol and aqueous root extracts was 54.72 µg/mL and 294.87 µg/mL concentration respectively which was compared to the IC_{50} of standard ascorbic acid of 2.88 µg/mL concentration. It can be inferred that the ethanol extract had a better free radical scavenging activity than the aqueous root extract. This is probably because the antioxidant compounds in root powder are better extracted in ethanol when compared to aqueous extract.

Superoxide Radical Scavenging Assay

Superoxide radical scavenging assay is based on the capacity of the ethanol and aqueous root extracts to inhibit blue formazan formation by scavenging the superoxide radicals generated in riboflavin-light-NBT system. The superoxide radical scavenging activity of root extract can be inferred by colour change from yellow to pale yellow colour showing the reduction of superoxide radical by the root extracts. The superoxide radical is ubiquitous in aerobic cells and transformed to the highly reactive and damaging hydroxyl radical which quickly break down the DNA helix **[17]**.

The maximum superoxide radical scavenging activity of ethanol root extract was 88.44±0.71% and aqueous extract was 58.75±0.23% at 60 µg/mL concentration **(Table 2)**. The IC_{50} of superoxide radical scavenging activity of ethanol and aqueous extracts were 36.51 µg/mL and 52.65 µg/mL concentration, as compared to the IC_{50} of 5.21 µg/mL concentration of the standard, ascorbic acid. Thus, inhibitory effects of the ethanol and aqueous root extracts on superoxide anion formation noted herein possibly render them as a promising antioxidant. It can be also inferred that the ethanol extract showed a better superoxide radical scavenging activity when compared to the aqueous extract.

Ferric (Fe^{3+}) Reducing Power Assay (FRAP)

Ferric ion reducing power activity determines the electron donating capacity of antioxidant components. The total antioxidant activity can be determined by the ferric reducing power assay (FRAP). The flavonoids and phenolic compounds present in medicinal plants demonstrate a

strong antioxidant activity which directly depends on their potential to form a complex with metal ions **[18]**. This method is based on the reduction of Fe^{3+} to Fe^{2+} and formation of green colour complex due to the electron donating ability of antioxidants **[19]**. Higher the absorbance read, higher is the reducing power.

Iron can catalyse the conversion of poorly reactive free radicals into highly active free radicals. Excess of iron seems to contribute initially to insulin resistance by decreasing glucose uptake by muscles and subsequently to decreased insulin synthesis and secretion in the pancreas which may possibly play a role in the development of diabetes **[20]**.

The maximum Fe^{3+} reduction activity of ethanol palm root extract was 64.55±0.38% at 300 µg/Ml concentration and the aqueous palm root extract was 64.52±0.68% at 300 µg/mL concentration **(Table 3)**. The RC_{50} (Reduction Concentration $_{50}$) of FRAP of ethanol and aqueous palm root extracts were 156.09 µg/mL concentration and 157.50 µg/mL concentration respectively, which were compared to the RC_{50} of 28.96 µg/mL concentration of standard ascorbic acid.

Phosphomolybdenum reduction assay

The phosphomolybdenum reduction method was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compounds present in the ethanol and aqueous palm root extracts and the formation of green phosphate/Mo (V) complex with a maximum absorption at 695nm **[21]**.

Table 1: DPPH radical scavenging activity of aqueous and ethanol root extracts of Palmyra Palm

Concentration µg/mL	Ethanol extract % of inhibition Mean±SD#	p value	Aqueous extract % of inhibition Mean±SD#	p value
50	45.69±0.20	0.000**	16.58±0.39	0.000**
100	66.83±0.25		23.37±0.35	
150	76.83±0.25		32.41±0.26	
200	82.54±0.36		38.47±0.19	
250	83.82±0.18		42.44±0.21	
300	85.71±0.21		50.87±0.12	

**Significant at p <0.01
#Values are the mean of triplicates

Table 2: Superoxide Radical Scavenging Activity of Aqueous and Ethanol root extracts of Palmyra Palm

Concentration µg/mL	Ethanol extract % of inhibition Mean±SD#	p value	Aqueous extract % of inhibition Mean±SD#	p value
10	10.52±0.43	0.000**	24.44±0.52	0.000**
20	37.49±0.64		27.64±0.37	
30	41.08±0.93		33.68±0.47	
40	76.44±0.83		43.14±0.98	
50	80.17±0.96		47.48±0.48	
60	88.44±0.71		58.75±0.23	

**Significant at p <0.01
#Values are the mean of triplicates

Table 3: Ferric (Fe^{3+}) reducing power activity of aqueous and ethanol root extracts of Palmyra Palm

Concentration µg/mL	Ethanol extract % of reduction Mean±SD#	p value	Aqueous extract % of reduction Mean±SD#	p value
50	28.40±0.13	0.000**	31.39±0.55	0.000**
100	36.46±0.42		37.38±0.32	
150	48.05±0.62		47.62±0.49	
200	53.27±0.99		56.53±0.49	
250	60.90±0.40		63.05±0.47	
300	64.55±0.38		64.52±0.68	

**Significant at p <0.01
#Values are the mean of triplicates

Table 4: Phosphomolybdenum reduction activity of aqueous and ethanol root extracts of Palmyra Palm

Concentration µg/mL	Ethanol extract % of reduction Mean±SD#	p value	Aqueous extract % of reduction Mean±SD#	p value
50	68.41±0.52	0.000**	32.29±0.28	0.000**
100	72.43±0.38		37.33±0.29	
150	73.46±0.41		43.71±0.49	
200	74.21±0.19		49.42±0.25	
250	75.14±0.12		61.5±0.46	
300	75.97±0.28		64.20±0.26	

**Significant at p <0.01
#Values are the mean of triplicates

The maximum phospho-molybdenum reduction activity of ethanol root extract was 75.97±0.28% and the aqueous root extract was 64.20±0.26% at 300 µg/mL concentration **(Table 4)**. The RC_{50} of ethanol root extract was 36.54 µg/mL concentration and the aqueous root extract was 202.35 µg/mL concentration, compared to the 5.54 µg/mL concentration of the standard ascorbic acid. It is obvious that ethanol extract had a better Phosphomolybdenum reducing power when compared to aqueous extract of lyophilized Palmyra palm root powder.

From the results it is evident that the free radical scavenging activity and reducing ability of both the extracts increased with increasing concentration. There was a significant difference ($p<0.01$) in all the free radical scavenging activity and reducing ability assays of both ethanol and aqueous extracts of Palmyra palm root powder at different concentrations.

The IC_{50} and RC_{50} values of standard ascorbic acid are lower than that of the extracts of Palmyra Palm root because standard ascorbic acid is an isolated antioxidant compound whereas Palmyra palm root is a plant food source containing nutrients and phytochemicals that in the combined form may have reduced potency when compared to isolated compounds like ascorbic acid used as the standard for comparison

DISCUSSION

A study of the antioxidant activity of *Borassus flabellifer L* (Palmyra palm root) assessed using DPPH and superoxide radical scavenging activity and showed the IC_{50} value to be 186 µg/mL, 221 µg/mL

and 347 µg/mL using DPPH assay; 196 µg/mL, not detected and 292 µg/mL respectively using superoxide assay for three different extracts namely methanol, chloroform and ethyl acetate **[22]**, indicating a limited effect when compared to the aqueous and ethanol extracts used in the present study. This also indicates that the extraction of bioactive compounds in Palmyra palm root was higher in aqueous and ethanol extract compared to ethyl acetate, chloroform and methanol extracts**.**

CONCLUSION

Palmyra palm root is an affordable, traditional and locally available food in Tamil Nadu which possesses good antioxidant property. Based on our findings of the present study it could be concluded that both aqueous and ethanol extracts of *Borassus flabellifer* L root, have significant antioxidant activity. Results observed from the antioxidant assay in ethanol and aqueous extracts of Palmyra palm root powder strongly suggest that both the extracts possess a good antioxidant potential but ethanol extract shows a better effect when compared to aqueous extract. Palmyra palm root can thus be considered a functional food with free radical scavenging phytochemicals.

ACKNOWLEDGEMENT

The authors are thankful to Dr. P. Arumugam, Director, ARMATS Biotek Training and Research Institute, Guindy, Chennai-600032 for providing guidance in the analysis of Antioxidant assays conducted at the ARMATS lab.

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