Available online at www.ijpsdr.com
International Journal of Pharmaceutical Sciences and Drug Research 2012; 4(2): 140-142

Research Article ISSN 0975-248X

In-vitro Antioxidant Studies of *Pritchardia arecina*

Krishna Kumar HN*, Rakshitha HR, Navyashree SN, Jyoti Bala Chauhan

Department of Studies in Biotechnology & Microbiology, Pooja Bhagavat Memorial Mahajana Post Graduate Centre, Affiliated to University of Mysore, K. R. S. Road, Metagalli, Mysore-570 016, Karnataka, India

ABSTRACT
In the present study, antioxidant activities of aqueous and methanolic fruit extracts of *Pritchardia arecina* were investigated. The antioxidant properties of the extracts were assessed using 1, 1,-diphenyl-2-picryl-hydrazyl (DPPH) assay and reducing power assay. Both the extracts exhibited significant reducing power which was dose dependent. The IC_{50} value in DPPH assay was found to be 18 and 27µg/ml for methanolic and aqueous extract respectively. Phytochemical analysis revealed the presence of tannins, steroids, amino acids and carbohydrates. The total phenolic content observed for aqueous and methanolic extracts were 25 and 40 mg gallic acid equivalent per gram of extract respectively.

Keywords: *Pritchardia arecina,* antioxidant activities, total phenolic content, reducing power, DPPH assay.

INTRODUCTION

Reactive oxygen species (ROS), which include free radicals such as superoxide anion radicals, hydroxyl radicals and non free-radical species such as H_2O_2 and singlet oxygen are various forms of activated oxygen. These molecules are exacerbating factors in cellular injury and aging process. [1] The most reactive free radical is the hydroxyl radical which is known to initiate lipid peroxidation and cause fragmentation of DNA leading to mutation. [2] In recent years, much attention has been devoted to natural antioxidant and their association with health benefits. [3] Antioxidant-based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer. [4] Plants are potential sources of natural antioxidants; it produces various antioxidative compounds to counteract reactive oxygen species (ROS) in order to survive. [5] Plant phenolics are commonly found in both edible and non-edible plants, and have been reported to have multiple biological effects, including antioxidant activity. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, metal ion chelators and singlet oxygen quenchers. [6]

The genus *Pritchardia* commonly called fan palms belonging to the family Arecaceae (tribe: Corypheae). They are found in tropical Pacific Ocean islands in Fiji, Samoa, Tonga, Tuamotus and Hawaii. There are 29 species of which 19 are endemic to the Hawalian Islands. These palms vary in height, ranging from 20 to 130 ft. The leaves are fan-shaped (*costapalmate*) and the trunk columnar, naked, smooth or fibrous, longitudinally grooved, and obscurely ringed by leaf scars. The flowers and subsequent fruit are borne in a terminal cluster with simple or compound branches of an arcuate or pendulous inflorescence that (in some species) is longer than the leaves. [7] *Pritchardia arecina* is a handsome ornamental palm. The medicinal use of this palm is not known.

The aim of this study was to evaluate the antioxidative activity of *Pritchardia arecina* using different in vitro methods.

***Corresponding author: Dr. H.N. Krishna Kumar,** Assistant Professor, Department of Studies in Biotechnology & Microbiology, Pooja Bhagavat Memorial Mahajana Post Graduate Centre, Affiliated to University of Mysore, K. R. S. Road, Metagalli, Mysore-570 016, Karnataka, India;
E-mail: hnkrishnakumar@yahoo.co.in

MATERIALS AND METHODS

Chemicals

All the chemicals and solvents used were of analytical grade. Methanol, ferric chloride, potassium ferricyanide, ascorbic acid, BHA (Butylated Hydroxy Anisole) and trichloroacetic acid (TCA) were purchased from Merck India ltd., Mumbai. DPPH (1, 1,-diphenyl-2-picryl-hydrazyl) was obtained from Sigma Aldrich Co. St. Louis, USA.

Plant material collection and preparation of extracts

The plant material consisting of mature fruits of *Pritchardia arecina* Becc. was collected from Mysore, Karnataka, India. The materials were identified and authenticated by Department of Studies in Botany, University of Mysore. The fruits were cleaned and washed under running tap water then dried at 40ºC in an oven for 3 days. The dried plant materials were powdered using a grinder. About 20 g of dried fruit powder was soaked in methanol (100 ml) and water (200 ml) separately for 24 h with intermittent shaking. Then extracts

were passed through Whatman No. 1 filter paper. The filtrates obtained were concentrated under reduced pressure and stored at 4°C for further use.

Preliminary phytochemical screening and Determination of total phenolic content

The methanolic and aqueous extracts were tested for different phytoconstituents such as glycosides, tannins, carbohydrates, proteins, saponins, flavonoids, steroids and free amino acids using the method described by Jigna *et al.* [8] The amount of total phenolics in extract was determined with Folin-Ciocalteu reagent according to the method of Singleton and Rossi [9] with slight modification. Briefly, 1.0 ml of extract solution (1 mg/ml) in a volumetric flask was diluted with 60 ml distilled water. Then, 5 ml of Folin-Ciocalteu reagent was added and the content of the flask was mixed thoroughly. After 1-8 min, 15 ml Na_2CO_3 (20 %) was added and the volume was made up to 100 ml using distilled water. The mixture was allowed to stand for 2 hours with intermittent shaking. The absorbance was measured at 760 nm. The total phenolic content was determined as mg of gallic acid equivalent per gram of extract using an equation obtained from the standard gallic acid calibration graph.

DPPH free radical scavenging activity

The antioxidant activity of the extracts was assessed according to the method described by Braca *et al.* [10] Ascorbic acid was used as standard. 0.004% of DPPH solution was prepared in methanol and 1 ml of this solution was mixed with 1 ml of sample solution and standard solution (at different concentration) separately. These solution mixtures were kept in dark for 30 minutes and optical density was measured at 517nm. The % inhibition was calculated using the formula given below.

$$\% \text{ inhibition} = [(A - B)/A] \times 100$$

Where A = optical density of the blank and B = optical density of the sample.

Reducing power assay

The reducing power of the extracts was determined by the method of Oyaizu. [11] Different concentrations of plant extracts (100-500µg/ml) in 1 ml of distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and 1% potassium ferricyanide (2.5 ml). The mixture was incubated at 50°C for 20 min. Then, 2.5 ml of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged for 10 min at 3000 rpm. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and 0.1% $FeCl_3$ (0.5 ml). The absorbance was measured at 700 nm against a blank. Increasing absorbance of the reaction mixture indicated increased reducing power. BHA was used as a standard.

Statistical analysis

All analyses were run in triplicates. Results were expressed as mean ± SD. The IC_{50} was graphically determined by a linear regression method.

RESULTS AND DISCUSSION

Preliminary phytochemical screening and Determination of total phenolic content

The phytochemical tests indicated the presence of tannins, steroids, amino acids and carbohydrates in the extracts (Table 1). Several of such compounds are known to possess potent antioxidant activity. [12] The polyphenolic content of the aqueous extract observed was 25 mg/g equivalent of gallic acid. Likewise, the methanolic extract showed 40 mg/g equivalent of gallic acid. Phenolic constituents are very important in plants because of their scavenging ability due to their hydroxyl groups. [13] It has been suggested that up to 1g polyphenolic compounds (from diet rich fruits or vegetables) consumed daily have remarkable inhibitory effects on mutagenesis and carcinogenesis in humans. [14]

DPPH free radical scavenging activity

DPPH is a stable free radical having paramagnetic property conferred by its odd electron. Methanolic solution of DPPH is densely coloured showing absorption band at 517nm. It can react with antioxidants resulting in decolorization of solution. This decolorization is stoichiometric with respect to the number of electrons taken up. [15] Both the extracts showed antioxidant activity by DPPH method with IC_{50} values of 18µg/ml (methanolic extract) and 27µg/ml (aqueous extract) (Fig. 1). The IC_{50} value for ascorbic acid was 4µg/ml (Fig. 1). The results indicated that the extracts reduce the radical to the corresponding hydrazine when it reacts with the hydrogen donors in the antioxidant principles. [16]

Table 1: Showing phytochemical constituents of fruit extracts of *Pritchardia arecina*

Test	Methanolic extract	Aqueous extract
Flavonoids	--	--
Carbohydrates	++	++
Tannins	++	++
Saponins	--	--
Proteins	--	--
Steroids	++	--
Amino acids	--	++
Glycosides	--	--

++ Presence of constituent; -- Absence of constituent

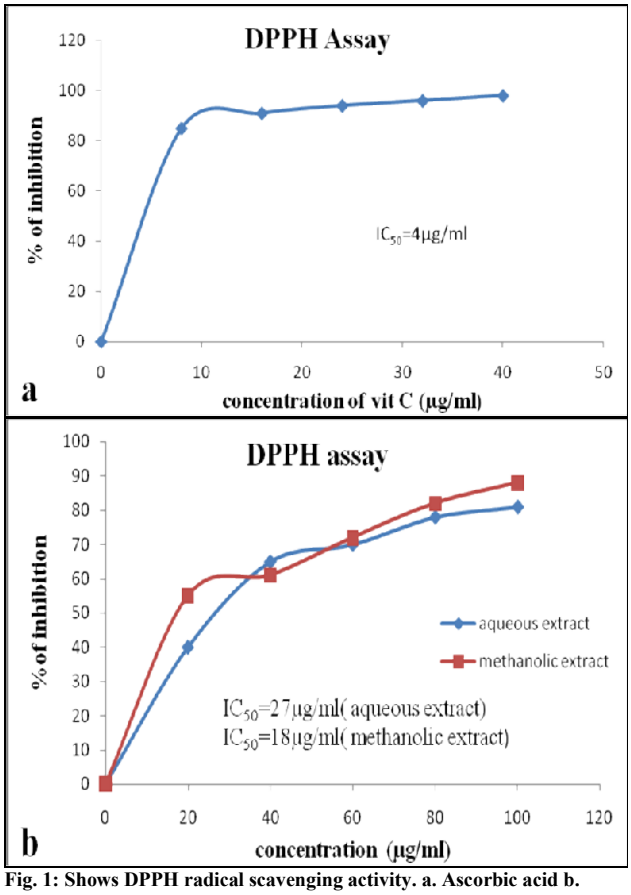

Fig. 1: Shows DPPH radical scavenging activity. a. Ascorbic acid b. Aqueous and methanolic extracts of *Pritchardia arecina*. Each value represents mean ± SD of three replicates.

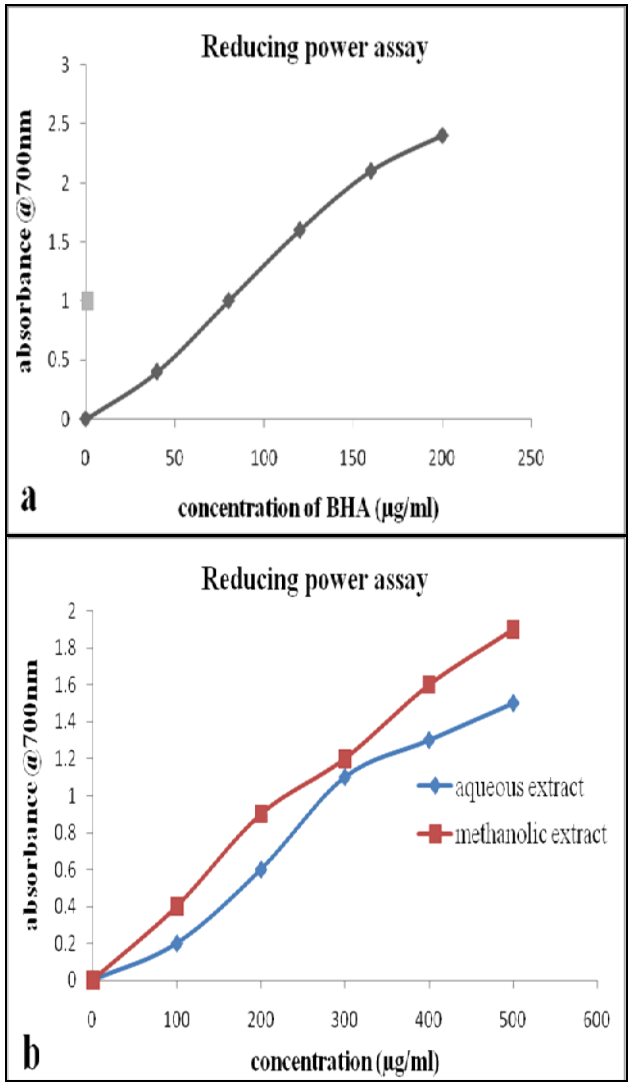

Fig. 2: Shows reducing power assay. a. BHA b. Aqueous and methanolic extracts of *Pritchardia arecina*. Each value represents mean ± SD of three replicates.

Reducing power assay

Reducing power is to measure the reductive ability of antioxidant and it is evaluated by the transformation of Fe^{3+} to Fe^{2+} in the presence of the sample extracts. [1] The reducing power of extracts and standard are summarized in Fig. 2. The reducing power of the extracts increased with increasing the concentration. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. [17] The ability to reduce Fe^{3+} may be attributed by hydrogen donation from phenolic compounds [18] which are also related to presence of reductant agent. [19] The results of the present study showed that both aqueous and methanolic extracts of *Pritchardia arecina* fruit possess antioxidant activity. The antioxidant potential of the extract may be due to the presence of phenolic compound.

ACKNOWLEDGEMENTS

The authors are thankful to Prof. C. K. Renukarya, Director, Pooja Bhagavat Memorial Mahajana Post Graduate Centre, Mysore for facilities to carry out this research work.

REFERENCES

1. Gülçin I, Oktay M, Kıreçci E, Küfreviolu I. Screening of antioxidant and antimicrobial activities of anise (*Pimpella anisum* L.) seed extracts. Food Chem. 2003; 83: 371-382.
2. Halliwell B. Oxygen radicals and Disease process. Hardwood Academic Publisher, Netherlands. 1997, pp. 1-14.
3. Arnous A, Makris DP, Kefalas P. Effect of principal polyphenolic components in relation to antioxidant characteristics of aged red wines. J Agric Food Chem. 2001; 49: 5736-5742.
4. Devasagayam TPA, Tilak JC, Boloor KK. Free radical and antioxidants in human health. Curr Stat Fut Pros JAPI. 2004; 53: 794-804.
5. Lu F, Foo LY. Toxicological aspects of food antioxidants. New York, Marcel Dekker, 1995.
6. Rice-Evans CA, Miller NJ, Bolwell PG, Bramley PM, Pridham J.B. The relative antioxidant activities of plant-derived polyphenolic flavonoids. Free Radical Res. 1995; 23: 375-383.
7. Riffle, Robert Lee, Paul Craft. An Encyclopedia of Cultivated Palms. Timber Press. 2003, pp. 419-422.
8. Jigna P, Nehal K, Sumitra C. Evaluation of antibacterial and phytochemical analysis of *Bauhinia variegate* L. bark. Afri J Biomed Res. 2006; 9(1): 53-56.
9. Singleton VL, Rossi JA. Colorimetry of total phenolic substances. US: American Chemical Society Symposium series 1965; 26: 47-70.
10. Braca A, Sortino C, Politi M. Anti-oxidant activity of flavonoids from *Licania licaniaeflora*. J Ethnopharmacol. 2002; 79: 379-381.
11. Oyaizu M. Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucoamine. Jpn J Nutr. 1986; 4: 307-315.
12. Lee J, Koo N, Min DB. Reactive oxygen species, aging and antioxidative neutraceuticals. Comp Rev Food Sci Food Safety. 2004; 3: 21-33.
13. Hatano T, Edamatsu R, Mori A, Fujita Y, Yasuhara E. Effect of interaction of tannins with co-existing substances. VI. Effects of tannins and related polyphenols on superoxide anion radical and on DPPH radical. Chemical and Pharmaceutical Bulletin 1989; 37: 2016–2021.
14. Tanaka M, Kuei CW, Nagashima Y, Taguchi T. Application of antioxidative maillrad reaction products from histidine and glucose to sardine products. Nippon Suisan Gakkaishi. 1998; 54: 1409–1414.
15. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature 1958; 181: 1199-1201.
16. Sanchez-Moreno C. Methods used to evaluate the free radical scavenging activity in foods & biological systems. Food Sci Tech Int. 2002; 8: 121-137.
17. Meir S, Kanner J, Akiri B, Hadas SP. Determination and involvement of aqueous reducing compounds in oxidative defense system of various senescing leaves. J Agric Food Chem. 1995; 43: 1813-1815.
18. Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of xanthan on the autoxidation of soyabean oil in cyclodextrin emulsion. J Agric Food Chem. 1992; 40: 945-948.
19. Duh PD. Antioxidant activity of Budrock (*Arctium laooa* Linn.) its scavenging effect on free radical and active oxygen. J Am Oil Chem Soc 1998; 75: 455-461.