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Free radical scavenging activity of *Roystonea regia*

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ABSTRACT

The aim of this study was to assess the antioxidant potential of aqueous and methanolic fruit extracts of Roystonia regia. (Kunth) O.F. Cook. The extracts were assessed for their antioxidant activity using DPPH assay and reducing power assay. Inhibition of DPPH free radicals by aqueous and methanolic extracts showed an IC_{50} of 16 and 14 µg/ml respectively. The reducing power was found to be concentration dependent. Methanolic extract showed higher reducing power activity when compared to aqueous extract. Phytochemical screening revealed the presence of tannins, steroids, amino acids and carbohydrates. Total phenolic content was measured by Folin-Ciocalteau method. Methanolic extract showed higher phenolic content (110.5mg) than the aqueous extract (26.9 mg) gallic acid equivalent per gram of extract.

Key words: *Roystonia regia*, Antioxidant activity, DPPH assay, reducing power, phytochemicals.

INTRODUCTION

A free radical is a molecule with one or more unpaired electrons in the outer orbital. These free electrons are referred to as oxidizing agents since they cause other molecules to donate their electrons [1]. Reactive oxygen species (ROS) such as superoxide radical, hydroxyl radical, peroxyl radical and nitric oxide radical, attack biological molecules, such as lipids, proteins, enzymes, DNA and RNA, leading to cell or tissue injury associated with aging, atherosclerosis, carcinogenesis [2]. Antioxidants are free-radical scavengers which can provide protection to living organisms from damage caused by uncontrolled production of reactive oxygen species [3]. The most commonly used synthetic antioxidants are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), Propylgallate (PG) and butylated hydroquinone. However, these synthetic antioxidants have side effects such as liver damage and carcinogenesis [4, 5]. Therefore, there is a need for isolation and characterization of natural antioxidant having less or no side effects, for use in foods or medicinal materials in order to replace synthetic antioxidants.

Roystonea regia, commonly known as the Cuban royal palm, Florida royal palm, or simply the royal palm is native to Southern Florida, Mexico and parts of Central America and the Northern Caribbean. It is a large and attractive palm planted throughout the tropics and subtropics as an ornamental tree. The plant reaches a height of 20–30 metres tall and stem diameter of about 47 centimetres. The trunk is stout, very smooth and grey-white in colour with a characteristic bulge below a distinctive green crownshaft. Trees have about 15 leaves which can be up to 4 m long. The flowers are white with pinkish anthers. The fruits are spheroid to ellipsoid in shape, 8.9–15 millimeters long and 7–10.9 mm wide. They are green when immature, turning red and eventually purplish-black as they mature

[6]. Best known as an ornamental, the plant is also used as a source of thatch, construction timber and as a medicinal plant. The fruit is eaten by birds and bats and fed to livestock. It serves as a roosting site and food source for a variety of animals. The seed is used as a source of oil and for livestock feed. The roots are used as a diuretic. They are also used as a treatment for diabetes [7]. An extract from *R. regia* fruit known as D-004 reduces benign prostate hyperplasia (BPH) in rodents. D-004 is a mixture of fatty acids and is being studied as a potential alternative to finasteride for the treatment of BPH [8].

MATERIALS AND METHODS

Plant material and extract preparation

The fruits of *Roystonea regia* was collected from Mysore, Karnataka, India in the month of September 2010 and authenticated by the DOS in Botany, University of Mysore. The collected fruits were washed under running tap water followed by washing with distilled water to remove surface impurities. The fruits were oven dried at 40º C for 3 days and pulverized using a mixer grinder. Twenty grams of powder was homogenized with 200 ml of distilled water and the homogenate was kept in a shaker at 40°C for 24 h. Then, the extract was filtered over Whatman No. 1 paper and the filtrates were frozen and lyophilized. For methanol extraction, 20 g of powder was mixed with 100 ml of methanol and the homogenate was kept in a shaker for 24 h. The resulting extract was filtered over Whatman No. 1 paper and the filtrate was collected and solvent was removed by a rotary evaporator at 50°C to obtain dry extract. Both extracts were stored in refrigerator until used.

Chemicals

All chemicals and solvents used in the study were of analytical grade. 1, 1-diphenyl-2-picryl hydrazyl (DPPH) was purchased from Sigma Aldrich Co. St. Louis, USA. Methanol, Trichloroacetic acid, Ascorbic acid, Potassium ferric cyanide, ferric chloride, butylated hydroxyl anisole (BHA), Folin-Ciocalteau reagent etc. were procured from Sd Fine chem. Ltd, India.

Phytochemical investigation and Determination of the total phenolics

Standard phytochemical screening tests were performed to identify the different constituents such as glycosides, tannins, carbohydrates, proteins, saponins, flavonoids, steroids and free amino acids present in the extracts [9]. Total phenolic contents in the extracts were determined by the modified Folin-Ciocalteau method [10]. An aliquot of the extracts (1 ml) was mixed with 0.5 ml Folin-Ciocalteu reagent and 1.5 ml of sodium carbonate (20%). Tubes were allowed to stand at 40°C for 30 min. Absorbance was measured at 765 nm. Total phenolic content was expressed as mg/g gallic acid equivalent.

DPPH free radical scavenging assay

The potential antioxidant activity of the extract was assessed on the basis of the scavenging activity of the stable DPPH free radical according to the previous described procedure [11]. Different concentrations of both aqueous and methanolic extracts were mixed with 3ml of 0.1mM methanolic solution of DPPH separately. The tubes were incubated for 30 minutes at room temperature in the dark. The absorbance was measured at 517 nm against a blank. Ascorbic acid was used as the standard. Free radical scavenging activity was expressed as inhibition percentage and was calculated using the following formula,

$$\% \text{ Inhibition} = [(A_0 - A_1)/A_0] \times 100$$

Where, A_0 is the absorbance of the control, A_1 is the absorbance of test samples.

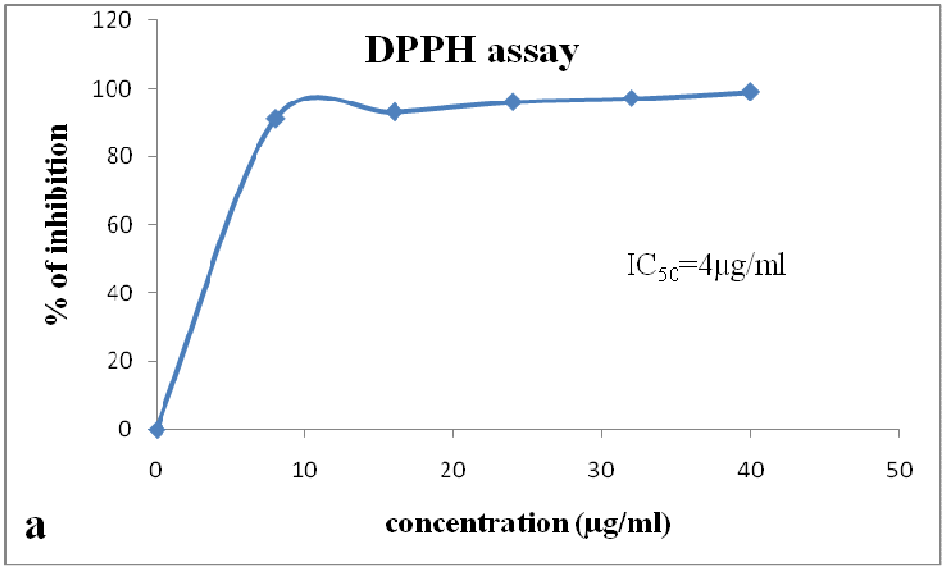
Reducing power assay

The reducing power of the extracts was determined according to the method of [12]. Different concentrations of extracts (100-500µg/ml) were mixed with 2.5 ml of 0.2M phosphate buffer (pH 6.6) and 2.5 ml of 1% Potassium ferrycyanide. This mixture was incubated at 50°C for 20 min. Then, 2.5 ml of 10% Trichloroacetic acid was added to mixture which was then, centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. Increase in absorbance of the reaction mixture indicated increased reducing power.

RESULTS AND DISCUSSION

Phytochemical investigation and Determination of the total phenolics

Phytochemical screening of the extracts showed the presence of carbohydrates, amino acids, steroids and tannins (Table 1). Phenolic content in the methanolic extract was higher when compared to aqueous extract i.e., 110.5 and 26.9 mg gallic acid equivalent per gram respectively. Polyphenols are the major plant compounds that are characterized by antioxidant activity. This activity is believed to be mainly due to their redox properties [13]. The results of the present work strongly suggest that phenolic compounds are important components of this plant and some of their pharmacological effects could be attributed to the presence of these valuable constituents. The methanol extract showed the highest phenolic content and the highest resulted antioxidant activity than the aqueous extract. Thus, the antioxidant activity of an extract could be predicted from its total phenolic content. It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans [14]. Phenolic compounds are known as powerful chain breaking antioxidants and may contribute directly to antioxidative action [15].



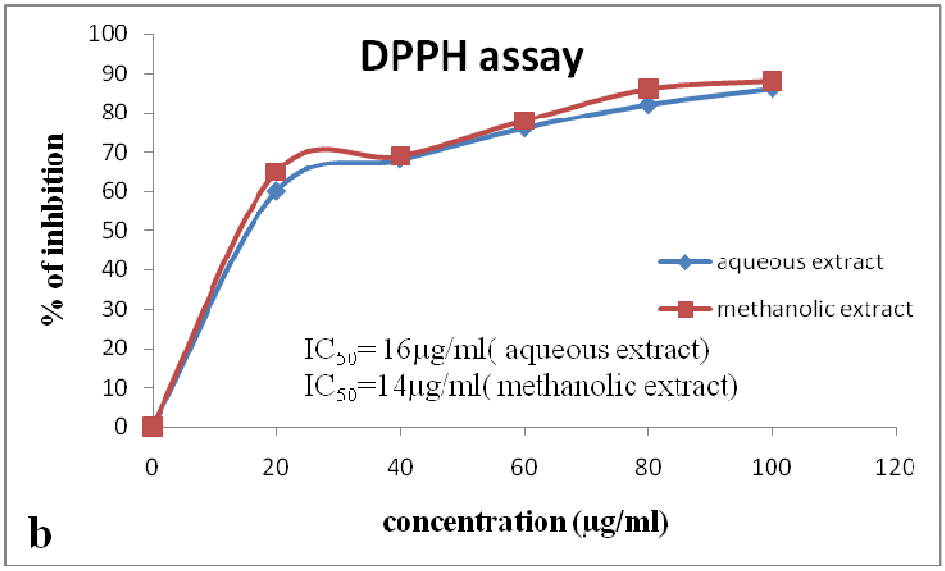


Fig.1 Shows DPPH radical scavenging activity. a. Ascorbic acid b. Aqueous and methanolic extracts of *Roystonea regia*
Each value represents mean ±SD of three replicates.

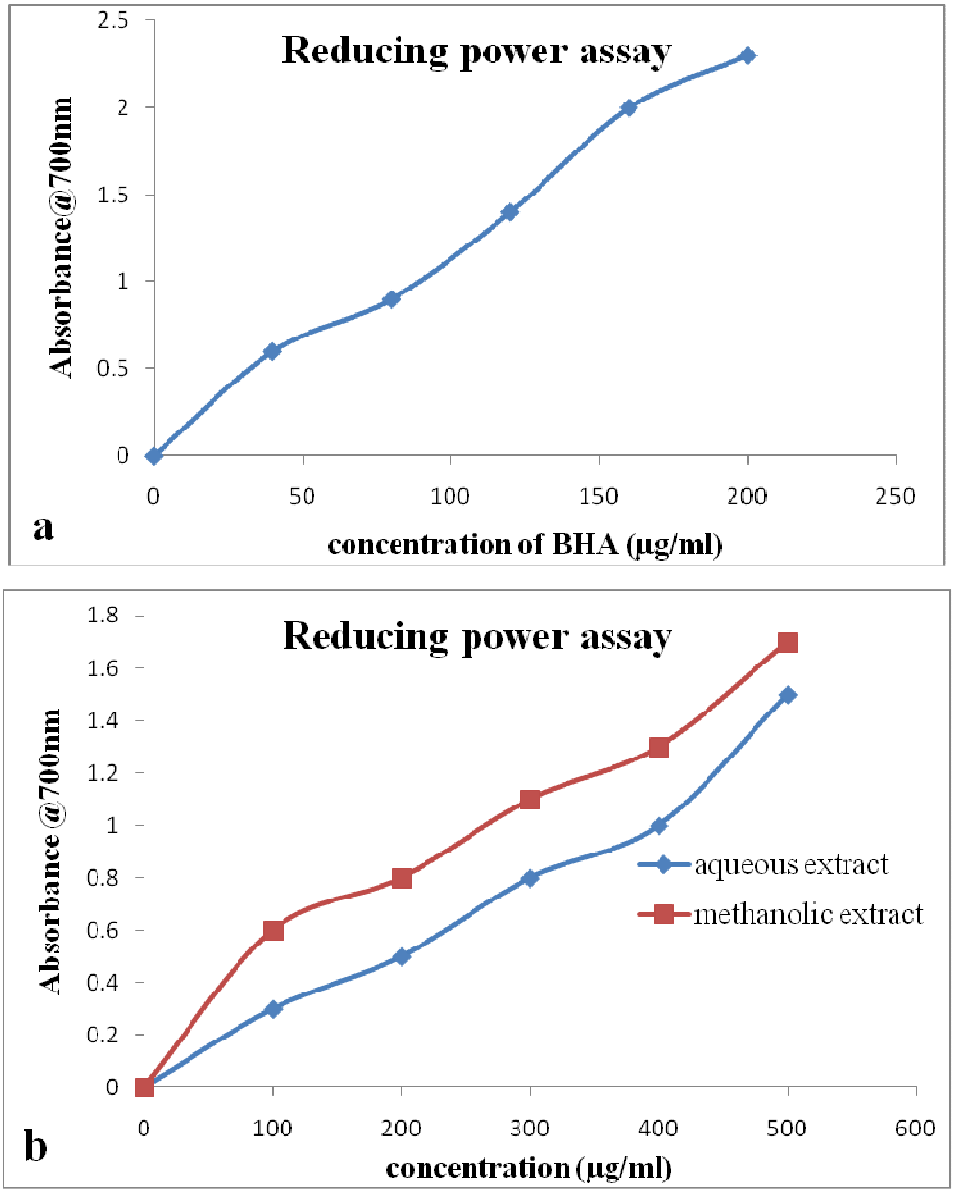


Fig.2 Shows reducing power assay. a. BHA b. Aqueous and methanolic extracts of *Roystonea regia*
Each value represents mean ± SD of three replicates

DPPH free radical scavenging assay

This assay is based on the theory that a hydrogen donor is an antioxidant. The antioxidant effect is proportional to the disappearance of DPPH radical in test samples. A freshly prepared DPPH solution exhibit a deep purple color with absorption maximum at 517nm. The purple color generally fades or disappears when an antioxidant is present in the medium [16, 17]. DPPH radical scavenging activity of the extracts was measured along with standard ascorbic acid. The IC_{50} values of methanol and aqueous were found to be 14 and 16µg/ml respectively while the similar activity for standard was 4µg/ml **(**Fig 1**).** The results revealed dose dependent radical scavenging activity in terms of IC_{50} values.

Reducing power assay

The extracts showed the dose dependent increase in the absorbance indicated that the fruits of Roystonea regia possess concentration dependent reducing power (Fig.2). The reducing capacity of methanolic extract was higher

than the aqueous extract. The consumption of foodstuffs rich in antioxidants provides protection against cancer, cardio and cerebrovascular diseases. This protection can be explained by the capacity of these active compounds to scavenge free radicals, which are responsible for the oxidative damage of lipids, proteins and nucleic acids [18, 19]. The existence of reductones are the key of the reducing power, which exhibit their antioxidant activities through the action of breaking the free radical chain by donating a hydrogen atom. The reducing capacity of Fe3+/ ferricyanide complex to the ferrous form by the extracts may serve as a significant indicator of its antioxidant capacity [20, 21]. The reduction of the Fe3+ / ferricyanide complex to the ferrous form occurs due to the presence of reductants in the solution. Absorbance of Fe3+ can be observed by measuring the O.D. values at 700nm the reduction power of the extract increases with increase in concentration [22].

Table 1: Showing phytochemical constituents of fruit extracts of *Roystonea regia*

Sl.No.	Test	Methanolic extract	Aqueous extract
1	Flavonoids	--	--
2	Carbohydrates	++	++
3	Tannins	++	++
4	Saponins	--	--
5	Proteins	--	--
6	Steroids	++	--
7	Amino acids	--	++
8	Glycosides	--	--

++ Presence of constituent; -- Absence of constituent

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