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Research Article

STUDIES ON THE ANTIOXIDANT ACTIVITY OF *ACTINORHYTIS CALAPPARIA*

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ABSTRACT: In the present study, preliminary phytochemical screening and *in-vitro* antioxidant activity of aqueous and methanolic fruit extracts of *Actinorhytis calapparia* H.Wendl. & Drude was investigated. The antioxidant activity was studied by using *in vitro* antioxidant models viz., DPPH radical scavenging activity and reducing power assay. Both the extracts showed antioxidant activity by inhibiting DPPH free radicals and also showed reducing power ability in ferric reducing model which was a dose dependent. The IC_{50} value was found to be 15 and 26 µg/ml for methanolic and aqueous extract respectively. Phytochemical screening of the extract revealed the presence of tannins, steroids, carbohydrates and amino acids. The total phenolic content of the extract was determined using the Folin-Ciocalteu method. The total phenolic content observed for aqueous and methanolic extracts were 56 and 64.3 mg/g equivalent of gallic acid respectively.

Key words: *Actinorhytis calapparia,* Antioxidant activity, DPPH assay, Phytochemicals, reducing power.

INTRODUCTION

Oxygen is essential for the survival of all on this earth. During the process of oxygen utilization in a normal physiological and metabolic process, approximately 5% of oxygen gets univalently reduced to oxygen derived free radicals like superoxide, hydrogen peroxide, hydroxyl and nitric oxide radicals. All these radicals known as reactive oxygen species (ROS) exert oxidative stress towards the cells of human body rendering each cell to face about 10,000 oxidative hits per second (Mondal et. al., 2006). Free radicals play an important role in the pathogenesis of several human diseases, such as cancer, rheumatoid arthritis, and cardiovascular diseases (Hertog *et al.*, 1997). It has been suggested that fruits, vegetables, natural plant products contain a large variety of substance called phytochemicals are the main source of antioxidant in the diet, which could decrease the potential stress caused by reactive oxygen species (Dell Agli *et al.*, 2004; Scoorbrate *et al.*, 2005). The natural antioxidants may have free-radical scavengers, reducing agents, potential complexers of prooxidant metals, quenches of singlet oxygen etc (Ebadi, 2002). The antioxidants can interfere with the oxidation process by reacting with free radicals (Gupta et.al., 2004). Recently interest has increased considerably in finding natural antioxidants for use in foods or medicinal materials to replace synthetic antioxidants like butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT) etc. which are being restricted due to their side effects such as carcinogenicity (Kumaran and Karunakaran, 2007).

Actinorhytis is a monotyphic genus of flowering plant in the palm family found in Oceania. The lone species *Actinorhytis calapparia* is a rain forest inhabitant and has the largest fruit of any palm in the Iguanurinae (Uhl et.al., 1987). This palm is endemic to New Guinea and the Solomon Islands thriving in lowland rain forest from sea level to 1000m.

The species is solitary trunked, reaching12-14m in height and is relatively slender, usually no wider than 20cm. The leaf crown is sparse but spherical, each arching leaf is around 3m long with pinnately arranged, 45 cm leaflets which are dark green in color. The leaflets are closely and regularly arranged along the rachis and the abaxially rounded petiole is usually long in young but shorter in maturity. The much branched monoecious inflorescence forms below the leaf bases, ringing the trunk with cream colored male and female flowers. Both sexes carry three sepals and three petals and in both cases the sepals are two or three times longer than the petals. The inflorescence becomes pendent as the large fruit set; the beaked, ovoid fruit are red to purple to green; each fruit contains one seed (Riffle et.al., 2003). *Actinorhytis calapparia* is widely cultivated in Southeast Asia and Malaysia where villagers attribute it magical or medicinal powers or as a substitute to betel (Uhl et.al., 1987).
The aim of the present study was to evaluate antioxidant activity of the aqueous and methanolic extracts of *Actinorhytis calapparia* fruits using different in vitro assays.

MATERIALS AND METHODS

Chemicals

Ascorbic acid, gallic acid, potassium ferricyanide, trichloroacetic acid, ferric chloride, folin-ciocalteu reagent, butylated hydroxyl anisole (BHA) etc. were purchased from Merck India Ltd., Mumbai and 1,1-diphenyl-2- picryl hydrazyl (DPPH) was obtained from Sigma (St. Louis, USA). All the reagents and solvents used were of analytical grade.

Plant material and extraction

The fruits of *Actinorhytis calapparia* were collected from Sirsi, Uttara Kannada District, Karnataka, India. The collected fruits were washed thoroughly in water and oven-dried for 3 days at 40ºC. The dried fruits were pulverized in electric grinder. 20g of power was extracted in 100ml of methanol by maceration process (24 hrs). The solvent was removed under the vacuum at temperature below 50ºC. For aqueous extract, 20g of powder was homogenized with 200ml of water and the homogenate was kept in a shaker at 40ºC for 24 hrs and then filtered using whatman No.1 filter paper. The filtrate was concentrated in a lyophilizer. Both the extracts were stored in a deep freezer until use.

Preliminary Phytochemical Screening and Determination of total phenolic compounds

Preliminary phytochemical screening of the extracts was carried out for the detection of the various plant constituents such as tannins, steroids, saponins, flavonoids, glycosides, amino acids, proteins and carbohydrates according to the method described by Khandelwal (2004). Total phenolic compounds present in the aqueous and methanolic extracts were determined by using Folin-Ciocalteu reagent as described by Slinkard and Singleton (1977) with slight modification. Briefly, to 0.1 ml of extract (1mg/ml) one ml of Folin-Ciocalteu reagent was added. After three minutes 3ml of 2% Na_2Co_3 was added. The mixture was incubated for 2 hrs at room temperature with intermittent shaking. The absorbance was recorded at 760nm. The standard graph was prepared using different concentrations of gallic acid. The concentration of total phenolic compounds in a sample was determined as mg of gallic acid equivalent per gram of dry extract.

DPPH radical scavenging activity

The method described by Kumaran and Karunakaran (2007) was used to investigate the DPPH radical scavenging activity. Briefly, 0.1 ml of plant extracts and standard ascorbic acid solution at different concentrations were mixed with 3 ml of 0.004% methanol solution of DPPH. After 30 minutes of incubation in the dark, absorbance was recorded at 517 nm. The percentage inhibition was calculated using the following fomula

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$$[(A_0-A_1)/A_0]\times100$$

Where A_0 is the absorbance of the control. A_1 is the absorbance of the extract.
The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} value was defined as the concentration (in µg/ml) of extracts that inhibits the formation of DPPH radicals by 50%.

Reducing power assay

Reducing power of the extracts were determined as described by Oyaizu (1986) with slight modification. Different concentrations of plant extract and standard BHA solutions were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50° C for 20 min. After incubation, 2.5 ml of 10% trichloroacetic acid was added and the mixture was then centrifuged at 3000 rpm for 10 minutes. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and 0.1% ferric chloride (0.5 ml). The absorbance of the mixture was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing capacity.

Statistical analysis

Experimental results were mean ± SD of three parallel measurements. Linear regression analysis was used to calculate the IC_{50} value.

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening and Determination of total phenolic compounds

The phytochemical screening of the extracts revealed the presence of tannins, steroids, carbohydrates and amino acids (Table 1). Phenolic compounds are a class of antioxidant agents, which act as free radical scavengers (Shahidi and Wanasundara, 1992). The content of the total phenolics in the aqueous and methanolic extracts were 56 and 64.3 mg/g equivalent of gallic acid respectively. The interests in phenolic compounds, particularly flavonoids and tannins have considerably increased in recent years because of their broad spectrum of chemical and diverse biological properties which include the antioxidant effects (Larson, 1988). Further, phenolic compounds are effective hydrogen donors which make them antioxidant (Rice-Evans et.al., 1993).

Table 1: Showing phytochemical constituents of fruit extracts of *Actinorhytis calapparia*

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

DPPH radical scavenging activity

DPPH is relatively stable nitrogen centered free radical that easily accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Yayaprakash et.al., 2001). The strong free radical scavenging effect of both the extracts were confirmed in a DPPH assay (Fig. 1).

The methanolic extract showed the stronger scavenging effect than the aqueous extract which is comparable to standard antioxidant ascorbic acid. The IC_{50} value of methanolic extract, aqueous extract and Ascorbic acid were found to be 15, 26 and 4µg/ml respectively. DPPH radicals react with suitable reducing agents as a result of which the electrons become paired off forming the corresponding hydrazine (Blois, 1958). Hence, the free radical scavenging capacity of an extract may serve as a significant indicator of its potential antioxidant activity.

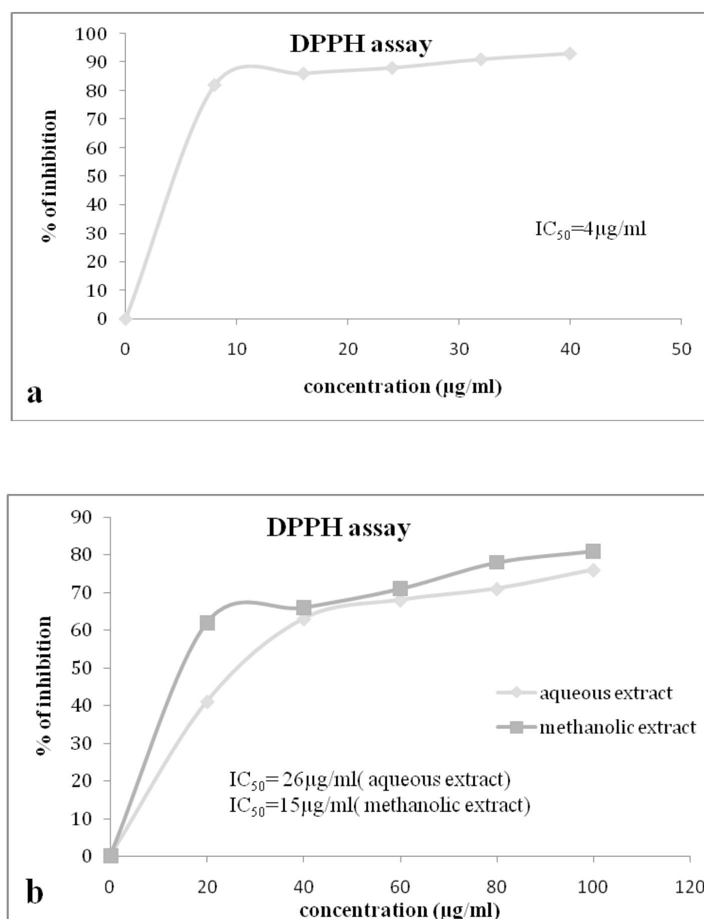

Fig.1 Shows DPPH radical scavenging activity. a. Ascorbic acid b. Aqueous and methanolic extracts of *Actinorhytis calapparia*. Each value represents mean ± SD of three replicates.

Reducing power assay

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Gulcin and Oktay, 2003). The present study revealed that reducing power of extract was significant (Fig. 2). The result clearly indicated that the reducing power of the extracts increased with increasing concentration and is comparable with the standard antioxidant ascorbic acid. The study showed some compounds in the extracts were electron donors and could react with free radicals to convert them in to more stable products and terminate the radical chain reactions.

The extracts showed antioxidant activity by inhibiting DPPH and reducing power ability which may be due to presence of phenolic compounds. Further studies are needed to evaluate the *in-vivo* antioxidant potential of this plant in various animal models.

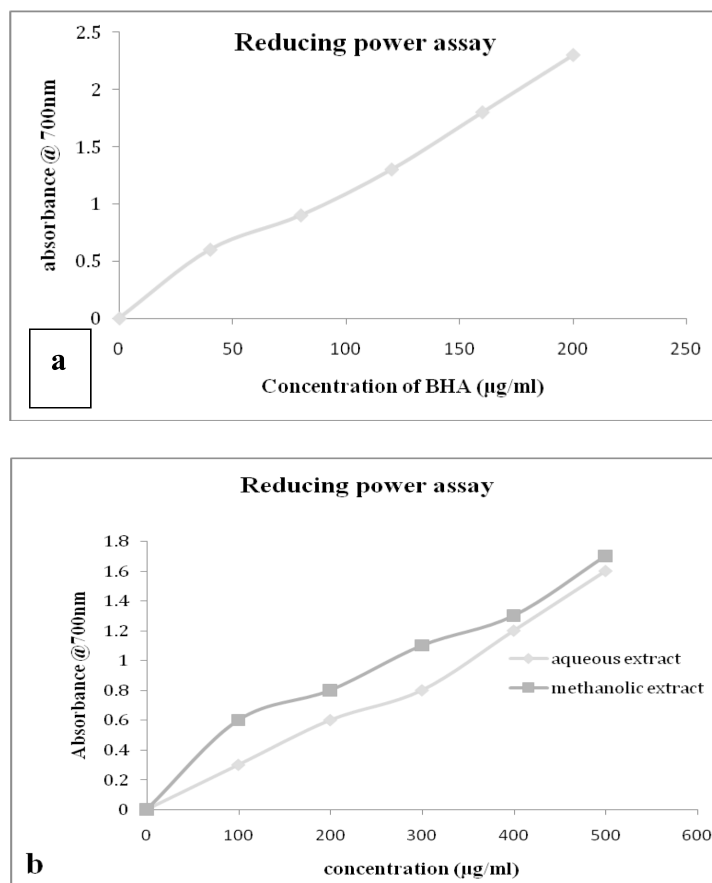

Fig.2 Shows reducing power assay. a. BHA b. Aqueous and methanolic extracts of *Actinorhytis calapparia*. Each value represents mean ± SD of three replicates.

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