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Total Phenolic content and antioxidant activity of aqueous and methanol extracts of *Dioscorea alata* tuber

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ABSTRACT

Antioxidant activity of aqueous extract (DAAE) and methanol extract (DAME) of *Dioscorea alata* tuber were evaluated using DPPH radical scavenging and reducing power assay. DAME contained significantly higher ($p \leq 0.05$) phenolics compared to DAAE and showed significantly higher ($p \leq 0.05$) radical scavenging activity (84%) than DAAE (12.6%). The radical scavenging activity of DAME was comparable with that of BHT (85%) at 100 µg/ml concentration. DAME also showed higher reducing power than DAAE. A significant ($p \leq 0.01$) correlation was observed between the phenolic content and the radical scavenging activity of the extracts indicating the contribution of phenolic compounds for the observed antioxidant effect.

Keywords: *Dioscorea alata*, radical scavenging activity, reducing power, phenolics, oxidative stress

INTRODUCTION

Free radicals arising from either the normal metabolism or induced by environmental sources interact continuously in the biological systems. Oxidants/antioxidants must be kept in balance to minimize molecular, cellular and tissue damage. Reactive oxygen species (ROS) such as superoxide, hydrogen peroxide and hydroxyl radicals are formed in the course of cellular metabolism. A series of antioxidant compounds are present in the cells, they react with oxidizing agents and disarm them[1]. Oxidative stress is implicated in several diseases including cardiovascular diseases, ischemic reperfusion injury, cancer, hypertension and diabetes[2]. Recently, attention has been focused on the relationship between reactive oxygen species (ROS) and several disorders including aging, various inflammatory diseases, carcinogenesis, neurodegenerative diseases, and diabetes. In fact, diabetes is usually accompanied by increased production of ROS and impaired antioxidant defense, indicating a central contribution of ROS to the onset, progression, and pathological consequences of diabetes[3]. An antioxidant is a useful chemical material for preventing oxidative deterioration of biomolecules and is widely applicable as an ingredient of food supplements, cosmetics and medicines. Many researchers have found efficient antioxidants in plants grown as food crops, vegetables, spices and medicinal herbs/plants[4]. Phenolics have been reported to have a capacity to scavenge free radicals[5]. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers. In addition, they have a metal chelation potential[6].

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Dioscorea alata is wide spread in distribution being grown in tropics and subtropics of Africa, America, Asia and Caribbean[7-8]. The tubers are nearly always single and very large weighing upto 60kg and measuring upto 2 meter in length[9]. Yam tuber may be stored for several months but sprouting is accompanied by considerable loss of dry matter and water[10]. The tuber is used in number of ways such as soup thickener, as fried chips and as fried mashed yam balls, it is also used in baked products as reconstituted dough and yam flakes[11]. In the present investigation, the antioxidant capacity of aqueous and methanol extract of the *Dioscorea alata* tuber was evaluated using DDPH radical scavenging and reducing power assay.

MATERIALS AND METHODS

Plant material

Dioscorea alata tuber was collected from Western Ghats, India and subsequently identified by Dr Ravishankar Rai, Dept of Applied Botany, University of Mysore, Mysore. The tuber was washed and the non edible portion (peel) was discarded. The edible portion was dried at 50°C, powdered, passed through 60 mesh sieve (BS) and stored in an air tight container at 4°C till further use.

Preparation of extracts

Dioscorea alata powder was extracted with hot (70°C) distilled water in a mechanical shaker for 24 h, filtered and freeze dried to yield aqueous extract (DAAE,1.4 g). Methanol extract (FRME, 1.5 g) was prepared by extracting bark powder with methanol in a mechanical shaker for 24 h, filtered and evaporated to dryness under reduced pressure in a rotary evaporator.

Estimation of total phenolics

The total Phenolic (TP) content was estimated according to Folin Ciocalteu micro method[12]. Briefly, extract solution (20 µl) was mixed with distilled water (1.58 ml) and Folin Ciocalteu reagent (100 µl) followed by the addition of Na_2Co_3 (20 %), after 1 min and before 8 min. Subsequently, the mixture was incubated at 40°C for 30 min and

the absorbance was measured at 760 nm. Gallic acid was used as standard for calibration curve ant total Phenolics content is expressed as Gallic acid equivalents.

DPPH radical scavenging activity

The hydrogen atom or electron donation ability of various extracts and some pure compounds (a- Tocopherol) was measured from the bleaching of purple colored methanol solution of DPPH. This spectrophotometric assay uses stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) as a reagent[13].The radical DPPH is reduced to the corresponding colorless hydrazine upon reaction with hydrogen donors[14]. Various concentrations of the extracts in 3 ml methanol were added to 1 ml of a 0.1 mM solution of DPPH. After a 30 min incubation period at room temperature the absorbance was read against a blank (methanol) at 517 nm using a semi auto analyzer. The percent RSA was calculated using the following formula:

$$\% RSA = \frac{Abs\ control - Abs\ sample}{Abs\ control} \times 100$$

Where, *Abs control* is the absorbance of the control reaction (containing all reagents except the test compound), and *Abs sample* is the absorbance of the test compound. a-tocopherol was used as positive controls and all tests were carried out in triplicates.

Reducing power assay

Reducing power of the extracts was determined by the method of Yildirm et al[15]. Various concentrations of the extracts and fractions(25-100 µg) in 1 ml of corresponding solvent of each extract was mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml (10 g/l) potassium ferricyanide. The mixture was incubated at 50°C for 30 min followed by addition of 2.5 ml trichloroacetic acid (100 g/l) and centrifugation at 1650×*g* for 10 min. From the upper layer solution 2.5 ml was taken and mixed with 2.5 ml ferric chloride (1 g/l). The absorbance was measured at 700 nm against reagent blank. Higher absorbance indicates higher reducing power. Ascorbic acid was used as reference compound. All tests were carried out in triplicate

Statistical analysis

The data was analyzed by ANOVA followed by Tukey's test for significant differences and the correlations between total phenolics and antioxidant activity were calculated by Pearson correlation using SPSS 11.0 computer software. Maximum inhibition and the IC_{50} values were calculated by Boltzmann's dose response analysis using Origin 6.1 computer software.

RESULTS

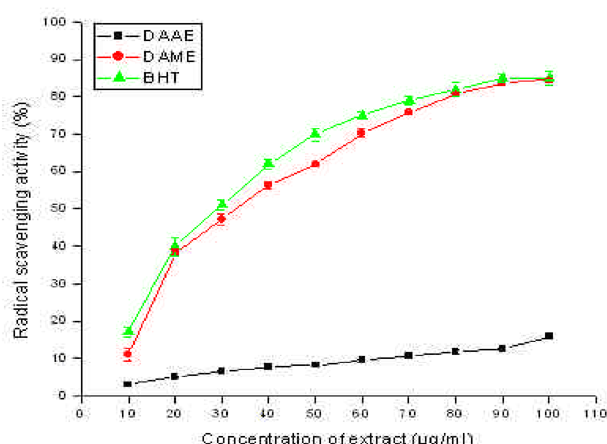
The data on total phenolic content is shown in Table 1. Methanol extract contained significantly higher ($p = 0.05$) phenolics and consequently exhibited significantly higher ($p = 0.05$) radical scavenging activity than the aqueous extract. The radical scavenging activity was dose dependent and the activity of DAME was comparable with that of butylated hydroxyltoluene (BHT), a synthetic antioxidant (Figure 1). The IC_{50} value obtained for BHT was significantly low ($p = 0.05$) compared to DAME while, IC_{50} value for DAAE could not be calculated as it did not attain 50% activity at the concentrations studied. Further, a significant correlation was observed between the total phenolic content and the radical scavenging activity of both extracts ($r = 0.948$, $p = 0.01$). Although, DAME exhibited significantly higher (p

Table 1. Total phenolic content and antioxidant activity of *Diascorea alata*

Sample	Total Phenolics* µgGAE/mg extract	RSA IC_{50} µg/ml
DAAE	12 ± 1.4[a]	NA
DAME	94.1 ± 7.7[b]	45[b]
BHT	ND	26[a]

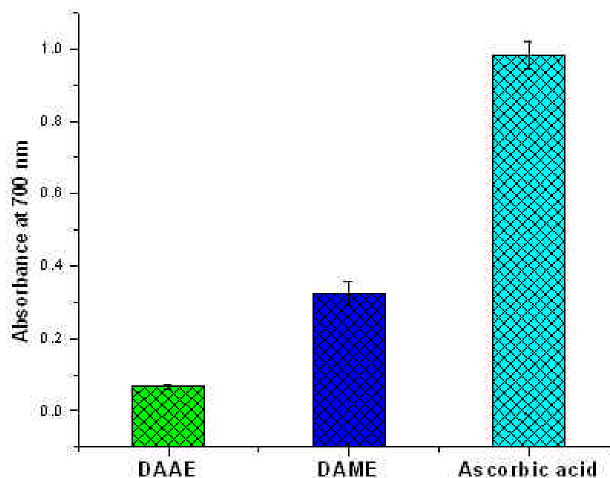
DAAE: aqueous extract, DAME: methanol extract, BHT: butylated hydroxy-toluene ND: not determined, NA: not attained,IC_{50}: extract concentration providing 50% radical scavenging activity,#Mean values carrying different superscript letters a, b, c….., in columns differ significantly (p = 0.05).*

Figure 1. Radical scavenging activity of aqueous and methanol extracts of *Dioscorea alata*



**DAAE: aqueous extract, DAME: methanol extract, BHT: butylated hydroxy-toluene*

Figure 2. Reducing power of aqueous and methanol extracts of *Dioscorea alata*



**DAAE: aqueous extract, DAME: methanol extract*

= 0.05) reducing power than DAAE it was significantly lower (p = 0.05) than the reducing power exhibited by ascorbic acid.

DISCUSSION

Numerous diseases are induced by free radicals via lipid peroxidation, protein peroxidation and DNA damage. It has been known that a variety of plant extracts have antioxidant activities to scavenge free radicals[5]. Both radical scavenging activity and reducing power indicate the electron donating capacity of the compounds which in turn indicates their antioxidant potential[16-17].

To determine the antioxidant activity of *Dioscorea alata* DPPH radical scavenging and reducing power assay was performed. The results raveled the methanol extract to exhibit strong radical scavenging activity comparable to that of BHT and also strong reducing power. The antioxidant activity of *Dioscorea alata* could be attributed to the presence of the phenolic compounds as phenolics have been known to scavenge free radicals and have multiple biological effects, including antioxidant activity[13,15].

It is reported that, no significant correlations could be found between the total phenolic content and antioxidant activity of plant extracts as different phenolics have different responses in the Folin Ciocalteu method[16] and their molecular antioxidant response varies markedly depending on the chemical structure[18] and hence, antioxidant activity of an extract cannot be predicted on the basis of its total phenolic content. On the contrary, in the present study a significant correlation was found between the total phenolic content and antioxidant activity.

CONCLUSION

From the results of the present study it is concluded that, *Dioscorea alata* extracts possess potent antioxidant effect *in vitro* and phenolic compounds are primarily responsible for the observed antioxidant effect. Further studies are needed to characterize these phenolic compounds for their effective utilization. The purified components may exhibit higher antioxidant activity.

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