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Antioxidant property and polyphenols evaluation of aqueous root extract of *Decalepis hamiltonii* Wight & Arn.

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ABSTRACT

Antioxidant activity, total phenol and flavonoids estimation of the aqueous extract of the tuberous root of *Decalepis hamiltonii* was evaluated by free radical scavenging activity using 2,2-diphenylpicryl-1-picrylhydrazyl (DPPH), metal chelating activity, superoxide scavenging activity, ferric reducing antioxidant power assay (FRAP) and 2,2'azinobis (3-ethylbenzothiozoline-6-sulfonic acid) disodium salt ($ABTS^{\bullet+}$) assay. The content of polyphenols in the extract was determined by spectrophotometrically, calculated as gallic acid and catechin equivalent. The present investigation revealed that this plant has rich source of antioxidant properties. Naturally, tuber of this plant has been traditionally used as food by the tribal communities. Hence it is inferred that this plant is not only being as food and it can also be a good source of health tonic.

Key Words: *Decalepis hamiltonii*, free radical scavenging activity, gallic acid, catechin.

INTRODUCTION

Decalepis hamiltonii Wight & Arn (Asclepiadaceae) is a monogeneric climbing shrub native of Deccan peninsula and forest areas of Western Ghats of India. The rhizome is largely used for pickling along with curd or limejuice (Anon, 1952). People procure and habitually carry the roots with them and chew the same whenever the digestion may seek relief. Besides treating indigestion the roots have been used locally to stimulate the appetite and to relieve flatulence and act as a general tonic (Vedavathy, 2004). In *Decalepis hamiltonii* the tuberous root extract contain the flavor compound 2-Hydroxy 4-methoxybenzaldehyde as a major compound (97%) which is extractable by steam distillation method followed by using dichloro methane (Giridhar *et al.*, 2004). The flavonoids are a category of natural substances belonging to the family of polyphenols. The main function seems to be the coloration of plants (just like chlorophyll and carotenoids). The presence of flavonoids in the plant is sometimes concealed under their leuco shape, which explains their commercial interest in the food industry (Fiorucci, 2006).

The natural antioxidants may have free-radical scavengers, reducing agents, potential complexes of peroxidant metals, quenchers of singlet oxygen (Ebadi, 2002). The antioxidants can interfere with the oxidation process by reacting with free radicals (Gupta *et al.*, 2004). Recently interest has been increased considerably in finding natural occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants which are being restricted due to their side effects such as carcinogenicity (Kumaran and Karunakaran, 2007).

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MATERIALS AND METHODS

Chemicals

All the chemicals and solvents were of analytical grade and were obtained from Himedia chemicals, Mumbai, India. 2, 2 - azinobis 3-ethylbenzothiozolin-6-sulphonate (ABTS) was obtained from Sigma chemicals, USA. The other chemicals used were Gallic acid, α-Tocoperol, Rutin, 2,2-diphenylpicryl-1-picrylhydrazyl (DPPH), Tert-

butylated hydroxytoluene (BHT), Ethylene diamine tetra acetic acid (EDTA), Trolox, Potassium persulphate.

Plant material

The tubers of *D. hamiltonii* were collected from tribal inhabitat from above 700 to 1000m of Kolli Hills, Namaakkal, Tamilnadu. The freshly harvested tubers were methodically washed and air dried in our laboratory. They were authenticated at the Botanical Survey of India (Southern part of Coimbatore). The voucher specimen was deposited at the Institute's herbaria.

Preparation of aqueous extract

25g of root powder was dissolved in 100ml of boiled distilled water in a conical flask kept on rotary shaker for 12 hours under 80rpm, residue was filtered using No. 1 Whatman filter paper. The residues were then collected and dried to dryness first on a water bath and then in an oven. After drying, the residue was weighed and scraped out and different aliquots were dissolved in 5ml of sterile water and stored at 4°C for further analysis.

Total Polyphenol content

The total phenol content was determined by the method described by Siddhuraju and Becker (2003). Aliquots (250 µl) of each extracts were taken in test tubes and made up to the volume of 1 ml with distilled water. Then 0.5ml of Folin-Ciocalteu phenol reagent (1:1 with water) and 2.5ml of sodium carbonate solution (20%) were added sequentially in each tube. Rapidly after vortexing the reaction mixture, the test tubes were placed in dark for 40 minutes and the absorbance was recorded at 725nm against reagent blank. All determinations were carried out in triplicate. The total phenolic compound in the extract in gallic acid equivalent (GAE) was calculated.

Flavonoid contents were determined according to the method of Zhishen *et al.* (1999). An aliquot (250 µl) of each extract or standard solution was mixed with 1.25 ml of DD H_2O and 75 µl of 5% $NaNO_2$ solution. After 6 min, 150 µl of 10% $AlCl_3.H_2O$ solution was added. After 5 min, 0.5 ml of 1 M NaOH solution was added and then the total volume was made up to 2.5 ml with double distilled water. Following thorough mixing of the solution, the absorbance against blank was determined at 510 nm. The results were expressed in mg catechin equivalent (CE).

DPPH free radical scavenging activity

DPPH scavenging activity of this plant tuber extract was measured according to the method of Blios (1958). IC_{50} values of the extracts and concentration of the extracts necessary to decrease the initial concentration of DPPH by 50% were calculated.

Metal chelating activity

The chelating activity of ferrous ions by aqueous extract was estimated by the method (Dinis *et al.*, 1994). Briefly the extract sample (250µl) was added to a solution of 2 mmol/L of $FeCl_2$ (0.005ml). The reaction was initiated by the addition of 5 mmol/L ferrozine (0.2ml) and the mixture was shaken vigoursly and left standing at room temperature for 10 to 15 min. Absorbance of the solution was then measured spectrophotometrically at 562nm. The chelating activity of the extracts was evaluated using EDTA as the standard. The results were expressed in mg EDTA equivalent/g extract.

Superoxide radical scavenging activity

The scavenging activity towards the superoxide radical (O_2) was measured in terms of inhibition of generation of Oxygen (Sanchez–Moreno, 2002). The reaction mixture consisted of phosphate buffer (50mM, pH 7.6) riboflavin (20µg/0.2ml). EDTA (12mm), NBT (0.1mg/3ml) and Sodium cyanide (3µg/0.2ml). The aqueous extract was added in various concentrations of 50-200µg/ml to make a total volume of 3ml. The absorbance was read at 530nm before and after illumination under UV lamb for 15 min against a control instead of the sample. The percentage of inhibition was calculated by using the same formula as given above.

Ferric reducing antioxidant power assay

Antioxidant capability of solvent extract of samples was estimated as described (Pulido *et al.*, 2000). FRAP reagent (900µl), prepared freshly and incubated at 37°C was mixed with 90µl of distilled water and 30µl of test sample or methanol (for the reagent blank). The test samples and reagent blank were incubated at 37°C for 30 min in a water bath. The final dilution of the test sample in the reaction mixture was 1/34. The FRAP reagent contained 2-5ml of 20µmol/l 2,4, 6-tripyridyl -2-triazine (TPTZ) solution

Table 1: Extraction yield and content of total polyphenols of root extract of *Decalepis hamiltonii.*

Sample	Extraction yield (%)	Content	
		Total phenols (mg GAE/g extract)	Total Flavonoids (mg CE/g extract)
Aqueous extract	5.81[a]	12.62±2.20[b]	14.08±2.40[c]

Values (mean ± SD, $n = 3$) in the same column followed by a different letter are significantly different ($p < 0.05$)
[a]Extraction yield (%) = (sample extract weight/sample weight) x 100%. [b]GAE, Gallic acid equivalents, [c]CE, Catechin equivalents.

in 40µmol/l HCl plus 2.5ml of 20µmol/l $Fecl_3.6H_2O$ and 25ml of 0.3 mol/l acetate buffer (pH 3.6) as described by (Siddhuraju and Becker, 2003). At the end of incubation the absorbance readings were taken immediately at 593nm using spectrophotometer. Methanolic solutions of known Fe II concentration ranging from 100 to 2000 µmol/l, $FeSO_4.7H_2O$ were used for the preparation of the calibration curve. The FRAP value is expressed as mmol Fe (II) equivalent/mg extract.

ABTS·+ cation radical scavenging activity

The total antioxidant activity of the samples was measured by ABTS radical cation decolourization assay according to the method (Re *et al.*, 1996). In this improved version, ABTS the oxidant is generated by per sulfate oxidation of 2, 2-azinobis (3-ethylbenzoline-6-Sulfonic acid) – ($ABTS^{2-}$). ABTS radical cation ($ABTS^{+}$) was produced by reacting ABTS solution (7mM) with 2.45 mM ammonium per sulfate and the mixture were allowed to stand in dark at room temperature for 12 to 16 h before use. For this study different concentrations that ranges from 25 to 100µg/ml of aqueous extract were added to 0.3 ml of ABTS solution and the final volume was made up with aqueous to 1 ml. The absorbance was read at 734nm and the percentage of inhibition was calculated.

Statistical analysis

The data were subjected to a one-way analysis of variance (ANOVA) and the significance of the difference between means was determined by Duncan's multiple range test ($p<0.05$) using statistical (Stat soft Inc., OK, USA). Values expressed are means of three replicate determinations ± standard deviation.

RESULTS AND DISCUSSION

Determination of Polyphenol

The total phenolics and flavonoids content of the root aqueous extracts were found to be 12.62±2.20 g/100g and 14.08±2.40mg/g respectively (Table 1) compared with gallic acid, catechin equivalents. These phytochemical compounds are known to support bioactive activities in medicinal plants and thus responsible for the antioxidant activities of this plant extract used in this study.

DPPH· radical scavenging activity

The results on DPPH· radical scavenging activity of the aqueous tuber extracts along with the reference standards α-tocopherol, rutin and butylated hydroxyl toluene (BHT) are shown in Figure 1. Concentration of the sample necessary to decrease initial concentration of DPPH· by 50% (IC_{50}) under experimental condition was calculated. Therefore lower value of IC_{50} indicates higher antioxidant activity of 135.59µg/ml. In this study indicates that the plant was potently active and these plant extract contain polyphenol compounds that are capable of donating hydrogen to a free radical in order to remove odd electron which is responsible for radical's reactivity. Free radicals are chemical entities that can exist individually with one or more unpaired elec-

Table 2: Metal chelating activity, FRAP and $ABTS^{\cdot+}$ assay of root extract of *Decalepis hamiltonii*.

Sample	Metal chelating mg EDTA/g sample	FRAP (µmol Fe (II)/mg extract)	TAA (µmol/g extract)
Aqueous extract	108±1.0[a]	746.89±4.90[b]	2620.8±1566.8[c]

Values (mean ± SD, $n = 3$) in the same column followed by a followed by different superscript letters indicate significant statistical difference ($p<0.05$). [a]EDTA equivalent, [b]Ferric-TPTZ reducing ability expressed as mmol Fe(II) equivalents, [c]Total antioxidant activity (µmol equivalent Trolox performed by using ABTS·+ radical cation)

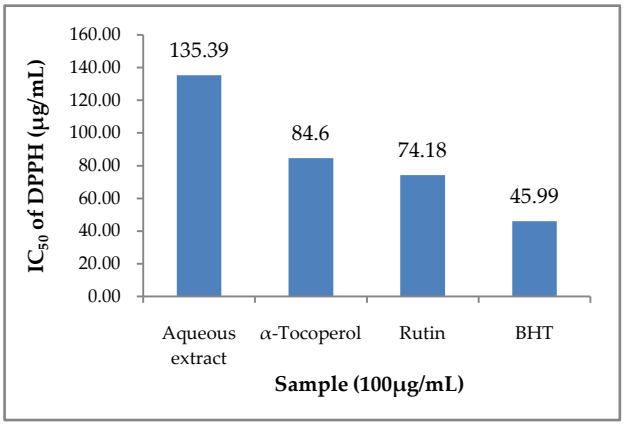

Figure 1: DPPH Radical scavenging activity of aqueous root extract of *Decalepis hamiltonii*

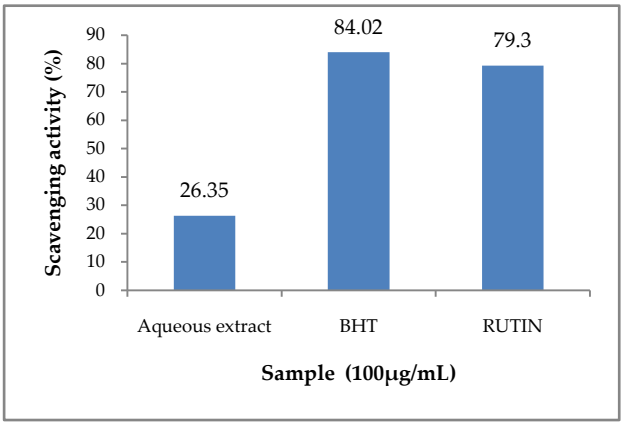

Figure 2: Superoxide radical scavenging activity of aqueous root extract of *Decalepis hamiltonii*

trons. The generation of free radicals can bring about thousands of reactions and thus cause extensive tissue damage. Lipids, proteins and DNA are all susceptible to attack by free radicals (Cotran *et al.*, 1999).

Metal chelating activity

Presence of transition metal ions in biological system could catalyse the Haber-Weiss and Fenton-type reactions, resulting in generation of hydroxyl radicals (OH^*). However, these transition metal ions could form chelate with the antioxidants, which result in the suppression of OH* generation, and inhibit the peroxidation process of biological molecules. The chelating effect of *D. hamiltonii* root extract on ferrous ion is shown in Table 2. It was observed that the maximum inhibition (108.0mg EDTA/g extract) was observed in the 50µg/ml concentration and the inhibitory effect started to decrease after this concentration. The chelate might be due to high concentration of phenolic compounds that can chelate metal ions. Metal chelating capacity was significant as they reduced the concentration of the catalyzing transition metal in lipid peroxidation (Duh *et al.*, 1999).

Superoxide radical scavenging activity

Superoxide anion plays an important role in plant tissues and is involved in the formation of other cell-damaging free radicals (Duan *et al.*, 2007). The relative scavenging effects of *D. hamiltonii* and BHT towards superoxide anion radicals are shown in Figure 2. Plant sample extract exhibited excellent superoxide anion scavenging activity as compared with BHT and Rutin. The effect values were found to be (26.35%) followed by BHT (84.2%) and Rutin (79.3%) respectively. It is known that the hydroxyl group of the phenolics contributes to superoxide anion scavenging activity by their electron donation (Bravo, 1998).

Ferric reducing antioxidant power assay

FRAP assay directly measured antioxidant or reductants in a sample that react with ferric tripyridyltriazine (Fe^{3+} TPTZ) complex and produce coloured ferrous tripyridyltriazine (Fe^{2+} TPTZ). The antioxidant ability of sample extract of *D. hamiltonii* varied significantly ($p<0.05$) (Table 2) and the root extract showed higher FRAP antioxidant activity (746.89 mmol/Fe II/mg). The phenolic compounds exhibited reduction properties by acting as reducing agents, hydrogen donators and singlet oxygen quenchers (Rice-Evans *et al.*, 1997).

ABTS·+ cation radical scavenging activity

ABTS·+ cation radical scavenging activity decolorization assay applicable to both lipophilic and hydrophilic antioxidants including flavonoids, hydroxycinnamates, carotenoids, and plasma antioxidants. The pre-formed radical monocation of 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) is generated by oxidation of ABTS with potassium persulfate and is reduced in the presence of such hydrogen-donating antioxidants. The activity of tested sample extract is expressed as a micromolar equivalent of Trolox solution, having an antioxidant ability equivalent to 1g of dry matter of the extract under the experimental investigation.

The effect of aqueous extract of *D. hamiltonii* on ABTS·+ cation radical scavenging activity is shown in Table 2 and the root extract exhibited higher total antioxidant activity of 2620.8 µmol/g. The scavenging activity of ABTS+ radical by the plant extract was found to be appreciable; this implies that the plant extract may be useful for treating radical related pathological damage especially at higher concentration (Wang *et al.*, 1998). Polyphenols are the major plant compounds with antioxidant activity, although they are not the only ones. The antioxidant activity of phenolic compounds is reported to be mainly due to their redox properties (Galato *et al.*, 2001; Zheng and Wang, 2001), which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides.

CONCLUSION

In conclusion, the present study shows that polyphenols content in the aqueous root extracts of *Decalepis hamiltonii* is high and these extracts exhibit strong antioxidant activities compared to that of the standard compounds such as α-Tocoperol, Rutin and Butylated hydroxytoluene (BHT). Hence, this investigation suggested that the plant naturally having rich source of antioxidants could be used in the prevention of free radical diseases and general health tonic.

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