Journal of Applied Pharmaceutical Science 02 (05); 2012: 106-110

Journal of Applied Pharmaceutical Science

Available online at www.japsonline.com

ISSN: 2231-3354
Received on: 14-04-2012
Revised on: 21-04-2012
Accepted on: 29-04-2012
DOI: 10.7324/JAPS.2012.2518

Nutritional assessment, polyphenols evaluation and antioxidant activity of food resource plant *Decalepis hamiltonii* Wight & Arn

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ABSTRACT

The most important objective of this study was to estimate the nutritional contents like total carbohydrate, lipids, protein, fiber, phenol, flavonoids and moisture content and to evaluate the properties of antioxidant activity. The content of total phenols in the extract was determined spectrometrically according to the Folin-Ciocalteu procedure and calculated as gallic acid equivalent. The achievable antioxidant activity of 2,2-diphenylpicryl-1-picryl-hydrazyl (DPPH) radical scavenging, 2,2'azinobis (3ethyl-benzothiozoline-6-sulphonic acid) disodium salt (ABTS·+) assay, ferric reducing antioxidant power (FRAP) assay, metal chelating activity, superoxide radical scavenging activity was investigated and the result suggested that this freshly harvested plant tubers have more nutritionally potential constituent and antioxidant ability to restore the cell wall damage, mortify and malnutrition.

Keywords: Decalepis hamiltonii, Protein, Gallic acid equivalent, Antioxidant activity.

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). These 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). With ever-increasing population pressure and fast depletion of natural resources, it has become extremely important to diversify the present day agriculture in order to meet various human needs (Janardhanan *et al.*, 2003). Information regarding the chemical and nutritional content of Indian wild edible tubers, rhizomes, corms, roots and stems is meager (Gopalan *et al.*, 1976; Babu *et al.*, 1990; Nair and Nair, 1992; Rajyalakshmi and Greevani, 1994; Shanthakumari *et al.*, 2008; Udensi *et al.*, 2008). In Decalepis hamiltonii the tuberous root extract contain the flavor compound 2-hydroxy 4-methoxy benzaldehyde major compound (97%) which is extractable 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. Their main function seems to be the coloration of plants (just like chlorophyll and carotenoids). Their presence in the plant is sometimes concealed under their leuco shape, which explains their commercial interest in the food industry (Fiorucci, 2006). The oxidative deterioration of lipid containing food is responsible for the stale odours and flavours during processing and storage, consequently decreasing the nutritional quality and safety of foods due to the formation of secondary, potentially toxic compounds (Zainol *et al.*, 2003).

Reactive oxygen and nitrogen species play key roles in normal physiological processes, including cellular life/death processes, protection from pathogens, various cellular signaling pathways, and regulation of vascular tone (Valko *et al.*, 2007). Oxidative stress is caused by an insufficient capacity of biological systems to neutralize excessive free radical production, which can contribute to human diseases and aging (Flora, 2007), including cardiovascular disease (Victor and Rocha, 2007), neurodegenerative disease and age related cognitive decline (Swerdlow, 2007), obesity and insulin resistance (Martinez, 2006), as well as immune system dysfunction (Larbi, 2007). Oxidative stress also contributes to the accumulation of damaged macromolecules and organelles, including mitochondria (Swerdlow, 2007; Terman *et al.*, 2007).

MATERIALS AND METHODS

Plant materials

In this opportunity the tubers of *Decalepis hamiltonii* were collected from field at foothills of Kolli hills on track at deciduous forest area near by tribal settlement. The samples of plants and fruits were identified self and binomially by Botanical Survey of India (Southern part Coimbatore, Tamilnadu, India) and voucher specimens were deposited at the Herbarium Department of Botany, Kongunadu Arts and Science College (Autonomous), Coimbatore, Tamilnadu, India.

Proximal Composition

Moisture, Lipid, fibre and ash were determined according to the standard method (AOAC, 1995). The nitrogen content was estimated by the micro Kjeldahl method (Humphries, 1956) and the crude protein content was calculated (Nx6.25). Samples were analyzed in triplicate and the results reported are the mean values±standard deviation (SD).

Preparation for Extraction

Well grown and healthy fresh tuberous root of *Decalepis hamiltonii* were collected. The plant root rinsed, dried in shade at room temperature (27˚C), powdered with mechanical grinder and were subjected to solvent extraction of petroleum ether and methanol using soxhlet apparatus and the air dried.

Aqueous extract

25g of root powder dissolved in 100ml of hot distilled water in a conical flask kept on rotary shaker for 12 hours under 80rpm, residue were 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 sterile water and were stored at 4°C for further analysis.

Determination of total Polyphenol

The total phenol content was determined by the method (Siddhuraju and Becker, 2003). Aliquots 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 min and the absorbance was recorded at 725nm against reagent blank. The analysis was performed in triplicate and the results were expressed as the gallic acid equivalents (GAE).

Flavonoid contents were determined according to the method (Zhishen *et al.*, 1999). Aliquots of each extract or standard solution was mixed with 1.25 ml of distilled water and 75 µl of 5% $NaNO_2$ solution. After 6 minutes 150 µl of 10% $AlCl_3.H_2O$ solution was added. After 5 minutes 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 gallic acid equivalents (GAE).

DPPH· radical scavenging activity

The 2, 2-diphenylpicryl- 1-picryl-hydrazyl (DPPH·) radical scavenging activity of root extracts were measured according to the method (Blios, 1958). IC_{50} values of the extract i.e., concentration of extract necessary to decrease the initial concentration of DPPH by 50% was calculated.

Ferric reducing antioxidant power (FRAP) assay

The antioxidant capacity of solvent extracts 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 minutes 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 20 µmol/L 2, 4, 6-tripyridyl-2-triazine (TPTZ) solution in 40µmol/L HCl plus 2.5mL of 20 µmol/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 a spectrophotometer. Methanolic solutions of known Fe (II) concentration, ranging from 100 to 2,000µmol/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.

Metal chelating activity

The chelating of ferrous ions by various root extracts was estimated by the method (Dinis *et al.*, 1994). Briefly the extract samples (250µL) were added to a solution of 2mmol/L $FeCl_2$ (0.05mL). The reaction was initiated by the addition of 5 mmol/L ferrozine (0.2mL) and the mixture was shaken vigorously and left standing at room temperature for 10 minutes. Absorbance of the solution was then measured spectrophotometrically at 562nm. The chelating activity of the extracts was evaluated using Ethylenediamine tetraacetic acid (EDTA) as standard. The results were expressed as 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 O_2 (Sanchez-Moren, 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 sample. The percentage of inhibition was calculated by using the same formula as given above.

Antioxidant activity by the ABTS·+ assay

The 2, 2'Azinobis (3-ethyl-benzothiozoline-6-sulfonic acid) disodium salt (ABTS) was dissolved in water to a 7mM concentration. ABTS radical cation (ABTS·+) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. Prior to assay, the solution was diluted in ethanol (about 1:89 v/v) and equilibrated 30ºC to give an absorbance at 734 nm of 0.70±0.02 in a 1-cm cuvette (Re *et al.*, 1999). The concentration of the extracts that produced between 20-80% inhibitions of the blank absorbance was determined and adapted. After the addition of 1 mL of diluted ABTS·+ solution to 10µL of root extracts or Trolox standards (Final concentration 0-15 µM) in ethanol, optical density (OD) was taken at 30ºC exactly 30 minutes after the initial mixing. The unit of total antioxidant activity (TAA) is defined as the concentration of Trolox having equivalent antioxidant activity expressed as µmol/g sample extracts on dry matter.

Statistical analysis

All analyses were carried out in triplicate and the data were reported as means±SD. Where there were significance of the difference between means was determined by Duncan's multiple range test ($p<0.05$) using statistical.

RESULT AND DISCUSSION

The proximate composition of fresh tuberous root (g/100 g) examined is shown in table 1. Tuber yielded an energy level of 1,650 KJ, with a high content of carbohydrate (2.39g), protein (2.37g), lipid (1.24g) and fiber (10.51g). The presence of fiber in the diet is necessary for digestion and for elimination of wastes. The contraction of muscular walls of the digestive tract is stimulated by fiber, thus counteracting constipation (Narasing *et al.*, 1989). The World Health Organization (WHO) has recommended an intake of 22-23kg of fiber for every 1000 K. calorie of diet (Kanwar *et al.*, 1997). The higher moisture content was recorded (84.05%) and it makes a stable food for prolonged period of time. The roots are highly aromatic sweetener and act as a general health tonic (Vedavathy, 2004; Samydurai and Thangapandian, 2012). This signifies that the sample is accomplished with essential nutrients required for human malnutrition deficiency. The percentage of extract yield, total phenol and flavonoids content of the tuberous root extracts obtained from *Decalepis hamiltonii* are shown in table 2. These polyphenol inferences were compared with gallic acid equivalents.

Table. 1: Proximate Composition of Edible Tubers of *Decalepis hamiltonii.*

Sample	Energy	Moisture	Lipid	Carbohydrate	Protein	Fibre
	KJ/100g	%	(g/100 g)			
Tuber-ous root	1,650	84.05	1.24	2.39	2.37	10.51

All the values are means of triplicate determinations expressed on dry weight basis

Table. 2: Extraction yield and content of total polyphenols of root extract of *Decalepis hamiltonii.*

Sample	Extract yield (%)	Total phenol	Total flavonoids
PET	11.2	6.95±1.63	9.58±0.47
MeOH	38.9	13.05±1.81	18.65±0.80
Aqueous	5.81	12.62±2.20	14.08±2.40

All the values are expressed as mean±SD (n=3).

DPPH· radical scavenging activity

The results on DPPH· radical scavenging activity of the different solvent extracts along with the reference standards quercetin, α-tocopherol, rutin, butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) are shown in fig. 1. The model of stable DPPH free radicals can be used to evaluate the antioxidative activities in a relatively short time. The absorbance decreases as a result of a color change from purple to yellow as radical is scavenged by antioxidants through donation of hydrogen to form the stable DPPH molecule. Concentration of the sample necessary to decrease initial concentration of DPPH· by 50% (IC_{50}) under the experimental condition was determined. Therefore, lower value of IC_{50} indicates a higher antioxidant activity. Both solvent extracts showed excellent DPPH· radical scavenging activity and also aqueous extract. MeOH extract of (55.19µg/mL) tuberous roots showed higher levels of free radical scavenging activity among the solvent extract tested. The DPPH· radical scavenging activity was found to be the least in petroleum ether and aqueous extracts of root (IC_{50} 81.1 and 135.39µg/mL, respectively). The radical scavenging activity of the extracts could be related to the nature of phenolics and their hydrogen donating ability (Shimada *et al.*, 1992).

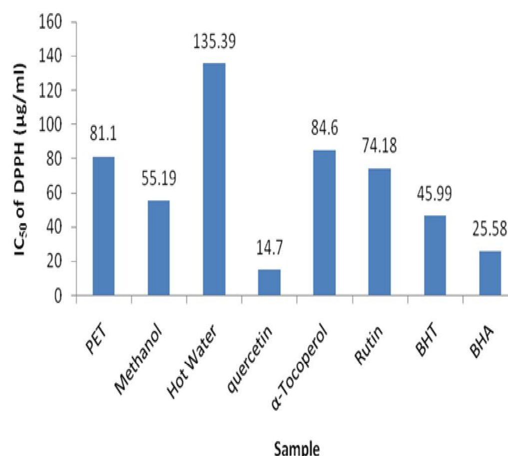

Fig. 1: DPPH radical scavenging activity of various solvent extracts from tuberous root of *Decalepis hamiltonii*

Ferric reducing antioxidant power assay

Antioxidants, explained as reductants and inactivation of oxidants by reductants, are involved in redox reactions in which one reaction species is reduced at the expense of the oxidation of another antioxidant. The antioxidant potential of various extracts of the plants were estimated from their ability to reduce TPTZ-Fe (III) complex to TPTZ-Fe (II) complex and the results are expressed as concentration of substance having ferric-TPTZ reducing ability equivalent that of 1 mmol concentration of Fe (II). The FRAP values for different solvent extracts of the plant is depicted in table 3. Among the various solvent extracts, methanol extract of roots (3955.56 mmol Fe (II)/mg extract) registered higher antioxidant activity. All extracts showed reducing power but not at the same level. The order of FRAP activity of various solvent root extracts is as follows: RM>RW>RP. The phenolic compounds exhibited reduction properties by acting as reducing agents, hydrogen donators and singlet oxygen quenchers (Rice–Evans *et al.*, 1997).

Table. 3: Metal chelating activity, Frap and TAA by $ABTS^{·+}$ assay of root extracts.

Sample extracts	Metal chelating (mg EDTA/g sample)	FRAP (µmol Fe (II)/mg extract)	TAA (µmol/g extract)
Petroleum ether	60.06±0.58	483±55.49	1974.9±1192.58
Methanol	101.17±0.54	3955.56±21.70	10108.9±3193.3
Hot water	108.0±1.0	746.89±4.90	2620.8±1566.8

All the values are expressed as mean±SD (n=3).

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. All the sample extracts exhibited the ability to chelate metal ions. Among the different sample extracts, the aqueous and methanol extract of root showed higher activity (108.0 and 101.17 mg EDTA/g extracts). Further, the activity decreased in petroleum ether extract of root (60.06 mg EDTA/g). 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. The effect values were found to be methanol, water and petroleum ether extracts (32.4, 26.35 and 26.34%) respectively, followed by Rutin (79.3%). It is known that the hydroxyl group of the phenolics contributes to superoxide anion scavenging activity by their electron donation (Bravo, 1998).

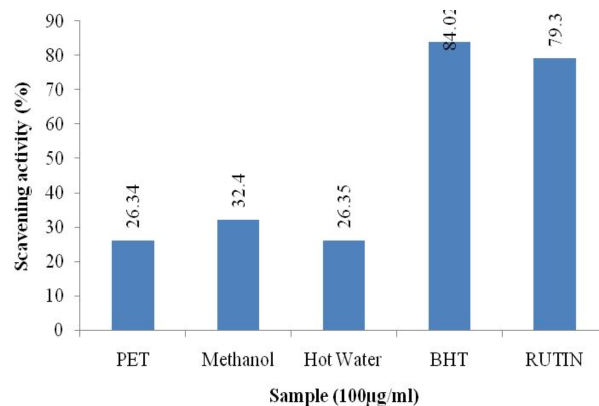

Fig. 2: Superoxide radical scavenging activity of petroleum ether, methanol and water extracts of *Decalepis hamiltonii.*

Total antioxidant activity by the ABTS·+ assay

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) ($ABTS•^{+}$) 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 dry matter of the extract under the experimental investigation. The effect of petroleum ether, methanol and aqueous root extract of *D. hamiltonii* on ABTS·+ cation radical scavenging activity is shown Table 3 and the methanol and aqueous root extract exhibited higher total antioxidant activity 10108.9 µmol/g and 2620.8 µmol/g respectively. 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). Antioxidants are substances that delay the oxidation process, inhibiting the polymerization chain initiated by free radicals and other subsequent oxidizing reactions (Halliwell and Aruoma, 1991). This may provide protection against chronic diseases, including cancer and neurodegenerative diseases, inflammation and cardiovascular disease (Prior *et al.*, 2005).

CONCLUSION

In the present study, the nutritional composition of this plant tuber revealed that rich source of carbohydrate, protein, fiber and energy. In addition, the tuberous root extracts proved to be higher in polyphenol compounds and an antioxidant property. This plant derived extract has great potential to be industrialized into resource food and other health products.

ACKNOWLEDGEMENT

The authors are greatly thankful to University Grants Commission, New Delhi, India for providing Rajiv Gandhi

National Fellowship scheme [(Ref. No. F. 14-2(ST)/2010 SA (III)] fund during this research work carried out successfully.

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