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Antioxidant property of *Decalepis hamiltonii* Wight & Arn*

K N Chidambara Murthy, T Rajasekaran, P Giridhar & G A Ravishankar[†]
Plant Cell Biotechnology Department, Central Food Technological Research Institute, Mysore 570 020, India

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Aromatic edible root of *D. hamiltonii* was subjected to the extraction of the antioxidant rich fraction. Different parts of root namely whole tuber, peel, tuber without peel and medullary portion were extracted with dichloromethane (European Patent No. W02005063272). The extract was found to contain flavor compound 2-hydroxy-4-methoxybenzaldehyde (2H4MB), which was identified by TLC and GC. Medullary portion was found to be rich in 2H4MB, (73.73 mg g^{-1}dry tissue) followed by peel, containing 68.34 mg g^{-1} 2H4MB. Different concentration of dichloromethane extracts were subjected for antioxidant assay by DPPH (1,1 dihydroxy 2-picryl hydrazyl) method, this has shown 44, 46.7% radical scavenging activity in case of medullary, peel extracts and 67.3% in case of pure 2-hydroxy-4-methoxybenzaldehyde at 100 ppm concentration, whereas ascorbic acid used as standard showed 94.3% activity. In β-carotene linoleate model system (b-CLAMS) 43.46 and 45.7% antioxidant activity was observed in medullary and peel extracts at 100 ppm concentrations respectively, whereas standard 2-hydroxy-4-methoxybenzaldehyde exhibited 69.64% at 100 ppm and BHA (butylated hydroxyl anisole) 90.1% activity also at 100-ppm level. Similarly hydroxyl radical scavenging activity was found to be 48.36, 46.86, 48.26 and 73.60% in whole tuber, medullary, peel and standard 2-hydroxy-4-methoxy benzaldehyde respectively at 100 ppm levels. This is the first report on the antioxidant activity of *D. hamiltonii*. Results have shown that 2H4MB is one of the major constituents responsible for antioxidant activity. Hence the extract of *D. hamiltonii* can be utilized for the production of antioxidant rich fractions required for various health benefits.

Keywords: Antioxidant activity, *Decalepis hamiltonii*, 2-Hydroxy-4-methoxy benzaldehyde.

Decalepis hamiltonii Wight & Arn, (Asclepiadaceae, commonly known as swallow root;) is a monogeneric climbing shrub native of the Deccan peninsula and forest areas of Western Ghats of India. It finds use as a culinary spice due to its high priced aromatic roots. The roots markedly fleshy, cylindrical (1-6 cm diameter) are characterized by a *sarasaparilla* like taste accompanied by a tingling sensation on the tongue as described in Wealth of India[1]. The roots of *D. hamiltonii* are used as a flavouring agent[2] and appetizer[3]. Similarly the roots of this taxon are considered as *"Sariva Bheda"* in Ayurveda which finds use as an alternative to roots of *Hemidesmus indicus* in the preparation of several herbal drugs like *Amrutamalaka taila, Drakshadi churna, shatavari rasayana and yeshtimadhu taila*[4]. Of late the highly aromatic roots have been subjected to overexploitation by destructive harvesting that has endangered the survival of this plant. We have reported for the first time that the aromatic root of *D. hamiltonii* has a potent bioinsecticidal property effective against storage pests[5]. The supercritical extracts of these roots proved to be potent antimicrobial agents[6] were able to regenerate plantlets of *D. hamiltonii* W & A from leaf callus. Similarly a method for *in vitro* rooting of *Decalepis hamiltonii* for field transfer was reported earlier[7,8]. In *D. hamiltonii* the tuberous root extracts contain the flavour compound 2-hydroxy-4-methoxy benzaldehyde (2H4MB; Fig. 1) as a major compound (97%), which is extractable by steam distillation method and followed by using dichloro methane[9].

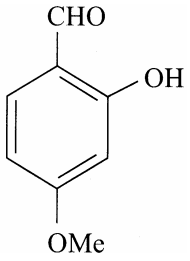

Fig. 1—Structure of active constituent of root, 2-hydroxy-4-methoxy benzaldehyde

[†]Correspondent author
Phone: +91 821 2516 501
Fax: +91 821 2517 233
E.mail: pcbt@cftri.res.in

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Giridhar *et al.*[10] have reported the presence of 2-hydroxy-4-methoxy benzaldehyde a flavor compound in plants developed *in vitro*. George *et al.*[11] have shown the production of *D.hamiltonii* through micro propagation and the same has produced similar content of constituents as that of conventional roots. Thangadurai *et al.*[12] have reported antibacterial activity of volatile constituents of *D. hamiltonii* which include 2H4MB 2-hydroxybenzaldehyde, 4-O-methylresorcylaldehyde, benzyl alcohol and atlantone as major constituents along with aromatic aldehydes. For the first time antioxidant potentials of root extracts of *Decalepis hamiltonii* Wight & Arn as an edible plant source for health food application is reported in the present communication.

Materials and Methods

All the solvents used for experiments were of analytical grade and purchased from Ranbaxy Fine Chemicals, India. UV visible spectrophotometer used was Shimadzu UV-160A (Japan). 2-hydroxy-4-methoxybenzaldehyde (2H4MB) standard was purchased from Fluka Chemicals, Switzerland. GC used was from Shimadzu, Japan. DPPH (1, 1 dihydroxy 2-picryl hydrazyl) was purchased from Sigma Chemicals, St Louis, USA.

Plant material and preparation of extract—Fresh tubers of *Decalepis hamiltonii* collected from Biligiri Rangana Hills near Mysore and they were sorted out to a required size (approximately 10.0 ± 1.0 cm lengths and 2.0±0.5 cm diam). The tubers were cleaned off extraneous matter and soil with cold water (15° ± 2°C). Later they were surface sterilized first with 200 ml of 70% (v/v) alcohol for 5 sec followed by washing with sterile water thrice. The surface of the tubers was blotted with sterilized blotting paper. Different parts namely, skin and medullary portions (100 g each) were separated and were grounded into paste in mixer and used for extraction in dichloromethane. Dichloromethane was utilized for extraction since it is permitted for extraction of oleoresins and other food constituents[13]. Extracts were pooled and were separated with water in separating funnel. This extract was dried in vacuum and re-suspended in ethanol (1 mg mL^{-1}) and used for further experiment at desired concentration.

Quantification of active principles by TLC and GC—Analysis of 2H4MB was done by spotting the root extracts on TLC plate along with standard (Fluka Chemicals, Switzerland) and run in a solvent system comprising of hexane: benzene (1:1). The concentrated volatiles were separated by GC, flame ionization detector (FID) with capillary column and GC-MS analysis using a Shimadzu, GC-14B coupled with QP 5000 MS system under the following conditions-SPB-1 column (Supelco, USA, 30 m × 0.32 mm, 0.25 μM film thickness) with a oven temperature programme of 60°C for 2 min, rising at 2°C/min to 250°C, held for 5 min; injection port temperature 225°C; detector temperature 250°C; carrier gas helium, flow rate 1ml min^{-1}. The quantity of extract injected was 1 μl for analysis[10]. The absorption maximum of sample was at 278 nm, which matched with that of standard.

Antioxidant activity of extract—The dichloromethane extract dissolved in ethanol was subjected to antioxidant activity by various model systems, including DPPH (1, 1 dihydroxy 2-picryl hydrazyl), β-Carotene Linoleate model System (b-CLAMS) and Hydroxyl radical scavenging activity.

(a) DPPH assay

Aliquots of extracts of *D. hamiltonii* and ascorbic acid 0.025, 0.05 and 0.1 ml were taken in separate tubes (from 1 mg ml^{-1} stock), and volume was made up to 0.5 ml with methanol. Samples were incubated with 50 μ*M* DPPH solution in methanol for 20 min and optical density was measured at 517 nm and per cent inhibition was calculated in comparison with OD of blank i.e., methanol[15]. The degree of discoloration indicates the scavenging potentials of the extracts. The antioxidants react with DPPH, which is a stable free radical, and convert in to 1, 1 dihydroxy 2-picryl hydrazine.

(b) β-carotene linoleate model system (β CLAMS)

Different aliquots (0.025, 0.05 and 0.1 ml) of *D. hamiltonii* extract of and 0.05 ml of BHA solution (from 1 mg mL^{-1} stock) of concentration of 25, 50 and 100 ppm were taken in separate test tubes and volume was made up to 0.5 ml with ethanol. β Carotene Linoleic acid emulsion (4 ml) was added to each tube. Absorbance of all samples was taken at 470 nm at Zero time and tubes were placed at 50°C in water bath. Measurement of absorbance was continued at an interval of 15 min, till the colour of β carotene disappeared in the control reaction (t=180 min). A mixture prepared as above without β Carotene emulsion served as blank and mixture without extract

served as control. Dose response of antioxidant activity for various extracts was determined at different concentrations. The antioxidant activity (%AA) of extracts was evaluated in terms of bleaching of β Carotene[15].

(c) Hydroxyl radical scavenging activity

Extracts of *D. hamiltonii* (0.025, 0.05 and 0.1 ml) prepared in 2% alcohol were taken in different test tubes and evaporated on a water bath. To these, 1 ml of Iron-EDTA solution, 0.5 ml of EDTA and 1ml of DMSO were added and the reaction was initiated by adding 0.5 ml of ascorbic acid to each of the test tubes. Test tubes were capped tightly and heated on water bath at 80°-90°C for 15 min. Then the reaction was terminated by the addition of 1ml of ice-cold TCA (17.5%, w/v) to all the test tubes, kept aside for 2 min and the formaldehyde formed was determined by the adding 3 ml of Nash reagent (75 g of ammonium acetate, 3 ml glacial acetic acid, 2 ml acetyl acetone mixed and raised to 1 L with distilled water) which was left for 10-15 min for colour development[16]. Intensity of yellow colour formed was measured spectrophotometrically at 412 nm against reagent blank. Percentage scavenging of hydroxyl radicals was calculated by comparison of the results of the samples with that of the blank[17].

Statistical analysis—All the analysis were done in triplicate and results were expressed as mean ± SD. Results of samples were compared with standards by one-way ANOVA test and represented in results.

Results and Discussion

Analysis of constituent by TLC and GC-MS—The extracts were analysed for 2H4MB content by spotting the root extracts on TLC plate along with standard. R_f (0.47) of sample coinciding with that of standard 2H4MB was eluted in solvent and UV spectrum was measured in spectrophotometer UV-160.

Quantification of dichloromethane extracts was done by GC in comparison with retention time of standard sample (R_T is 4.42 min, Fig. 2) and by the peak area measurement. The content of 2H4MB in dichloromethane extract of medullary portion was found to be 73.73 ± 1.10 and peel was about 68.34 ± 0.79 mg kg^{-1} (Table. 1).

Analysis of antioxidant activity—DPPH assay of ethanol dissolved dichloromethane extract indicate the radical scavenging potentials, which was 44 and 46.7% in medullary and peel extracts and 67.3% in pure 2H4MB (Fig. 3).

β-CLAMS showed similar trend of results, wherein the activity was significant in medullary portion and peel compared to standard 2H4MB. The activity was 43.46 and 45.7% in medullary and peel extracts in 100 ppm concentration, whereas standard 2H4MB exhibited 69.64% activity (Fig. 4). β-carotene in this system undergoes rapid discoloration in the absence of antioxidant and *vice versa* in presence of

Table 1—2-Hydroxy-4-methoxy benzaldehyde (2H4MB) content (mg/100 g fresh weight) in various parts of *D. hamiltonii* root in dichloromethane extract [Values are mean ± SD].

Part of root	2H4MB
Whole tubers	62.6 ± 1.21
Tubers without skin	54.7 ± 0.89
Central core or medullary portion	73.7 ± 1.10
Peel	68.3 ± 0.79

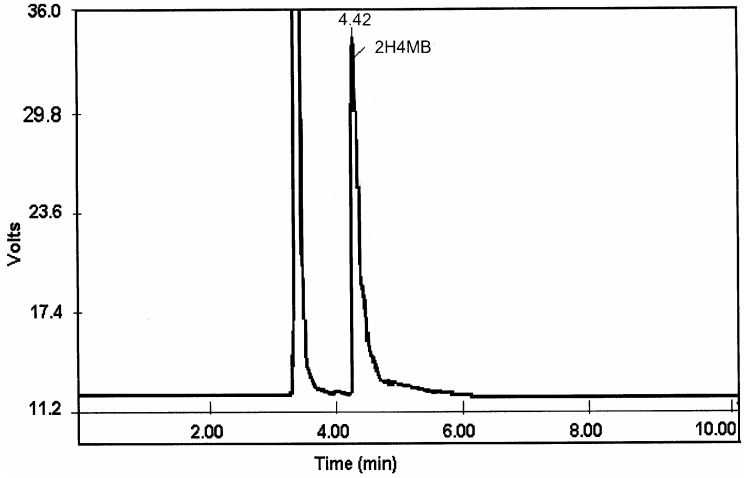

Fig. 2—GC Chromatogram of dichloromethane extract of *D. hamiltonii*

antioxidant. The linolenic acid free radical formed upon the abstraction of hydrogen atom from one of its diallylic methylene groups attacks the highly unsaturated β-carotene molecule. As β-carotene molecules lose their double bond by oxidation, the compound looses its chromophore and turns into characteristic orange colour, which can be monitored spectrophotometrically[18].

In hydroxyl radical scavenging activity the activity was significant in extracts at higher concentration in different parts of the tuber. The same was 48.36, 46.86, 48.26 and 73.60% in whole tuber, medullary, peel and standard 2H4MB respectively (Fig. 5).

Hydroxyl radicals have been implicated as highly damaging species in free radical pathology. This radical has the capacity to join nucleotides in DNA, cause strand breakage, which contributes to carcinogenesis, mutagenesis and cytotoxicity[19]. In addition, this species is considered to be one of the initiators of lipid peroxidation process. Hence in the present method hydroxyl radical scavenging activity of the selected natural products were assessed by generating the hydroxyl radicals using ascorbic acid-Iron EDTA[15]. The hydroxyl radicals formed by the oxidation react with DMSO to yield formaldehyde. The formaldehyde production from DMSO provides a

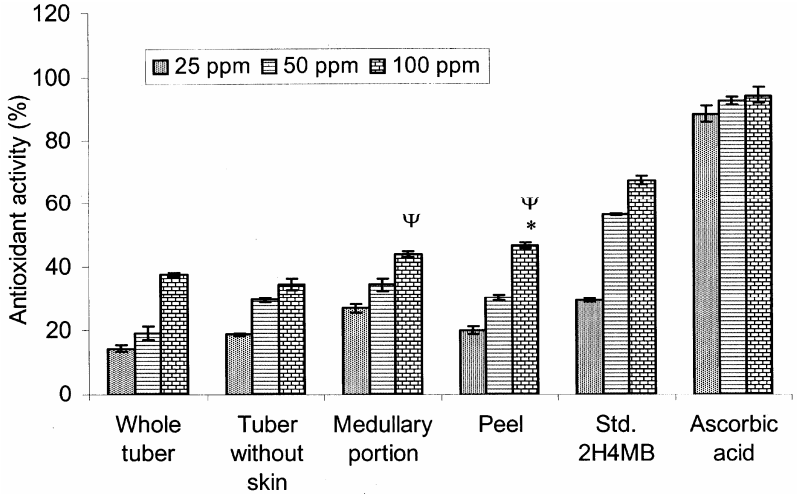

Fig. 3—Antioxidant activity of various portions of *D.hamiltonii* roots by DPPH model. (P<0.05 as compared to *ascorbic acid; $^{\Psi}$2H4MB)

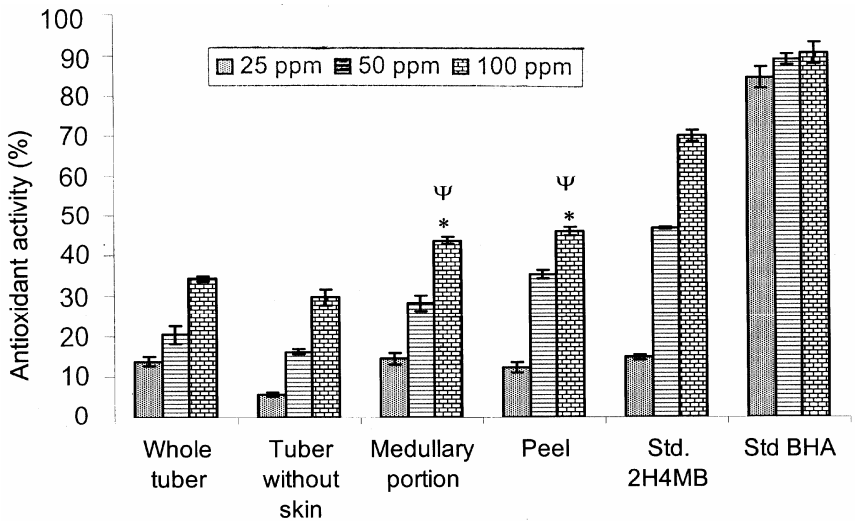

Fig. 4—Antioxidant activity of various portions of roots of *D. hamiltonii* by β-CLAMS model. (P<0.05 as compared to *ascorbic acid; $^{\Psi}$2H4MB)

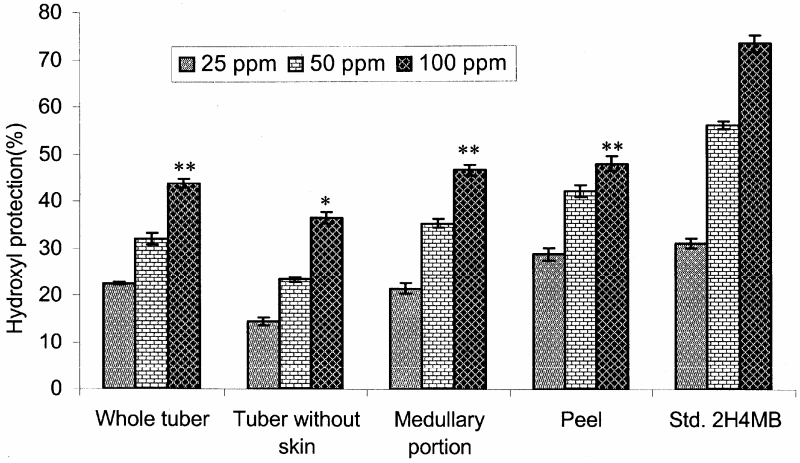

Fig. 5—Hydroxyl radical scavenging activity of various portions of roots of *D. hamiltonii*. *(P*: *<0.05; **<0.1 as compared to 2H4MB)

convenient method to detect hydroxyl radicals formed during the oxidation of DMSO by the Fe^{3+}/ascorbic acid system which was used to detect hydroxyl radicals[17].

Antioxidants are the compounds that when added to food products, especially to lipids and lipid-containing foods, can increase the shelf life by retarding the process of lipid peroxidation, which is one of the major reasons for deterioration of food products during processing and storage. Synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoulene (BHT), are restricted for use in foods as these synthetic antioxidants are suspected to be carcinogenic[20]. In living systems, varieties of antioxidant mechanisms play an important role in combating ROS (reactive oxygen species)[21]. The antioxidants may act by easing the levels of endogenous defenses by up-regulating the expression of genes encoding the enzymes such as super-oxide dismutase (SOD), catalase or glutathione peroxidase[22].

It was observed that the peel, having lesser content of 2H4MB has shown higher activity in contrast to medullary, which, may be due to the reason that peel apart from 2H4MB, is also known to contain phenolics and other components responsible for the antioxidant activity. Recent reports have shown DHA (4-hydroxyisophthalic acid), 4-(1-hydroxy-1-methyl-ethyl)-1-methyl-1, 2-cyclohexanediol and analogues have been reported to be the potent antioxidant in *Decalepis*. IC_{50} of these compounds are in the range of 11.74 to 508.24 nmol/mL[23]. Apart from 2H4MB, *p*-anisaldehyde, vanillin, borneol, salicylaldehyde, and bis-2, 3, 4, 6-galloyl-α/β-D-glucopyranoside are also reported to be the responsible compounds for radical scavenging activity[24]. However the results of the present study indicates much more potential with IC_{50} in the range of 35-40 ppm and easy process for extraction of radical scavenging principles.

Conclusion

The study unraveled the bioactive property of root extracts of *D. hamiltonii* as a potent source of antioxidant for food application. This edible plant exhibited antioxidant activity in the range of 60.0 % and radical scavenging activity in the range of 50 % of standard antioxidants. This report undoubtedly elucidated antioxidant potential of *D. hamiltonii* substantiating the already known utility of this wonder herb.

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