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Cytoprotective and antioxidant activity of free, conjugated and insoluble-bound phenolic acids from swallow root (*Decalepis hamiltonii*)

M.A. Harish Nayaka [1], U.V. Sathisha, Shylaja M. Dharmesh *

Department of Biochemistry and Nutrition, Central Food Technological Research Institute, Mysore 570 020, Karnataka, India

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ABSTRACT

Swallow root (*Decalepis hamiltonii*) was extracted for free (SRFP), conjugated (SRCP) and insoluble-bound phenolic acids (SRIBP), and evaluated for cytoprotectivity, 1,1,diphenyl-2-picrylhydrazyl (DPPH) scavenging ability, reducing power and protection to DNA damage. In addition, the constituent phenolic acids in the extracts were also analysed. Results indicated a total phenol content of 20.72, 7.97 and 11.52 mg gallic acid equivalents (GAE)/g for SRFP, SRCP and SRIBP extracts, respectively. At 0.12 μg/mL concentration SRCP showed 87% cytoprotection (on NIH 3T3 cells) compared to SRFP (47%) and SRIBP (65%). DPPH radical scavenging activity indicated an IC_{50} of 0.046, 0.06 and 0.128 μg/mL for SRCP, SRIBP and SRFP, respectively. Also, SRCP showed higher reducing power and DNA protectivity (80%). HPLC analysis of phenolic acid extracts showed the presence of hydroxybenzoate and cinnamate derivatives. Among the phenolics identified gallic, gentisic, protocatechuic and *p*-coumaric acids were the major contributors to antioxidant activity.

1. Introduction

Plant and plant products are recognised as safe and potential health promoting (Khan & Mukhtar, 2007), disease curing (Rathore, McCutchan, Sullivan, & Kumar, 2005), immune system boosting (Bin-Hafeez et al., 2003), detoxifying (Kaviarasan & Anuradha, 2007) and nutritive (Bovell-Benjamin, 2007) sources. The role of natural products are gaining more popularity in both developed and developing countries and much appreciated towards their applications as "alternatives" against chronic diseases such as diabetes, ulcer, and cancer, among others, particularly those diseases that require a long-term treatment, mainly due to complexities in their disease pathogenicity. Although synthetic drugs are required for immediate relief, long term use of these drugs not only causes side effects such as nausea, allergy, and immnosuppression, but leading themselves to be a causative factor for several disorders. Cells and tissues of living systems may become an easy target for exposure to free radicals generated during drug metabolism. In addition, exogenous sources like ozone, exposure to UV radiations and cigarette smoke also induce biomolecular changes, such as DNA damage, protein oxidation and generation of lipid peroxides leading to severity of chronic diseases.

Swallow root (*Decalepis hamiltonii*), which belongs to the Asclepiadaceae family is one of the most potent antioxidant source with varieties of biological activity (Anup & Shivanandappa, 2006; Georgea, Ravishankar, Keshava, & Udayasankar, 1999; Naik, Smitha, Harish Nayaka, Lakshman, & Shylaja, 2007; Sathisha, Smitha, Harish Nayaka, & Shylaja, 2007). The roots are largely used in making pickles along with lime and also in the preparation of health drink. Further, the roots are being used in Ayurveda, the ancient Indian system of medicine to stimulate appetite, relieve flatulence and as a general tonic. The roots are also used as a substitute for *Hemidesmus indicus* in Ayurvedic preparations (Nayar, Shetty, Mary, & Yoganarasimhan, 1978). Aldehydes, inositol, saponins, amyrins and lupeols (Murti & Seshadri, 1941) as well as volatile compounds such as 2-hydroxy-4-methoxybenzaldehyde (HMBA), vanillin, 2-phenylethyl alcohol and benzaldehyde (Nagarajan, Rao, & Gurudutt, 2001) have been reported in swallow root. In addition, 4-hydroxyisophthalic acid, 14-aminotetradecanoic acid, 4-(1-hydroxy-1-methylethyl)-1-methyl-1, 2-cyclohexanediol, 2-(hydroxymethyl)-3-methoxybenzaldehyde, 2,4,8-trihydroxybicyclo[3.2.1]octan-3-one, bis-2,3,4,6-galloyl-α/β-D-glucopyranoside, borneol and ellagic acid have also been identified in swallow root

Abbreviations: BHA, butylated hydroxyanisole; *D. hamiltonii*, *Decalepis hamiltonii*; DMSO, dimethyl sulphoxide; DPPH, 1,1,diphenyl-2-picrylhydrazyl; GAE, gallic acid equivalent; HBA, *p*-hydroxybenzoic acid; HMBA, 2-hydroxy-4-methoxybenzaldehyde; SRFP, swallow root free phenolic acids; SRCP, swallow root conjugated phenolic acids; SRIBP, swallow root insoluble-bound phenolic acids; RT, room temperature.

* Corresponding author. Address: Lab 114, Department of Biochemistry and Nutrition, Central Food Technological Research Institute, Mysore 570020, Karnataka, India. Tel.: +91 0821 2514876; fax: +91 0821 2517233.
E-mail address: cancerbiolab@yahoo.co.in (S.M. Dharmesh).
[1] Present address: Department of Studies in Sugar Technology, Sir. M. Vishvesvaraya Post Graduate Center, University of Mysore, Tubinakere, Mandya 571402, Karnataka, India.

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(Srivastava, Harish, & Shivanandappa, 2006; Srivastava, Rao, & Shivanandappa, 2007).

Even though some of the chemical components of swallow root have been reported in the literature, the chemical composition of free, conjugated (also called esterified) and insoluble-bound phenolic acids and their role in bioactivity have not been established. Since phenolic acids are known to possess antioxidant activity (Alasalvar et al., 2009; Shahidi & Wanasundara, 1992; Wijeratne, Abou-Zaid, & Shahidi, 2006), and the wide use of the plant roots in folklore and Ayurveda medicine, it is pertinent to study the *in vitro* biological activity of free, conjugated and insoluble-bound phenolic acids, which may lead to a better understanding of the medicinal properties attributed to swallow root. Hence, the present investigation was undertaken (a) to determine the cytoprotective ability of swallow root free (SRFP), conjugated (SRCP) and insoluble-bound phenolic acid (SRIBP) extracts on NIH 3T3 cells, (b) to evaluate antioxidant activity of phenolic acid extracts using different antioxidant assays and (c) to determine phenolic acid composition in free, conjugated and insoluble-bound phenolic acid extracts of swallow root.

2. Materials and methods

2.1. Chemicals

1,1,Diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteau reagent, ascorbic acid, butylated hydroxyanisole (BHA), Tris–HCl, phenolic acid standards, 2-hydroxy-4-methoxybenzaldehyde, vanillin, agarose, ethidium bromide, and tert-butyl hydroperoxide were purchased from Sigma Chemical Co. (St. Louis, MO). Lambda phage DNA was procured from Bangalore Geni, Bangalore, India. NIH 3T3 fibroblast cells were purchased from National Center for Cell Sciences, Pune, India. The other chemicals such as ferric chloride, trichloroacetic acid and solvents used in this experiment were purchased from Sisco Research Laboratories, Mumbai, India.

2.2. Sample

Fresh swallow roots were purchased from a local market (Devaraja market, Mysore, Karnataka, India), sun dried for 3 days, and powdered in a mixer (Gopi Lal Electrical and Mechanicals Co. Ambala, India) and preserved in dry condition at 4 °C until further extraction.

2.3. Extraction of free, conjugated, and insoluble-bound phenolic acids

Phenolic acid extracts of swallow root were isolated according to the method followed by Liyana-Pathirana and Shahidi (2006) with slight modification. Two grams of defatted swallow root powder (in triplicates, $n = 3$) were extracted six times (each extraction for 2 h) with 40 mL of methanol–acetone–water (7:7:6, v/v/v) at RT (25 ± 2 °C) with constant stirring. The mixtures were then centrifuged at 4000*g* for 20 min (Sigma, Osterode am Harz, Germany) and supernatants were collected and combined. The solvent was evaporated at 30 °C under vacuum in a flash evaporator (Buchi 011, Switzerland) to approximately 40 mL. Concentrated supernatant was acidified to pH 2 with 6 M hydrochloric acid, extracted six times with diethyl ether and phenolic acids so extracted were labeled as free phenolic acids (SRFP). The supernatants with conjugated phenolic acids were then hydrolysed with 30 mL of 4 M sodium hydroxide for 4 h at RT under nitrogen atmosphere. The resultant hydrolysate was acidified to pH 2 using 6 M hydrochloric acid and extracted six times with diethyl ether. The ether extracts were then combined and evaporated to dryness at 30 °C under vacuum and labeled as conjugated phenolic acids (SRCP). The leftover residue after extractions was treated with 20 mL of 4 M sodium hydroxide for 4 h at RT under nitrogen atmosphere and then acidified to pH 2 with 6 M HCl followed by centrifugation (4000*g*, 20 min). The mixture was extracted six times with diethyl ether. The ether extracts were combined and evaporated to dryness under vacuum at 30 °C and labeled as insoluble-bound phenolic acids (SRIBP). Free, conjugated and insoluble-bound phenolic acids were dissolved separately in 2 mL of methanol and stored at −20 °C until used within 1 week. The total phenol content of SRFP, SRCP and SRIBP was determined using Folin-Ciocalteau method (Singleton & Rossi, 1965) and expressed as mg gallic acid equivalents (GAE) per gram of sample.

2.4. Cytoprotective assay on cultured NIH 3T3 fibroblast cells exposed to tert-butyl hydroperoxide

Cytoprotective ability of SRFP, SRCP and SRIBP were conducted according to the method reported by Nardini et al. (1998). NIH 3T3 fibroblast cells (2.8×10^4 cells/mL) were cultured with or without phenolic acid extracts (0–0.15 μg/mL) along with standard butylated hydroxyanisole (BHA, 0–0.125 μg/mL) dissolved in PBS containing 0.25% dimethyl sulphoxide (DMSO) in a 96 well microplate (180 μL suspension/well). These concentrations of DMSO were found to have no effect on cell proliferation during 20 h incubation (data not shown). After 30 min of incubation, cells were treated with 500 μM tert-butyl hydroperoxide and incubated for 3 h. A negative control without test sample and oxidant was also maintained. Cell viability was assessed by microculture tetrazolium assay (Hansen, Nielsen, & Berg, 1989) and the results were expressed as percent cell viability.

2.5. Antioxidant activity

2.5.1. DPPH radical scavenging assay

The free radical scavenging ability of swallow root phenolic acid extracts was estimated using DPPH radical (Suresh Kumar, Harish Nayaka, Shylaja, & Salimath, 2006). Phenolic acid extracts (0–0.15 μg/mL) and standard phenolic acids including BHA (0–0.125 μg/mL) in 200 μL aliquot was mixed with 100 mM Tris–HCl buffer (800 μL, pH 7.4) and then added to 1 mL of 500 μM DPPH in ethanol (final concentration of 250 μM). The mixture was shaken vigorously and left to stand for 20 min at RT in dark. The absorbance of the resulting solution was measured spectrophotometrically at 517 nm. The capability to scavenge DPPH radical was calculated using the following equation:

$$\text{Scavenging effect}\ (\%) = [(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100$$

2.5.2. Measurement of reducing power

The reducing power of swallow root phenolic acid extracts was determined according to the method published earlier (Suresh Kumar et al., 2006). The phenolic acid extracts (0–0.15 μg/mL) and standard BHA (0–0.125 μg/mL) were mixed with an equal volume of 0.2 M phosphate buffer, pH 6.6 and 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. Then, an equal volume of 10% trichloroacetic acid was added to the mixture and centrifuged at 5000*g* for 10 min. The upper layer of the solution was mixed with distilled water and 0.1% ferric chloride at a ratio of 1:1:2 and the absorbance were measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

2.5.3. DNA protection assay

DNA protection ability of swallow root phenolic acid extracts was performed using lambda phage DNA (Suresh Kumar et al.,

2006). Briefly, λ phage DNA (0.6 μg) was subjected to oxidation using Fenton's reagent (0.3 mM hydrogen peroxide, 0.5 μM ascorbic acid and 0.8 μM ferric chloride) in presence and absence of phenolic acid extracts (0.6 μg/16 μL) and BHA (1 μg/16 μL) for 2 h at 37 °C. The samples were subjected for electrophoresis (Submarine electrophoresis system, Bangalore Geni, Bangalore, India) on 1% agarose for 2 h at 50 V DC. Gels were stained with ethidium bromide (0.5 μg/mL) and documented (Herolab GmbH Laborgeräte, Wiesloch, Germany).

2.6. HPLC analysis of phenolic acid extracts

Phenolic acid extracts of swallow root were analysed on a HPLC (Model LC-10A. Shimadzu Corporation, Kyoto, Japan) with reversed phase Shimpak C_{18} column (4.6 × 250 mm; Shimadzu Corporation, Kyoto, Japan) and a diode array UV-detector (operating at 280 nm). A solvent system consisting of water/acetic acid/methanol (Isocratic, 80:5:15, v/v/v) was used as mobile phase at a flow rate of 1 mL/min (Suresh Kumar et al., 2006). Phenolic acid standards such as caffeic, *p*-hydroxybenzoic, *p*-coumaric, ferulic, gallic, gentisic, protocatechuic, *trans*-cinnamic, syringic and vanillic acids including vanillin and 2-hydroxy-4-methoxybenzaldehyde were used for identification of phenolic acids. The identified phenolic acids were quantified on the basis of their peak area and comparison with a calibration curve obtained with the corresponding standards.

2.7. Characterisation of a major phenolic compound of swallow root insoluble-bound phenolic acid extract

The major phenolic compound in the bound phenolic acid extract was isolated by preparative HPLC using the same solvent system as used for the qualitative analysis of phenolic acids and characterised by UV absorption, infrared, mass and 2D NMR studies. Mass was obtained with Finnigan MAT 95 mass spectrometer, infrared spectra was recorded with Perkin–Elmer Spectrum RX I FT-IR and NMR spectra was recorded using a Bruker AVANCE-400 NMR spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany).

2.8. Statistical analysis

All the experiments were carried out in triplicates ($n = 3$) and the results are expressed as mean ± standard deviation (SD). The correlation (r) between antioxidant activity and phenolic content were calculated using Microsoft Excel software.

3. Results

3.1. Total phenol

The total phenolic content of swallow root phenolic acid extracts was determined by Folin-Ciocalteau method. Results indicated higher amount of total phenolics in the SRFP extract (20.72 ± 0.77 mg GAE/g) followed by SRIBP (11.52 ± 0.54 mg GAE/g) and SRCP extract (7.97 ± 0.5 mg GAE/g). In comparison with SRFP extract, 1.8-fold and 2.6-fold reduction in total phenolic content was observed in SRIBP and SRCP extracts, respectively.

3.2. Cytoprotective assay on cultured NIH 3T3 fibroblast cells

The phenolic acid extracts of swallow root protected NIH 3T3 cells from oxidative damage induced by tert-butyl hydroperoxide. From Fig. 1A it is evident that all phenolic acid extracts tested showed a dose dependent protection between 0.03 and 0.15 μg/mL concentration. Among the phenolic acid extracts tested, at equal concentration of 0.12 μg/mL, SRCP extract showed the highest cytoprotectivity with 87% protection. SRFP and SRIBP extract protected cells up to 47% and 65%, respectively. Standard antioxidant BHA showed 67% cytoprotectivity at 0.125 μg/mL.

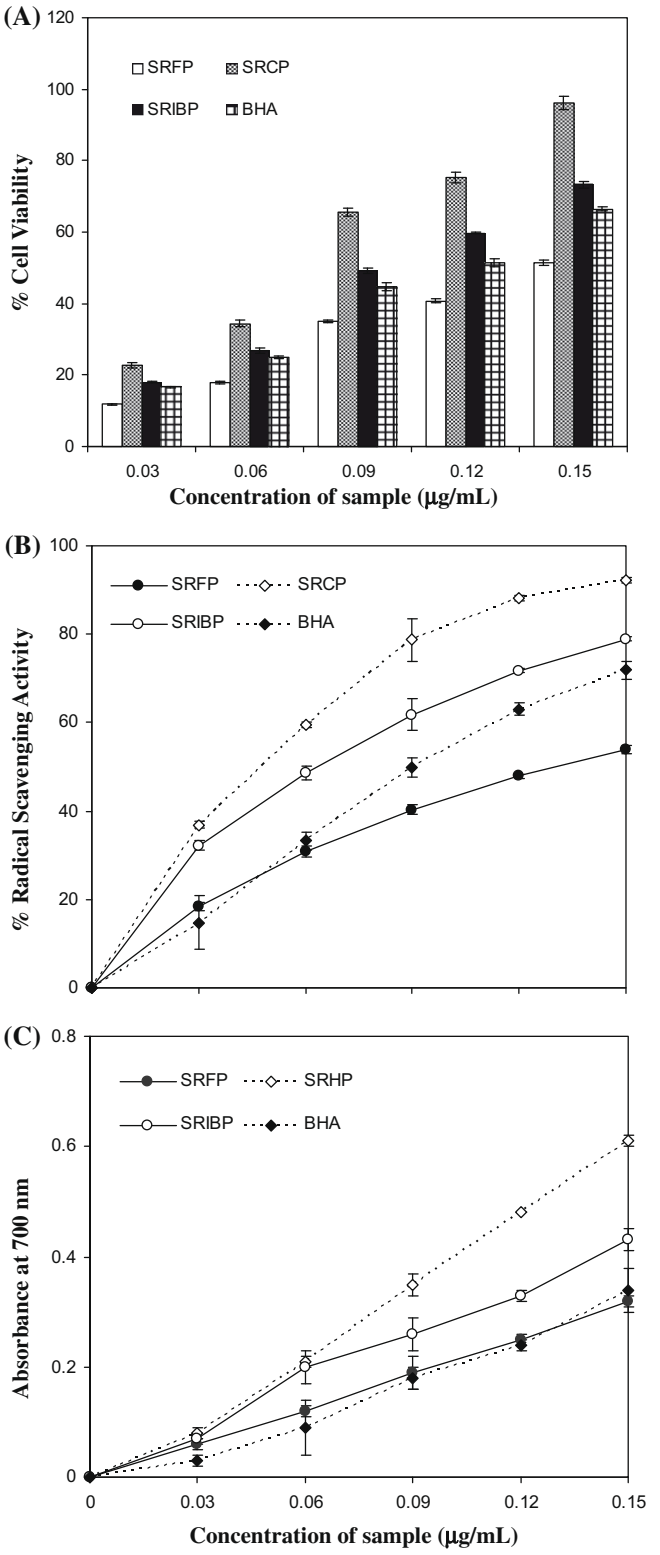


Fig. 1. (A) Cytoprotective effect on tert-butyl hydroperoxide induced cell damage of NIH 3T3 cells, (B) DPPH radical scavenging activity and (C) reducing power of free (SRFP), conjugated (SRCP) and insoluble-bound (SRIBP) phenolic acid extracts (0–0.15 μg/mL) of swallow root in comparison with BHA (0–0.125 μg/mL). Values are mean ± SD ($n = 3$).

3.3. Antioxidant activity of swallow root phenolic acid fractions

3.3.1. DPPH radical scavenging activity

Antioxidant activity of SRFP, SRCP and SRIBP extracts was evaluated by DPPH radical scavenging activity. Fig. 1B shows percent DPPH radical scavenging activity of phenolic extracts in addition to standard BHA. All phenolic extracts showed free radical scavenging activity with an IC_{50} of 0.046, 0.06, 0.128 and 0.08 μg/mL for SRCP, SRIBP, SRFP and BHA, respectively, suggesting that SRCP and SRIBP were more potent in free radical scavenging activity than BHA.

3.3.2. Reducing power

Further, to support the antioxidant activity exhibited by phenolic acid extracts in free radical scavenging assay, reducing power of the phenolic acid extracts of swallow root were evaluated. Fig. 1C indicates a dose dependent increase in activity of phenolic acid extracts of swallow root. The increased absorbance at 700 nm due to the reduction of potassium ferricyanide/ferric chloride complex indicates the presence of reducing power in all the phenolic acid extracts tested including the standard antioxidant BHA. At 12 μg/mL concentration approximately 2-fold higher activity was observed in SRCP compared to BHA. The reducing power of phenolic extracts tested correlates ($r = 0.9122$) well with their total phenolic content. However, the difference in the reducing power between the phenolic extracts may be due to their constituent phenolic acids.

3.3.3. DNA protection assay

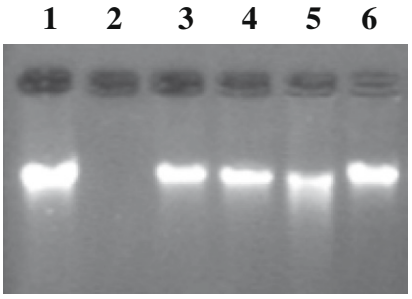
The efficiency of phenolic acid extracts of swallow root in preventing oxidative damage of DNA was also evaluated. Fig. 2 shows the DNA protective activity of SRFP, SRCP and SRIBP extracts including BHA. The hydroxyl radical generated by Fenton's reagent caused DNA fragmentation and increased its electrophoretic mobility. The fragmented DNA's have moved out of the gel easily and hence no band is evident in lane 2. This DNA fragmentation was recovered with the treatment of different phenolic acid extracts in addition to BHA to varying extent (lane 3–6). From the gel documentation analysis, higher protection (82%) was observed in BHA treated (1 μg/16 μL), while 80%, 67% and 42% protection were observed for SRCP, SRIBP and SRFP extracts at 0.6 μg/mL concentration each, respectively.

3.4. HPLC analysis of swallow root phenolic acid extracts

In swallow root phenolic acid extracts, a total of 12 phenolic compounds were detected (Table 1) of which five were hydroxybenzoate derivatives and four were cinnamate derivatives. Also, two hydroxybenzaldehyde derivatives were identified. In total 12, 9, and 9 phenolic compounds were identified in SRFP, SRCP and SRIBP extracts of swallow root, respectively. In SRFP extract, gentisic acid, 2-hydroxy-4-methoxybenzaldehyde (HMBA), vanillin, vanillic acid and *p*-hydroxybenzoic acid were the major phenolic compounds. In SRCP extract, gallic acid and gentisic acid were the abundant phenolic acids, while SRIBP extract was constituted by HMBA, vanillic acid and *p*-hydroxybenzoic acid contributing to more than 70% of phenolic acid content.

3.5. Characterisation of a major phenolic compound of SRIBP extract

The HPLC analysis of SRIBP showed the presence of a major peak and its retention time was matched with HMBA, a major component in methanol extract of swallow root as reported earlier (Nagarajan et al., 2001). Hence, to confirm the presence of HMBA in the insoluble-bound extract the major peak was subjected to spectroscopic analysis after isolating it through preparative HPLC.



	1	2	3	4	5	6
Native DNA	+	+	+	+	+	+
SRCP	-	-	+	-	-	-
SRIBP	-	-	-	+	-	-
SRFP	-	-	-	-	+	-
BHA	-	-	-	-	-	+
Fenton's Reagent	-	+	+	+	+	+

Fig. 2. Electrophoretic analysis of DNA protection by swallow root free (SRFP), conjugated (SRCP) and insoluble-bound (SRIBP) phenolic acid extracts (0.6 μg/16 μL each) in addition to standard antioxidant BHA (1 μg/16 μL). Lane 1 – native DNA; lane 2 – oxidised DNA; lane 3 – SRCP treated DNA; lane 4 – SRIBP treated DNA; lane 5 – SRFP treated DNA and lane 6 – BHA treated DNA.

Results indicated the presence of an aromatic compound with a mass of 152, λ_{max} of 213, 279 and 305 nm and the infrared measurements showed the presence of aldehydic (1718 cm^{-1}; C=O stretching), methoxy (1280 cm^{-1}; C–O–CH_3 stretching) and hydroxyl (3206 cm^{-1}; –OH stretching) functional group. From 2D NMR (^1H and ^{13}C) studies the compound was identified and confirmed as 2-hydroxy-4-methoxybenzaldehyde. Data thus provides evidence for the presence of HMBA in insoluble-bound phenolic acid extract.

4. Discussion

In the present investigation, detailed efforts have been put to identify phenolic acids that may be potentially involved in health beneficial properties of swallow root. Current paper addresses the role of phenolic acids of swallow root in exhibiting cytoprotective/DNA protective and antioxidant activity. Also, the study addresses the role of HMBA, a predominant component of swallow root as reported earlier (Nagarajan et al., 2001) in bioactivity. There were no reports available on phenolic acids and their contribution towards cytoprotective and antioxidant activity in swallow root. Hence, the investigation focused on the bioactivity of phenolic acid extracts employing various antioxidant assays and also to identify different phenolic components responsible for antioxidant activity. SRFP, SRCP and SRIBP were isolated from swallow root by differential extraction procedure. Since phenolic acids were known to possess biological activity (Hsu, Huang, & Yen, 2006), the isolated phenolic acid extracts were evaluated for cytoprotective effect, antioxidant activity and identification of their constituent phenolic acids. The phenolic acid extracts evaluated for cytoprotectivity on NIH 3T3 cells oxidation induced by tert-butyl hydroperoxide showed cytoprotection. Oxidative cell death of NIH 3T3 cells were effectively prevented by the phenolic acid extracts. At equal concentration (0.12 μg/mL), 87%, 65% and 47% cytoprotection (NIH 3T3 cells) were observed for SRCP, SRIBP and SRFP extracts, respectively (Fig. 1A). These results indicate that the phenolic acid extracts may quench tert-butyl hydroperoxide and free radicals generated during subsequent oxidation of lipid membrane of cells

Table 1
DPPH radical scavenging activity and phenolic acid composition of free, conjugated and insoluble-bound phenolic acid extracts of swallow root.

Compound	DPPH radical scavenging activity[a] (IC_{50} in μg/mL)	Amount of phenolic acid (in mg/g)[a]		
		SRFP	SRCP	SRIBP
1. Gallic acid	1.1 ± 0.04	0.54 ± 0.052	1.49 ± 0.11	0.38 ± 0.055
2. Protocatechuic acid	1.35 ± 0.08	1.16 ± 0.045	0.03 ± 0.004	0.03 ± 0.008
3. Gentisic acid	3.0 ± 0.11	6.54 ± 0.242	0.76 ± 0.036	–
4. Vanillic acid	49.5 ± 2.4	4.72 ± 0.104	–	1.04 ± 0.05
5. Caffeic acid	1.80 ± 0.05	0.15 ± 0.008	0.08 ± 0.008	–
6. Syringic acid	64.9 ± 5.42	0.21 ± 0.007	0.15 ± 0.010	0.05 ± 0.008
7. *p*-Coumaric acid	1.90 ± 0.041	0.08 ± 0.01	0.08 ± 0.01	0.25 ± 0.005
8. Ferulic acid	6.60 ± 0.21	0.08 ± 0.017	0.07 ± 0.003	0.10 ± 0.017
9. *trans*-Cinnamic acid	4.60 ± 0.34	0.02 ± 0.001	–	–
10. *p*-Hydroxybenzoic acid	33.75 ± 1.72	3.91 ± 0.141	–	0.83 ± 0.043
11. HMBA[b]	213.2 ± 5.12	5.06 ± 0.239	0.10 ± 0.003	1.14 ± 0.06
12. Vanillin	80.00 ± 2.01	4.61 ± 0.101	0.02 ± 0.002	0.25 ± 0.016

[a] Values are expressed as mean ± SD.
[b] 2-Hydroxy-4-methoxybenzaldehyde.

by tert-butyl hydroperoxide. The demonstrated cytoprotective ability may be attributed to swallow root phenolic acids and may work synergistically with other cytoprotective (Srivastava et al., 2007) and bioactive molecules reported earlier (Harish, Divakar, Srivastava, & Shivanandappa, 2005; Srivastava et al., 2006). In addition, the antioxidant potency as evaluated through studies on DPPH radical scavenging ability, reducing power and protection to DNA damage showed potent activity in SRCP. Extracts showed dose dependent antioxidant response; a correlation co-efficient was established between the phenolic content and their activity in each assay. Results indicated no significant correlation co-efficient suggesting that not only phenolic content but also the type of phenolic constituent present in each fraction may be responsible for the activity. Even though the total phenolic content is higher in SRFP extract, at equal concentration SRCP and SRIBP showed higher antioxidant activity up to 75% and 58% than SRFP. The fact that SRCP showed better antioxidant activity than SRFP and SRIBP in all assays could be due to the presence of higher levels of gallic acid, which is a potent antioxidant molecule. SRFP and SRIBP extracts had abundant poor antioxidant molecules like vanillin, HMBA, and *p*-hydroxybenzoic acid compared to SRCP (Table 1). The poor antioxidant potency of HMBA was also substantiated by other workers (Harish et al., 2005). The differences in antioxidant activity between the extracts may be attributed to the presence of different phenolic acids with different antioxidative potential and their synergistic effects. These results may strengthen the view of swallow root usage for their medicinal properties in Ayurveda and folklore medicine.

The presence of phenolic acids in bound form particularly in association with polysaccharides/lignin has been reported earlier (Iiyama, Tuyet Lam, & Stone, 1990; Lapierre, Pollet, Ralet, & Saulnier, 2001; Liyana-Pathirana & Shahidi, 2006; Madhujith & Shahidi, 2009). Cinnamic acid derivatives are usually seen bound to polysaccharides (Shyama Prasad Rao & Muralikrishna, 2004). However, in swallow root (Table 1), the presence of derivatives of hydroxybenzoate (gallic aicd) and hydroxybenzaldehyde (vanillin and HMBA) were found in the bound form. Vanillin, HMBA and *p*-hydroxybenzoic acid being poor antioxidant molecules, the presence of these compounds may have little contribution towards antioxidant activity. Hence, the cytoprotective and antioxidant properties may be attributed to the phenolic acids like gallic, protocatechuic, gentisic, and vanillic acid in SRFP; gallic and gentisic in SRCP and gallic and *p*-coumaric acid in SRIBP extracts. These phenolic acids were reported to possess good antioxidant activities (Miller & Rice-Evans, 1997). The presence of phenolic acids both in free and bound form attached to various polysaccharides is of significant interest in preventing oxidative stress induced diseases. The free phenolic acids are easily absorbed into the circulation while the phenolic acids bound to the polysaccharides are released by the intestinal enzymes as well as by the colonic microflora and can be absorbed into the circulatory system (Andreasen, Kroon, Williamson, & Garcia-Conesa, 2001). This may have a significant role in the reduction of oxidative stress in lower alimentary canal also.

Earlier studies have shown the presence of HMBA as a major constituent in water/methanol extracts and in the volatile oil fraction of swallow root (Harish et al., 2005; Nagarajan & Rao, 2003). The presence of HMBA in the SRFP extract is therefore obvious. Hence, the presence of HMBA in the SRIBP extract is an additional finding and the precise way in which HMBA is associated with the macromolecules especially polysaccharides needs to be addressed. The relative percent contribution of HMBA in SRIBP extract was 2-fold higher than in the SRFP extract.

In conclusion, swallow root phenolic acid extracts showed cytoprotectivity, reducing power, radical scavenging ability and protection to DNA damage induced by hydroxyl radical. Swallow root conjugated phenolic acid extract (SRCP) showed good cytoprotection followed by bound (SRIBP) and free (SRFP) phenolic acid extracts. All the phenolic acid extracts contained both hydroxybenzoic and cinnamic acid derivative as antioxidant molecules to different extent. Among the phenolic acids identified, gallic, gentisic, protocatechuic, and *p*-coumaric acids were the major contributors for antioxidant activity. Studies may thus support the use of swallow root in Ayurveda and folklore medicine and as a nutraceutical in the preparation of food and health drinks.

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References

Alasalvar, C., Karamać, M., Kosińska, A., Rybarczyk, A., Shahidi, F., & Amarowicz, R. (2009). Antioxidant activity of hazelnut skin phenolics. *Journal of Agricultural and Food Chemistry, 57*, 4645–4650.

Andreasen, M. F., Kroon, P. A., Williamson, G., & Garcia-Conesa, M. T. (2001). Intestinal release and uptake of phenolic antioxidant diferulic acids. *Free Radical Biology and Medicine, 31*, 304–314.

Anup, S., & Shivanandappa, T. (2006). Hepatoprotective effect of the aqueous extract of the roots of *Decalepis hamiltonii* against ethanol-induced oxidative stress in rats. *Hepatology Research, 35*, 267–275.

Bin-Hafeez, B., Haque, R., Parvez, S., Pandey, S., Sayeed, I., & Raisuddin, S. (2003). Immunomodulatory effects of fenugreek (*Trigonella foenum* graecum L.) extract in mice. *International Immunopharmacology, 3*, 257–265.

Bovell-Benjamin, A. C. (2007). Sweet potato: A review of its past, present, and future role in human nutrition. *Advances in Food and Nutrition Research, 52*, 1–59.

Georgea, J., Ravishankar, G. A., Keshava, U. N., & Udayasankar, K. (1999). Antibacterial activity of supercritical extract from *Decalepis hamiltonii* roots. *Fitoterapia, 70*, 172–174.

Hansen, M. B., Nielsen, S. E., & Berg, K. (1989). Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. *Journal of Immunological Methods, 119*, 203–210.

Harish, R., Divakar, S., Srivastava, A., & Shivanandappa, T. (2005). Isolation of antioxidant compounds from the methanolic extract of the roots of *Decalepis hamiltonii* (Wight and Arn.). *Journal of Agricultural and Food Chemistry, 53*, 7709–7714.

Hsu, C. L., Huang, S. L., & Yen, G. C. (2006). Inhibitory effect of phenolic acids on the proliferation of 3T3–L1 preadipocytes in relation to their antioxidant activity. *Journal of Agricultural and Food Chemistry, 54*, 4191–4197.

Iiyama, K., Tuyet Lam, T. B., & Stone, B. A. (1990). Phenolic acid bridges between polysaccharides and lignin in wheat internodes. *Phytochemistry, 29*, 733–737.

Kaviarasan, S., & Anuradha, C. V. (2007). Fenugreek (*Trigonella foenum* graecum) seed polyphenols protect liver from alcohol toxicity: A role on hSRHPtic detoxification system and apoptosis. *Pharmazie, 62*, 299–304.

Khan, N., & Mukhtar, H. (2007). Tea polyphenols for health promotion. *Life Sciences, 81*, 519–533.

Lapierre, C., Pollet, B., Ralet, M. C., & Saulnier, L. (2001). The phenolic fraction of maize bran: Evidence for lignin-heteroxylan association. *Phytochemistry, 57*, 765–772.

Liyana-Pathirana, C. M., & Shahidi, F. (2006). Importance of insoluble-bound phenolics to antioxidant properties of wheat. *Journal of Agricultural and Food Chemistry, 54*, 1256–1264.

Madhujith, T., & Shahidi, F. (2009). Antioxidant potential of barley as affected by alkaline hydrolysis and release of insoluble-bound phenolics. *Food Chemistry, 117*, 615–620.

Miller, N. J., & Rice-Evans, C. A. (1997). Cinnamates and hydroxybenzoates in the diet: Antioxidant activity assessed using the $ABTS^{\cdot+}$ radical cation. *British Food Journal, 99*, 57–62.

Murti, P. B. R., & Seshadri, T. R. (1941). A study of the chemical components of the roots of *Decalepis hamiltonii* (Makali veru). Part II. Chemical composition of the roots. *Proceedings of Indian Academy of Science, 13A*, 221–232.

Nagarajan, S., & Rao, L. J. (2003). Determination of 2-hydroxy-4-methoxy-benzaldehyde in roots of *Decalepis hamiltonii* (Wright and Arn.) and *Hemidesmus indicus* R.Br. *Journal of AOAC International, 86*, 564–567.

Nagarajan, S., Rao, L., & Gurudutt, K. N. (2001). Chemical composition of the volatiles of *Decalepis hamiltoii* (Wight and Arn). *Flavour and Fragrance Journal, 16*, 27–29.

Naik, Y., Smitha, J., Harish Nayaka, M. A., Lakshman & Shylaja, M. D. (2007). Gastroprotective effect of swallow root (*Decalepis hamiltonii*) extract: Possible involvement of H^+–K^+–ATPase inhibition and antioxidative mechanism. *Journal of Ethnopharmacology, 112*, 173–179.

Nardini, M., Pisu, P., Gentili, V., Naterlla, F., Felice, M. D., Piccolella, E., & Scaccini, C. (1998). Effect of caffeic acid on tert-butyl hydroperoxide-induced oxidative stress in U937. *Free Radical Biology and Medicine, 25*, 1098–1105.

Nayar, R. C., Shetty, J. K. P., Mary, Z., & Yoganarasimhan, S. N. (1978). Pharmacognostical studies on the root of *Decalepis hamiltonii* (Wt. and Arn.) and comparison with *Hemidesmus indicus* (L.). *Proceedings of Indian Academy of Science, 87B*, 37–48.

Rathore, D., McCutchan, T. F., Sullivan, M., & Kumar, S. (2005). Antimalarial drugs: Current status and new developments. *Expert Opinion and Investigation on Drugs, 14*, 871–883.

Sathisha, U. V., Smitha, J., Harish Nayaka, M. A., & Shylaja, M. D. (2007). Inhibition of galectin-3 mediated cellular interactions by pectic polysaccharides from dietary sources. *Glycoconjugate Journal, 24*, 497–507.

Shahidi, F., & Wanasundara, P. K. (1992). Phenolic antioxidants. *Critical Reviews in Food Science and Nutrition, 32*, 67–103.

Shyama Prasad Rao, R., & Muralikrishna, G. (2004). Non-starch polysaccharide–phenolic acid complexes from native and germinated cereals and millet. *Food Chemistry, 84*, 527–531.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*, 144–158.

Srivastava, A., Harish, R., & Shivanandappa, T. (2006). Novel antioxidant compounds from the aqueous extract of the roots of *Decalepis hamiltonii* (Wright and Arn.) and their inhibitory effect on low-density lipoprotein oxidation. *Journal of Agricultural and Food Chemistry, 54*, 790–795.

Srivastava, A., Rao, L. J., & Shivanandappa, T. (2007). Isolation of ellagic acid from the aqueous extract of the roots of *Decalepis hamiltonii*: Antioxidant activity and cytoprotective effect. *Food Chemistry, 103*, 224–233.

Suresh Kumar, G., Harish Nayaka, M. A., Shylaja, M. D., & Salimath, P. V. (2006). Free and bound phenolic antioxidants in amla (*Emblica officinalis*) and turmeric (*Curcuma longa*). *Journal of Food Composition and Analysis, 19*, 446–452.

Wijeratne, S. S., Abou-Zaid, M. M., & Shahidi, F. (2006). Antioxidant polyphenols in almond and its coproducts. *Journal of Agricultural and Food Chemistry, 54*, 312–318.