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Chemical and Antioxidant Evaluation of Indian Gooseberry (*Emblica officinalis* Gaertn., syn. *Phyllanthus emblica* L.) Supplements

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Indian gooseberry (*Emblica officinalis* Gaertn.) (Euphorbiaceae) has a distinguished history in Ayurveda medicine and is ascribed a number of medicinal properties and as a dietary supplement, its use is increasing in Western countries. It is thought that its beneficial properties are a function of its antioxidant potency. The study investigated the chemistry and antioxidant properties of four commercial *E. officinalis* fruit extracts in order to determine if there are any qualitative–quantitative differences. All extracts produced positive responses in the total phenol, total flavonoid and total tannin assays. The presence of predominantly (poly)phenolic analytes, e.g. ellagic and gallic acids and corilagin, was confirmed by RP-HPLC coupled with photodiode array detection. Despite ascorbic acid being a major constituent of *E. officinalis* fruits, the furanolactone could not be identified in one of the samples. The extracts demonstrated varying degrees of antioxidative efficacy. The extract designated IG-3 was consistently amongst the most effective extracts in the iron(III) reduction and 1,1-diphenyl-2-picrylhydrazyl and superoxide anion radical scavenging assays while the extract designated IG-1 demonstrated the best hydroxyl radical scavenging activity. All extracts appeared to be incapable of chelating iron(II) at realistic concentrations. Copyright © 2009 John Wiley & Sons, Ltd.

Keywords: botanicals; *Emblica officinalis*; composition; free radical scavenging; dietary supplements; antioxidant activity.

INTRODUCTION

Indian gooseberry (*Emblica officinalis* Gaertn., syn. *Phyllanthus emblica* L.) is a small to medium sized deciduous tree belonging to Euphorbiaceae which principally grows in subtropical and tropical areas of China, India, Indonesia and on the Malay Peninsula. Its fruits are spherical, light greenish, smooth and hard with six vertical stripes or furrows enclosing a stone. The fruits are used extensively in Ayurveda medicine as a potent *Rasayana* (Udupa and Singh, 1995) with it being a major component of the formulations known as *Chyavanprash* and *Triphala* (Govindarajan *et al.*, 2007; Naik *et al.*, 2005).

Indian gooseberry has been reported to contain a wide spectrum of components, including alkaloids (Khanna and Bansal, 1975), benzenoids (El-Mekkawy *et al.*, 1995; Pozharitskaya *et al.*, 2007), carbohydrates (Nizzamuddin *et al.*, 1982), coumarins (Desai *et al.*, 1977), diterpenes (Ram and Raja, 1978), flavonoids (Anila and Vijayalakshmi, 2002; Khanna *et al.*, 1982), furanolactones (Basa and Srinivasulu, 1987), triterpenes (Desai *et al.*, 1977; Hui and Sung, 1968) and steroids (Hui and Sung, 1968). Therefore, it should not be surprising that a wide range of health-promoting properties have been ascribed to Indian gooseberry extracts. Amongst these are adaptogenic (Kumar and Muller, 1999), antiatherogenic (Duan *et al.*, 2005), antitussive (Nosalova *et al.*, 2003), hepatoprotective (Tasduq *et al.*, 2005; Panda and Kar, 2003) and immunomodulatory (Sai Ram *et al.*, 2002; Ganju *et al.*, 2003) properties. It has been concluded that many of these properties may be associated with the potent *in vitro* and *in vivo* antioxidant properties of their constituents (Bhattacharya *et al.*, 2000; Naik *et al.*, 2005; Pozharitskaya *et al.*, 2007; Sabu and Kuttan, 2002; Tasduq *et al.*, 2005).

A search of the internet revealed that Indian gooseberry/fruit extracts are extremely commonly being marketed by herbal/medicinal plant retailers for the treatment of various conditions or ailments. Because of the medicinal significance – and thus economic importance – of *E. officinalis* fruit extracts, it was thought that it would be useful to investigate the chemistry and *in vitro* antioxidative properties of commercial extracts. Such a study may well be indicative of the variation in composition and activity of *E. officinalis* extracts available in the market place. Accordingly, phytochemical (total phenolic, flavonoid and tannin content and qualitative-quantitative composition) and antioxidant [iron(III) reducing, iron(II) chelating and 1,1-diphenyl-2-picrylhydrazyl (DPPH), superoxide anion and hydroxyl radical scavenging] data are presented for four water-soluble, commercially available *E. officinalis* fruit extracts.

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MATERIALS AND METHODS

Materials. Four different preparations described as water-soluble *E. officinalis* medicinal preparations were obtained by 'Diod' Ltd (Moscow, Russia) and were designated IG-1, IG-2, IG-3 and IG-4. All reagents and solvents were of either analytical or HPLC grade and were obtained from Sigma Chemical Co. (St Louis, MO). Chromatography standards were purchased from Sigma Chemical Co. (St Louis, MO). Ultra pure water (18.2 MΩcm) was prepared using a Millipore Milli-RO 12 plus system (Millipore Corp., Bedford, MA).

Total phenol content. The total phenol content was estimated as gallic acid equivalents (mg gallic acid/g extract) according to the Folin-Ciocalteu method (Singleton *et al.*, 1999). In brief, a 0.25 mL aliquot of dissolved extract was transferred to a 25.0 mL volumetric flask containing 6 mL of H_2O, to which was added 1.25 mL undiluted Folin-Ciocalteu reagent. After 1 min, 3.75 mL of 20% (w/v) aq. Na_2CO_3 was added, and the volume was made up to 25.0 mL with H_2O. The controls contained all the reaction reagents except the extract. After a 2 h incubation at 25 °C, the absorbance was measured at 760 nm and compared with a gallic acid calibration curve.

Total flavonoid content. The total flavonoid content was estimated as rutin equivalents (mg rutin/g extract) according to the method described in a USSR State Pharmacopoeia (Anon, 1989). In brief, a 1.0 mL aliquot of each extract dissolved in ethanol (10 mg/mL) was mixed with 1 mL $AlCl_3$ (20 mg/mL, in EtOH) and 1 drop of acetic acid, then diluted to 25 mL with EtOH. Blanks were prepared as above except $AlCl_3$ was replaced by EtOH. The absorption at 415 nm was measured after 40 min and compared with a rutin calibration curve.

Total tannin content. The total tannin content was estimated as tannin equivalents (mg tannin/g extract) according to the method described in a USSR State Pharmacopoeia (Anon, 1989). In brief, 2.0 g of each extract was dissolved in 250 mL of water. A 25 mL aliquot was mixed with 500 mL of water and 25 mL of indigo sulphuric acid then titrated using a 20 mM $KMnO_4$ solution.

High performance liquid chromatography analysis. The liquid chromatographic apparatus (Waters 600) consisted of an in-line degasser, pump and controller coupled to a 2996 photodiode array detector equipped with a Rheodyne injector (20 µL sample loop) interfaced to a PC running Millenium[32] chromatography manager software (Waters Corp., Milford, Massachusetts). Separations were performed on a reverse-phase Hypersil BDS-C18 analytical column (250 × 4.6 mm i.d., particle size 5 µm) (Agilent Technologies, Milford, Massachusetts) operating at room temperature with a flow rate of 0.7 mL/min. Detection was carried out with a sensitivity of 0.1 a.u.f.s. between the wavelengths of 200 to 550 nm. Elution was effected using a ternary non-linear gradient of the solvent mixture MeOH:H_2O:CH_3COOH (10:88:2, v/v/v) (solvent A), MeOH:H_2O:CH_3COOH (90:8:2, v/v/v) (solvent B) and MeOH (solvent C). The composition of the mobile phase was changed from 99:1:0 (A/B/C) to 98:2:0 (A:B:C) in 15 min, changed to 60:40:0 (A/B/C) in 3 min, changed to 50:50:0 (A/B/C) in 12 min, changed to 5:95:0 (A/B/C) in 5 min, 0:85:15 (A/B/C) in a further 2 min, and to 0:70:30 (A/B/C) in 11 min, then returned to initial conditions in 7 min. A 10 min equilibrium time was allowed between injections. The components were identified by comparison of their retention times to those of authentic standards under identical analysis conditions, and the UV spectra with our in-house PDA library. Stock solutions of the extracts and standards were prepared in water and 70% (v/v) aq. MeOH to final concentrations of 10 and 1 mg/mL, respectively. The concentration used for the calibration of reference compounds was 0.01–0.10 mg/mL. All standard and sample solutions were injected in triplicate.

Iron(III) reductive activity assay. Iron(III) reductive ability was assessed by the method of Oyaizu (1986). In brief, a 1 mL aliquot of each dissolved extract was mixed with 2.5 mL phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of a 1% (w/v) potassium hexacyanoferrate solution. After 30 min at 50 °C, 2.5 mL of 10% (w/v) aq. trichloroacetic acid (TCA) was added. The mixture was centrifuged for 10 min. A 2.5 mL aliquot was mixed with 2.5 mL H_2O and 0.5 mL of 0.1% $FeCl_3$ solution, and the absorbance was recorded at 700 nm. The mean absorbance values were plotted against concentration, and a linear regression analysis was carried out. The data are presented as ascorbic acid equivalent (AscAE) [mmol ascorbic acid/g (dry wt.) extract] values. Ascorbic acid and gallic acid was used as positive controls.

Iron(II) chelating activity assay. Iron(II) chelating ability was assessed by the method of Carter (1971). In brief, a 200 µL aliquot of each dissolved extract was mixed with 100 µL of 2.0 mM $FeCl_2 \cdot 4H_2O$ solution and 900 µL MeOH. After 5 min, the reaction was initiated by 400 µL of a 5.0 mM ferrozine solution. After a 10 min reaction period, the absorbance at 562 nm was recorded. The percentage iron(II) chelating values were calculated using Eq. (1) and the EC_{50} values were estimated by a nonlinear regression algorithm (SigmaPlot 2004 version 9.01). EDTA was used as a positive control.

$$\text{Chelation (\%)} = \frac{[(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})]}{\text{Abs}_{\text{control}}} \times 100 \quad (1)$$

DPPH free radical scavenging assay. The ability of the extracts to scavenge DPPH free radicals was assessed by the method of Gyamfi *et al.* (1999). In brief, a 50 µL aliquot of each dissolved extract was mixed with 450 µL of Tris-HCl buffer (50 mM, pH 7.4) and 1.0 mL of 0.1 mM DPPH (in MeOH). After a 30 min incubation in darkness at ambient temperature (23 °C), the resultant absorbance was recorded at 517 nm. The percentage inhibition values were calculated using Eq. (2) and the IC_{50} values were estimated by a non-linear regression algorithm (SigmaPlot 2004 version 9.01). Ascorbic acid and gallic acid were used as positive controls.

$$\text{Inhibition (\%)} = \frac{[(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})]}{\text{Abs}_{\text{control}}} \times 100 \quad (2)$$

Superoxide anion free radical scavenging assay. The superoxide anion free radical scavenging ability was

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assessed by the method of Lee *et al.* (2002). In brief, the reaction mixture contained 2.4 mL of NaH_2PO_4–NaOH buffer (50 mM, pH 7.4), 100 µL of dissolved extract (in NaH_2PO_4–NaOH buffer), 100 µL of 30 mM Na_2EDTA in NaH_2PO_4–NaOH buffer, 100 µL of 3 mM hypoxanthine and 200 µL of 1.42 mM nitroblue tetrazolium (NBT). After a 3 min incubation, the reaction was initiated by 100 µL of 0.50 U/mL xanthine oxidase solution. After a 20 min equilibrium period, the absorbance at 560 nm was recorded. The percentage superoxide anion free radical scavenging values using Eq. (1) and the IC_{50} values were estimated by a nonlinear regression algorithm (SigmaPlot 2004 version 9.01). Ascorbic and gallic acids were used as positive controls.

Xanthine oxidase generation of uric acid assay. Xanthine oxidase inhibitory activity was assessed by the method described by Lee *et al.* (2002). The reaction mixture contained the same proportion of components as in the superoxide anion free radical scavenging assay, except NBT was substituted by buffer.

Hydroxyl free radical scavenging assay. Hydroxyl radical scavenging was assessed by the method described by Halliwell *et al.* (1987). In brief, the reaction mixture contained 500 µL of dissolved extract, 100 µL of 28 mM 2-deoxy-D-ribose solution, 200 µL of a premixed 100 µM $FeCl_3$ and 104 mM EDTA (1:1 v/v) solution, 100 µL of 1.0 mM H_2O_2 and 100 µL of 1.0 mM ascorbic acid solution. After a 60 min incubation at 37 °C, 50 µL of 2% (w/v) butylated hydroxytoluene solution was added followed by 1 mL of 2.8% (w/v) TCA solution and 1 mL of 1% (w/v) 2-thiobarbituric acid solution. After vortexing, the samples were heated in a water bath (100 °C) for 20 min. The reaction was stopped by bathing the tubes in an ice–H_2O mixture. Two mL *n*-butanol was added and the mixture was vortexed vigorously. After centrifugation, the extent of oxidation was estimated from the absorbance of the organic layer at 532 nm. The percentage inhibition was calculated using Eq. (1) and the IC_{50} values were estimated by a nonlinear regression algorithm (SigmaPlot 2004 version 9.01).

Statistical analysis. Data were presented as mean values ± standard deviation. Analysis of variance was performed by ANOVA procedures. Significant differences between means were determined by Tukey's pairwise comparison test at a level of $p < 0.05$.

RESULTS AND DISCUSSION

The total phenol content ranged from 188.8 mg gallic acid/g (IG-4) to 237.0 mg gallic acid/g (IG-3), increasing in the order: IG-4 < IG-2 < IG-1 < IG-3 (Table 1). The estimated total flavonoid content ranged from 6.4 mg rutin/g (IG-1) to 20.1 mg rutin/g (IG-4), increasing in the order IG-1 < IG-2 < IG-3 < IG-4 (Table 1). The estimated total tannin content ranged from 375.2 mg tannin/g (IG-2) to 642.8 mg/g (IG-3), increasing in the order IG-2 < IG-4 < IG-1 < IG-3 (Table 1).

The Folin-Ciocalteu reagent-determined total phenol data showed a significant correlation with the iron(III) reduction index ($r^2 = 0.934$; $p < 0.05$), a positive association with the tannin content ($r^2 = 0.625$; $p > 0.200$) and

Table 1. Total phenolic, flavonoid and tannin content and HPLC-PDA analysis data

Sample	TP[b]	TF[c]	TT[d]	Identified component[a]					
				L-Ascorbic acid	Gallic acid	Corilagin	Ellagic acid	Phyllanthin	Σ
IG-1	204.9 ± 2.5	6.4 ± 0.4	566.1 ± 22.5	32.96 ± 1.32	25.77 ± 0.19	1.96 ± 0.10	2.18 ± 0.11	2.21 ± 0.10	65.08 ± 1.35
IG-2	199.3 ± 11.6	8.4 ± 0.5	375.2 ± 15.0	89.18 ± 3.55	45.22 ± 1.04	3.96 ± 0.19	1.29 ± 0.06	nd[e]	139.65 ± 3.70
IG-3	237.0 ± 5.4	10.1 ± 0.5	642.8 ± 31.1	42.18 ± 1.69	52.70 ± 4.01	3.59 ± 0.18	2.42 ± 0.12	nd	100.89 ± 4.36
IG-4	188.8 ± 7.3	20.1 ± 1.0	461.9 ± 20.3	nd	85.49 ± 0.10	nd	1.50 ± 0.08	nd	86.99 ± 0.13

[a] Values are expressed as mean ± standard deviation ($n = 3$).
[b] TP, total phenolic content expressed as gallic acid equivalents, mg gallic acid/g (dry wt) of extract ($n = 4$).
[c] TF, total flavonoid content expressed as rutin equivalents, mg rutin/g (dry wt) of extract ($n = 3$).
[d] TT, total tannin content expressed as tannin equivalents, mg tannin/g (dry wt) of extract ($n = 3$).
[e] nd, not detected.

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DPPH scavenging (r^2 = 0.845; p = 0.08); however, no association was identified between this index with the remaining indices ($0.000 \leq r^2 \leq 0.185$). Similarly, there was a significant correlation between the tannin content and iron(III) reduction ($r^2 = 0.848$, $p < 0.05$), a positive association with DPPH scavenging ($r^2 = 0.723$, $p > 0.01$) but no other positive associations were identified ($0.181 \leq r^2 \leq 0.366$). No correlations were identified for the $AlCl_3$-determined flavonoid content and the remaining indices ($0.000 \leq r^2 \leq 0.426$).

The qualitative–quantitative analysis of the different water-soluble *E. officinalis* fruit samples is presented in Table 1, while their chromatographic profiles are presented in Fig. 1. The components L-ascorbic acid, gallic acid, corillagin, ellagic acid and phyllanthin (Fig. 2) were identified in the samples. All contained relatively polar compounds (i.e. hydroxybenzoates and flavonoids), with gallic and ellagic acids ranging from 105.4 to 23.22 mg/g and 2.42 to 1.29 mg/g, respectively. L-Ascorbic acid and corilagin were identified in the IG-1, IG-2 and IG-3 extracts. Phyllanthin was identified only in the IG-1 (2.21 ± 0.10 mg/g) extract.

There was a significant association identified between the HPLC-determined content and superoxide anion ($r^2 = 0.929$; $p < 0.05$) and hydroxyl radical ($r^2 = 0.957$; $p < 0.05$) scavenging indices. No other significant associations were identified ($0.000 \leq r^2 \leq 0.326$).

The expression of antioxidant activity is thought to be concomitant with the development of reductones (Duh, 1998), which are reported to be terminators of free radical chain reactions (Gordon, 1990); thus, any antioxidant properties of the *E. officinalis* extracts may relate to their reductive activity. Therefore, it was considered important to assess the ability of the extracts to reduce iron(III). As can be seen in Fig. 3A, all the extracts possessed the ability to reduce iron(III) and did so in a concentration-dependent fashion (data not show). On the basis of the AscAE values, calculated from the plots of absorbance versus sample concentration, the IG-2 and IG-4 extracts were better iron(III) reducers when compared with the IG-3 and IG-1 extracts. However, it was not possible to statistically distinguish ($p > 0.05$) the activity observed for the IG-2 extract from that observed for the IG-1 and IG-4 extracts. Despite the ability of extracts to reduce iron(III), none of the extracts were significantly ($p > 0.05$) better than the reference substance, ascorbic acid, or the positive control, gallic acid. There was a significant correlation between the iron(III) reduction index and the total phenols ($r^2 = 0.934$, $p < 0.05$), total tannins ($r^2 = 0.848$, $p < 0.05$) and DPPH free radical scavenging ($r^2 = 0.879$, $p < 0.05$) indices; however, no association was identified for the remaining indices ($0.003 \leq r^2 \leq 0.128$).

The water-soluble *E. officinalis* extracts were capable of reducing iron(III), and thus, are capable of donating electrons. This property suggests that the extracts may act as free radical chain terminators, transforming reactive free radical species into more stable nonradical products (Dorman *et al.*, 2004).

An important antioxidant mechanism is transition metal chelation (Kehrer, 2000) as such species possess the ability to catalyse hydroperoxide decomposition and Fenton-type reactions (Gordon, 1990; Dorman *et al.*, 2003). Plant extracts rich in polyphenolic components should be able to complex and stabilize transition metals. Therefore, it was considered important to determine the iron(II)-chelating ability of the extracts.

None of the extracts chelated iron(II) even at a concentration of 30 mg/mL (data not shown). It is

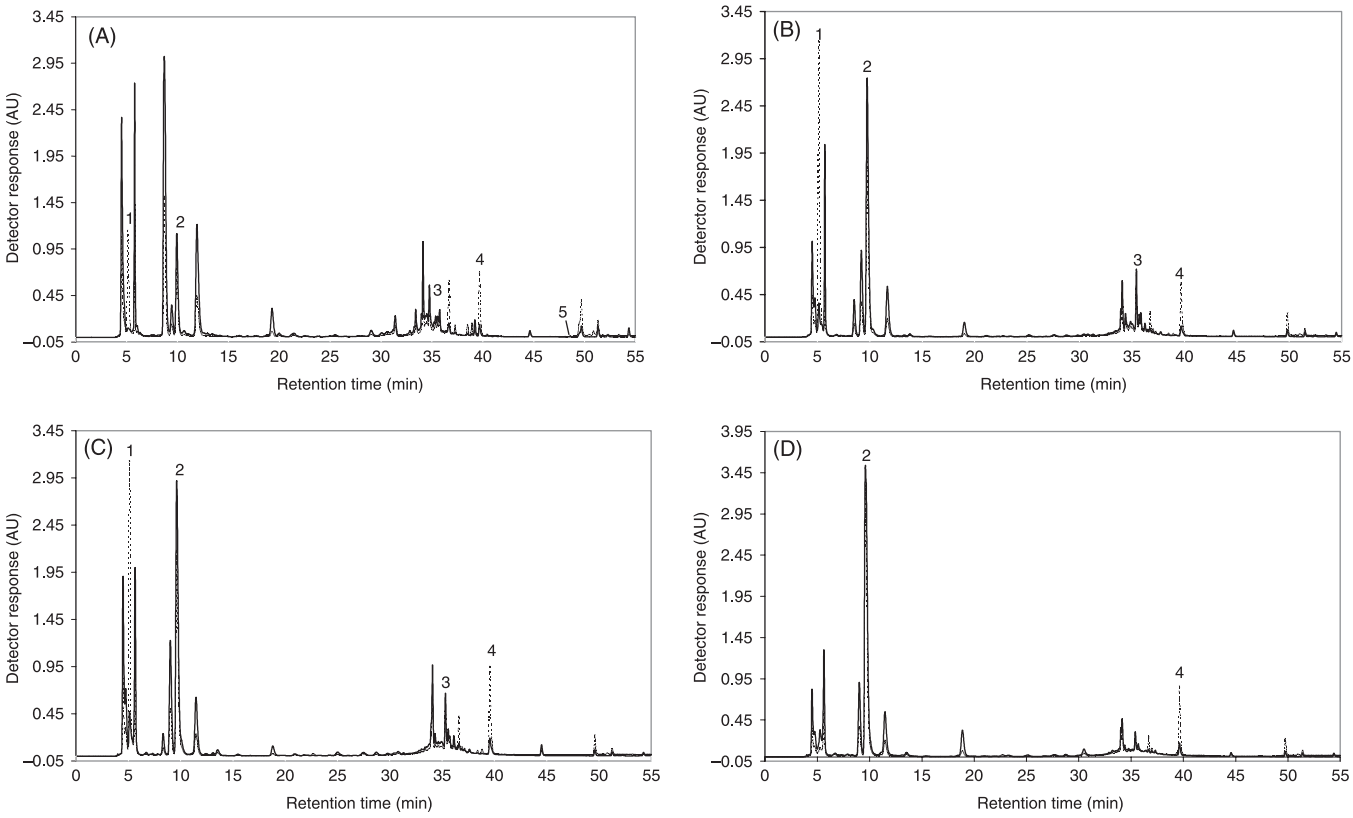

Figure 1. HPLC-PDA analysis of the (A) IG-1, (B) IG-2, (C) IG-3 and (D) IG-4 extracts with detector responses at 254 (dotted line) and 280 nm (straight line) overlaid. 1, L-ascorbic acid; 2, gallic acid; 3, corilagin; 4, ellagic acid; 5, phyllanthin.

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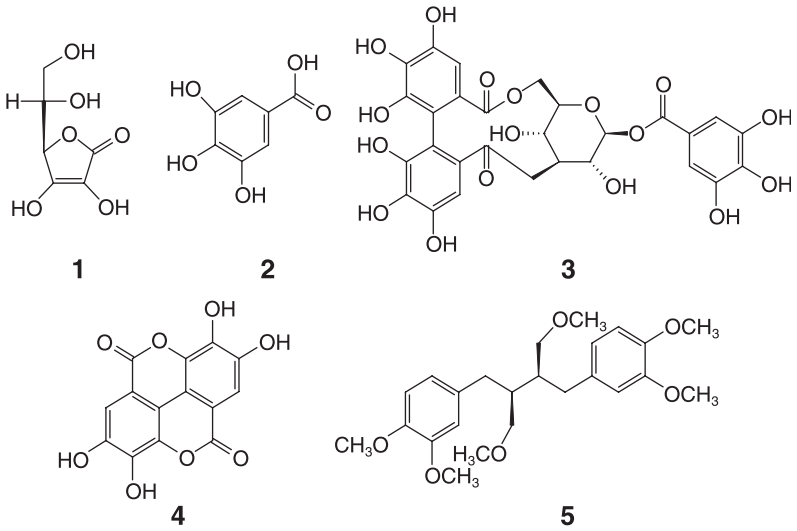

Figure 2. Structural formulae of the identified components within the extracts: 1, L-ascorbic acid; 2, gallic acid; 3, corilagin; 4, ellagic acid; 5, phyllanthin.

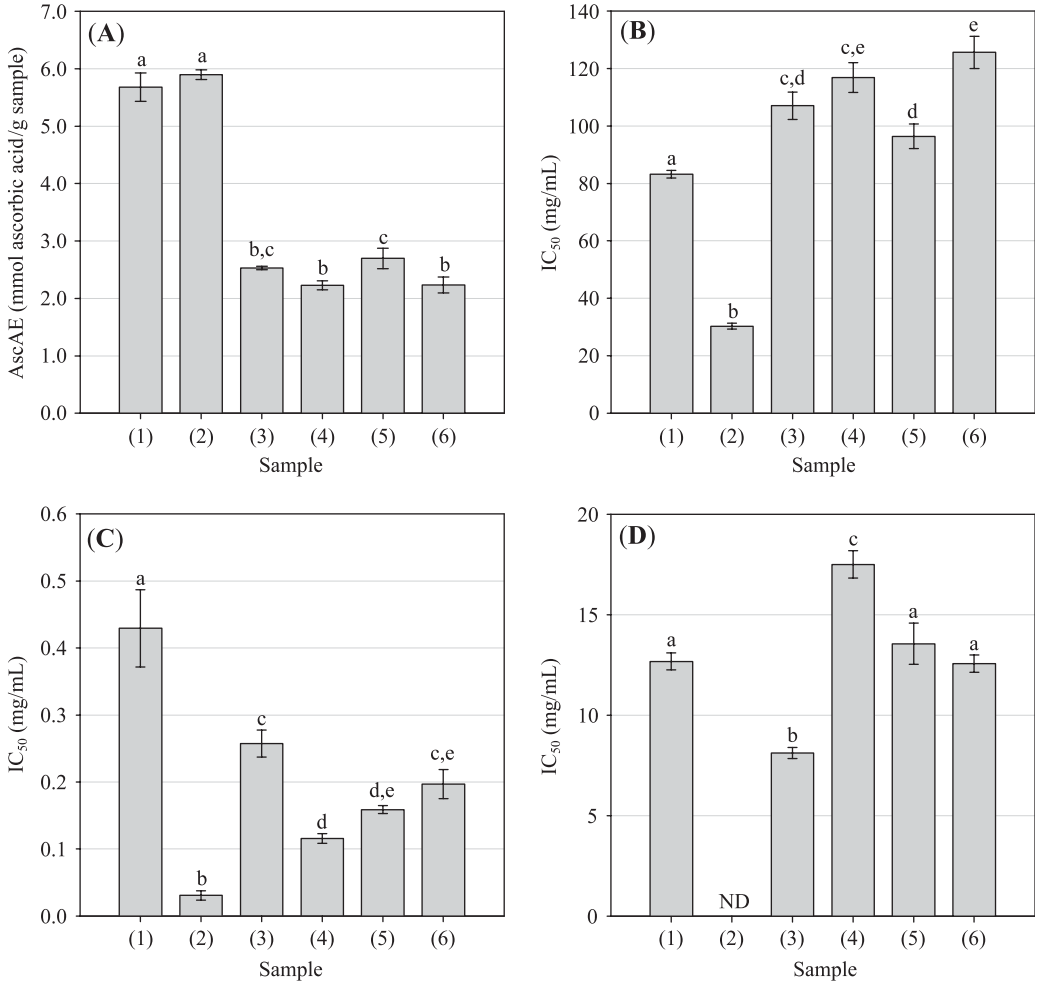

Figure 3. The effect of the extracts and positive controls upon (A) iron(III) reduction and (B) DPPH, (C) superoxide anion and (D) hydroxyl free radical scavenging. Data are presented as mean value ± standard deviation ($n = 3$). Bars with the same lowercase letter (a–e) are not significantly ($p > 0.05$) different. Samples: 1, ascorbic acid; 2, gallic acid; 3, IG-1; 4, IG-2; 5, IG-3; 6, IG-4. ND, not determined.

clear from the iron(II) chelation data that the extracts are not capable of a protective role against oxidative damage that may be caused by iron(II) ions catalysing Fenton-type reactions or participating in metal-catalysed hydroperoxide decomposition, at least at realistic concentrations.

A cardinal antioxidant property is the ability to scavenge free radicals, species involved in deleterious oxidative reactions, the pathogenesis of various chronic diseases and premature aging (Dorman *et al.*, 2003; Shikov *et al.*, 2006). Therefore, it was considered important to assess the free radical scavenging efficacy

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of the *E. officinalis* extracts using the nitrogen-centered free radical DPPH.

All the extracts were capable of scavenging DPPH free radicals and did so in a concentration-dependent fashion (data not shown). As can be seen from the IC_{50} values (Fig. 3B), the IG-3 extract was the most potent DPPH radical scavenger, however, it was statistically ($p < 0.05$) indistinguishable from the IG-1 extract. Similarly, there was no significant difference between the IG-2 and IG-4 extracts. Moreover, the activity of IG-2 did not significantly ($p > 0.05$) differ from that demonstrated by the IG-1 extract. There was a significant correlation between the DPPH free radical scavenging index and the total phenols ($r^2 = 0.845$, $p < 0.05$), total tannins ($r^2 = 0.733$, $p < 0.05$) and iron(III) reduction ($r^2 = 0.879$, $p < 0.05$) indices. No significant association was identified with either the total flavonoid content ($r^2 = 0.426$, $p > 0.300$), HPLC-determined content ($r^2 = 0.050$, $p > 0.700$) and superoxide anion radical scavenging ($r^2 = 0.016$, $p > 0.800$) indices.

The results of the DPPH scavenging assay suggest that the *E. officinalis* extracts are capable of scavenging free radicals at physiological pH and thus may be able to prevent the initiation and propagation of free radical-mediated chain reactions by stabilizing reactive species before they can participate in deleterious reactions, e.g. hydrogen abstraction from susceptible polyunsaturated fatty acids.

The superoxide anion radical is an electron adduct of molecular oxygen, principally generated via the NADPH oxidase system. This species can directly affect the physiological functioning of cellular organelles through its reactivity (i.e. initiate lipid peroxidation, react with carbonyls or nitric oxide to generate peroxyl radicals or peroxynitrite, respectively); however, its *in vivo* oxidative significance is due to its direct participation in the generation of hydroxyl radicals. Therefore, it was considered important to assess the ability of the *E. officinalis* extracts to scavenge this free radical.

The different water-soluble *E. officinalis* samples inhibited the reduction of NBT and did so in a concentration-dependent fashion (data not shown). Furthermore, the extracts had no significant ($p > 0.05$) effect upon the generation of uric acid during hypoxanthine oxidation (data not shown), which confirms that the components within the *E. officinalis* extracts did, in fact, scavenge superoxide anion radicals and did not inhibit enzyme-dependent superoxide anion free radical generation. From the estimated IC_{50} data presented in Fig. 3C, it can be seen that the IG-1 extract was the least effective superoxide anion radical scavenger, though it was not possible to distinguish it statistically ($p > 0.05$) from the IG-4 extract. The IG-2 extract appeared to be the most effective; however, it was statistically indistinguishable ($p > 0.05$) from the IG-3 extract. The activity of the IG-3 and IG-4 extracts was statistically ($p > 0.05$) identical. Despite none of the *E. officinalis* extracts being as potent superoxide anion radical scavengers as the positive control, gallic acid, all were more potent than ascorbic acid. There was a significant correlation between superoxide anion radical scavenging and the HPLC-determined content ($r^2 = 0.929$, $p < 0.05$) but no such association appeared to exist with the remaining indices ($0.000 \leq r^2 \leq 0.181$).

The results of the superoxide anion radical scavenging assay suggest that the *E. officinalis* extracts are capable of scavenging this radical species at physiological pH and thus may be able to interfere in the initiation and propagation of free radical-mediated chain reactions by preventing the Fenton chemistry type-generation of the highly reactive hydroxyl radical *in vivo*.

Although iron(III) reduction and DPPH scavenging are commonly used assays to identify potential antioxidants, neither method utilizes a food or biologically relevant oxidizable substrate. Therefore, it was considered useful to assess the ability of the *E. officinalis* extracts to scavenge the highly reactive and universal occurring hydroxyl radical. When ethylenediaminetetraacetic acid chelated iron(III) is incubated with ascorbic acid and H_2O_2, hydroxyl free radicals are generated (Dorman *et al.*, 2004). In this assay, an extract capable of inhibiting the formation of 2-thiobarbituric acid reactive species may be described as an antioxidant capable of scavenging hydroxyl radicals and protecting carbohydrates from oxidative degradation (Dorman *et al.*, 2004).

As can be seen from the estimated IC_{50} values presented in Fig. 3D, the extracts were capable of scavenging hydroxyl free radicals and did so in a concentration-dependent fashion (data not shown). The IG-1 extract was a statistically ($p < 0.05$) better hydroxyl free radical scavenger than either ascorbic acid or the other extracts. The IG-4 and IG-3 (statistically indistinguishable, $p < 0.05$) extracts were the second most potent extracts but were statistically indistinguishable ($p > 0.05$) from ascorbic acid. The IG-2 extract was the least effective hydroxyl free radical scavenger. A significant correlation was identified between hydroxyl radical scavenging and the HPLC-determined content ($r^2 = 0.957$, $p < 0.05$) and superoxide anion radical scavenging ($r^2 = 0.967$, $p < 0.05$) indices; however, no such association was found for the remaining indices ($0.000 \leq r^2 \leq 0.366$).

According to the presented data, the *E. officinalis* extracts were capable of scavenging hydroxyl free radicals before they could degrade the 2-deoxy-D-ribose substrate. Thus, the extracts contain compounds that may be capable of protecting substrates susceptible to hydroxyl radical-mediated oxidative degradation *in vivo* or in foods, cosmetics and pharmaceutical preparations.

CONCLUSION

E. officinalis fruit extracts are used chiefly in the preparation of Ayurveda-type 'Rasayanas', i.e. plant-derived drugs used to promote health and longevity. However, because of their high ascorbic acid content and the presence of complex polyphenolic components, a potential mainstream use could be in the functionalization of foods and beverages and as an ingredient in the formulation of novel nutraceuticals. Irrespective of its final utilization, sufficient and repeatable quality and quantity is essential if it is to be considered. Therefore, it was decided to assess four water-soluble *E. officinalis* fruit extracts obtained from commercial sources for differences in their phytochemical profiles and efficacy in a screening battery of five *in vitro* antioxidant-related assays. The estimated levels of total phenols, flavonoids and tannins varied quantitatively. From the HPLC-PDA qualitative-quantitative analysis data, it can be seen that

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the samples did differ. Ascorbic acid, corilagin and phyllanthin were absent from the IG-4 sample while phyllanthin was only identified in the IG-1 sample. All the extracts demonstrated antioxidant-related activities, though they were poorly active in the iron(II) chelation screen. The extract designated IG-3 was consistently effective in the iron(III) reduction and DPPH and superoxide anion radical scavenging assays while the extract designated IG-1 was the best hydroxyl radical scavenger. It has been suggested that the antioxidant activity of fruits is due to the high levels of ascorbic acid, however, in this case, IG-3 did not contain the highest ascorbic acid levels (indeed, neither did the IG-1 sample) yet it was consistently potent in the antioxidant screens. It did, however, contain high levels of total phenols and total tannins – confirming the hypothesis forwarded by Pozharitskaya *et al.* (2007) that the antiradical activity of *E. officinalis* fruit extracts is associated with the presence of hydrolysable tannins rather than ascorbic acid. Despite this, it is clear from the data that the composition of commercial extracts do vary, particularly in the content of ascorbic acid – a principal component of *E. officinalis* fruit. Clearly, even from this small-scale sampling, the need for standardization is apparent. Though beyond the scope of this study, it would be interesting to determine the variation between batches from each supplier whose extracts were used in this study.

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