ELSEVIER

Journal of Food Composition and Analysis 19 (2006) 446–452

JOURNAL OF FOOD COMPOSITION AND ANALYSIS

www.elsevier.com/locate/jfca

Original Article

Free and bound phenolic antioxidants in amla (*Emblica officinalis*) and turmeric (*Curcuma longa*)

G. Suresh Kumar[a], Harish Nayaka[a,b], Shylaja M. Dharmesh[a], P.V. Salimath[a,*]

[a]*Department of Biochemistry and Nutrition, Central Food Technological Research Institute, Mysore 570 020, Karnataka, India*
[b]*Department of Biochemistry, Yuvaraja College, University of Mysore, Mysore 570 005, Karnataka, India*

Received 29 December 2004; received in revised form 5 December 2005; accepted 21 December 2005

Abstract

The antioxidant activity of free and bound phenolics of amla (*Emblica officinalis*) and turmeric (*Curcuma longa*) was investigated. The *Emblica officinalis* free (EOFP) and bound phenolics (EOBP) showed between four- to 10-fold higher levels of antioxidant activity as evaluated by both free radical scavenging and reducing power assays compared to that of *Curcuma longa* free (CLFP) and bound phenolics (CLBP). Higher level of antioxidant activity in *E. officinalis* has been attributed to the phenolic content (12.9%, w/w, correlation coefficient $R = 0.74$) in them. The free and bound phenolics of *E. officinalis* showed high content of phenolic compounds (126 and 3.0 mg/g) compared to that of *C. longa* (29.7 and 1.6 mg/g). Gallic acid and tannic acid were identified as the major antioxidant components in phenolic fractions of *E. officinalis*. The antioxidant activity of CLFP could be attributed to curcumin and that of CLBP to ferulic acid and *p*-coumaric acid. Further, the extracts of both *E. officinalis* and *C. longa* also exhibited significant protection to DNA against oxidative damage as evidenced by migration of DNA on the agarose gel. Our results clearly suggest the presence of potent antioxidants such as gallic acid in *E. officinalis* and protocatechuic acid and ferulic acid in *C. longa*, in addition to the known ascorbic acid and curcumin in *E. officinalis* and *C. longa*, respectively.

Keywords: *Emblica officinalis*; *Curcuma longa*; Curcumin; Turmeric; Antioxidant activity; Phenolic acids; Ascorbic acid; Free radical scavenging activity; Reducing power; Gallic acid

1. Introduction

Oxidants and antioxidants in humans are maintained in balance in a normal physiological state, and overproduction of oxidants in certain conditions such as smoking, hazardous environmental exposures or infectious diseases can cause oxidative stress leading to oxidative damage to biomolecules and cells (Temple, 2000; Willett, 2002; Doll, 1990; Ames, 1979; Thompson, 1994). Human diets constituting of fruits, vegetables and spices have been shown to contain beneficial components, including phenolic compounds with antioxidant properties (Croft, 1998). Phenolic components have been known to act as antioxidants not only because of their ability to donate electrons, but also because of their stable radical intermediates, which can effectively prevent the oxidation at cellular and physiological level (Cuvrelier et al., 1992).

Amla (*Emblica officinalis*) and turmeric (*Curcuma longa*) have long been known in India and many other countries as important dietary sources in addition to their use in traditional medicine for wound healing, inflammation and stomach acidity. Several investigators have determined the efficacy of both amla and turmeric as anti-atherosclerotic (Thakur et al., 1988), antidiabetic (Tripathi et al., 1979), antimutagenic (Nagabhushan and Bhide, 1986; Sharma et al., 2000a; Polasa et al., 1991) and anticancer agents (Aggarwal et al., 2003; Jose et al., 2001). It has now been well established that oxidative stress plays an important role in these disorders. Notably, ascorbic acid and

Abbreviations: CLBP, *Curcuma longa* bound phenolics; CLFP, *Curcuma longa* free phenolics; DPPH, 1, 1-diphenyl-2-picryl hydrazyl; EOBP, *Emblica officinalis* bound phenolics; EOFP, *Emblica officinalis* free phenolics; GAE, gallic acid equivalent

*Corresponding author. Tel.: +91 0821 2514876; fax: +91 0821 2517233.
E-mail address: paramahans1954@yahoo.com (P.V. Salimath).

0889-1575/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.jfca.2005.12.015

curcumin have been shown to be the major active components responsible for the beneficial properties in amla and turmeric, respectively (Sharma et al., 2000b; Selvam et al., 1995). However, the precise phenolic acids and their antioxidant potency need to be determined in order to understand these health attributes. Phytochemicals other than ascorbic acid and curcumin with potential beneficial effects have already been reported in amla and turmeric (Bhattacharya et al., 1999; Yu et al., 2002). Although the role of phenolic acids in the maintenance of health and prevention of diseases seems positive, the evidence is still limited and conflicting. Moreover, the bioavailability of phenolic acids from various food sources is poorly known. Therefore, in the present study, we undertook an investigation on the free and bound phenolics present in amla and turmeric, as well as their antioxidant potency.

2. Materials and methods

2.1. Materials

Emblica officinalis fruit and *Curcuma longa* rhizome were purchased from a local market (Devaraja Market, Mysore, Karnataka, India). 1,1-Diphenyl-2-picryl hydrazyl (DPPH), Folin-Ciocalteau reagent, tannic acid, ascorbic acid, phenolic acid standards such as gallic acid, caffeic, *p*-coumaric, ferulic, gentisic, protocatechuic, syringic and vanillic acids, butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), agarose and calf thymus DNA were purchased from Sigma Chemical Co. (St. Louis, Missouri, USA). HPLC column (Shimpak C_{18}) was obtained from Shimadzu Corp. (Tokyo, Japan). The other chemicals such as ferric chloride, hydrogen peroxide, trichloro acetic acid and solvents used were of the analytical grade purchased from a local chemical company.

2.2. Methods

Fresh fruits of *E. officinalis* were deseeded, sun dried for 3 days, reduced to powder in a mixer (Gopi, C. Lal Electrical and Mechanicals Co., Ambala, India) and preserved under dry conditions at 4 °C until further extraction. *C. longa* rhizomes were freed from soil and other organic matter and processed as described for *E. officinalis*.

2.3. Isolation of free phenolic acids

Free phenolics were isolated according to the method followed by Subba Rao and Murlikrishna (2002). One gram each of *C. longa* and *E. officinalis* powder (in triplicate, $n = 3$) was extracted (1:50, w/v) in 70% ethanol (3×50 mL, 2 h each), and the supernatants were obtained by centrifugation (Sigma 3-16K, USA) at 3000*g* for 15 min and concentrated by flash evaporation (Buchi 011, Switzerland); the pH was adjusted to 1.5 with 4 N hydrochloric acid. Phenolic acids were separated by ethyl acetate phase separation (4×50 mL) and the pooled fractions were treated with anhydrous sodium sulphate, filtered and evaporated to dryness. Total phenolic acid was estimated spectrophotometrically by Folin-Ciocalteu method with gallic acid as the reference standard and expressed as gallic acid equivalent (GAE) in milligrams per gram dry weight (dw) of sample.

2.4. Isolation of bound phenolic acids

E. officinalis and *C. longa* samples (2 g, each) were defatted with petroleum ether and chloroform (1:1, v/v) and extracted with 70% ethanol (4×50 mL) to extract free phenolic acids. The dried samples were extracted with 1 M sodium hydroxide (2×100 mL) containing 0.5% sodium borohydride under nitrogen atmosphere, and the clear supernatants were collected by centrifugation (Nordkvist et al., 1984). The combined supernatants (bound phenolics) were acidified with 4 N hydrochloric acid to pH 1.5 and the phenolic acids were extracted and quantified colorimetrically in the same way as free phenolic acids.

2.5. Determination of antioxidant activity of bound and free phenolic fractions: measurement of reducing power

The reducing power of free and bound phenolics of *E. officinalis* and *C. longa* were determined according to the method of Yen and Chen (1995). The free and bound phenolic extract (2–10 μg) of *E. officinalis*, *C. longa* and standard BHA and BHT (2–10 μg) 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. An equal volume of 10% trichloroacetic acid was added to the mixture and centrifuged at 3000*g* for 10 min. The upper layer of the solution was mixed with distilled water and 0.1% $FeCl_3$ at a ratio of 1:1:2 (v/v) and the absorbance were measured in a spectrophotometer (Shimadzu UV-160 spectrophotometer, Tokyo, Japan) at 700 nm. An increase in the absorbance values compared to that of blank indicates higher reducing power.

2.6. Free radical scavenging effect

The effect of free and bound phenolics of *E. officinalis* and *C. longa* on DPPH radical was estimated according to the method of Lai et al. (2001). An aliquot of 200 μL of different phenolic fractions (2–10 μg, GAE) of *E. officinalis* and *C. longa* and 2–10 μg of synthetic antioxidants (BHA and BHT) were mixed with 100 mM Tris–HCl buffer (800 μL, pH 7.4). One millilitre of 500 μM DPPH in ethanol was added to the sample to a final concentration of 250 μM. The mixture was shaken vigorously and left to stand for 20 min at room temperature in the dark. The absorbance of the resulting solution was measured spectrophotometrically at 517 nm. The capability to scavenge the DPPH

radical was calculated using the following equation:

$$\text{Scavenging effect (\%)} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100.$$

2.7. DNA protection assay

The DNA protective effect of phenolic fractions was determined electrophoretically (Submarine electrophoresis system, Bangalore Genei, Bangalore, India) using calf thymus DNA (Rodriguez and Akman, 1998). Calf thymus DNA (1 μg) was subjected to oxidation by Fenton's reagent (30 mM H_2O_2, 50 μM ascorbic acid and 80 μM $FeCl_3$). Relative difference in the migration between the native and oxidized DNA was ensured on 1% agarose gel electrophoresis after staining with ethidium bromide. Gels were documented (Herolab, Germany) and the intensity of the bands was determined (Easywin software). Protection to DNA was calculated based on the DNA band corresponding to that of native in the presence and absence of 1.5 and 3.0 μg of *Emblica officinalis* free phenolics (EOFP), *Emblica officinalis* bound phenolics (EOBP), *Curcuma longa* free phenolics (CLFP) and *Curcuma longa* bound phenolics (CLBP).

2.8. Characterization of free and bound phenolics by HPLC

The active phenolic components in free and bound fractions of *E. officinalis* and *C. longa* were characterized by HPLC (model LC-10A. Shimadzu Corporation, Tokyo, Japan) analysis on a reverse phase Shimpak C_{18} column (4.6 × 250 mm) using a diode array UV-detector (operating at 280 nm). A solvent system consisting of water:acetic acid:methanol (isocratic; 80:5:15, v/v) was used as mobile phase at a flow rate of 1 mL/min. In addition, a gradient solvent system was also used in order to resolve ascorbic acid and gallic and tannic acid peaks in the sample (solvent A—water: acetic acid [90:10, v/v] and solvent B—15% methanol; 0–100% gradient; run time—5 min; flow rate: 1.0 mL/min). Standard phenolic acids such as caffeic, *p*-coumaric, ferulic, gallic, gentisic, protocatechuic, syringic and vanillic acid along with ascorbic acid and curcumin were used for identification of phenolic components present in both free and bound phenolic fractions of *E. officinalis* and *C. longa*. The percentage contribution of antioxidant activity in various fractions of *E. officinalis* and *C. longa* including the earlier reported antioxidants, and the phenolic acids as confirmed by HPLC were determined.

2.9. Statistical analysis

All the experiments were carried out in triplicate ($n = 3$) and the results expressed as mean ± standard deviation (S.D.). The significance of difference was calculated by Student's *t*-test, and values <0.05 were considered to be significant. The correlations (R) between antioxidant activity and phenolic contents were calculated using Analyse-it software for Microsoft Excel.

3. Results

Different proportions of free and bound phenolics were observed in *E. officinalis* and *C. longa*. The free phenolic content of EOFP was 126.0 mg GAE/g dw, five-fold higher than that of CLFP (28.3 mg GAE/g dw) as measured by the Folin-Ciocalteu method. In addition, two-fold higher bound phenolics were observed in EOBP (3.0 mg GAE/g dw) in comparison with that of CLBP (1.6 mg GAE/g dw).

3.1. Antioxidant activities in various phenolic fractions of E. officinalis and C. longa

To evaluate the antioxidant activity of different phenolic fractions, the reducing power and free radical scavenging activity were analysed. The protective effect of phenolic fractions on DNA strand scission induced by oxidative stress was also examined.

Reducing power ability: Fig. 1 indicates a dose-dependent increase in the activity of free and bound phenolic fractions of *E. officinalis* and *C. longa*. As indicated in Fig. 1A, at >20 μg GAE sample extract of EOFP, saturation in the activity was observed. Activity was therefore compared at 10 μg GAE, where an approximately five-fold increase in activity was observed in EOFP over that of CLFP (Fig. 1B), which correlates well with the total phenolic concentration in EOFP (correlation coefficient $R = 0.74$, $P = 0.015$), suggesting that *E. officinalis* is a better source of phenolics which possesses better antioxidant activity than *C. longa*. This difference could be due to the constituent phenolic acid present in each. Similarly, EOBP also showed higher activity than CLBP. Free and bound phenolics thus have the ability to donate electrons to free radicals, converting them to more stable radicals and thus terminating the oxidative stress caused by a free radical chain reaction.

Free radical scavenging effects of free and bound phenolics of E. officinalis and C. longa: In order to substantiate the results of reducing power assays of *E. officinalis* and *C. longa*, the phenolic fractions were tested for their ability to scavenge free radicals. Table 1 shows the differential free radical scavenging ability of *E. officinalis* and *C. longa* in addition to standard phenolic acids as evaluated by scavenging of DPPH radicals by the test solution, which was monitored by the characteristic absorption of DPPH radical at 517 nm. Both EOFP and EOBP showed an IC_{50} of 0.65 and 0.85 μg/mL, respectively, as against that of 7.13 and 3.5 μg/mL of CLFP and CLBP. The standard antioxidants BHA and BHT exhibited an IC_{50} of 3.9 and 8.5 μg/mL, respectively. These results indicate that

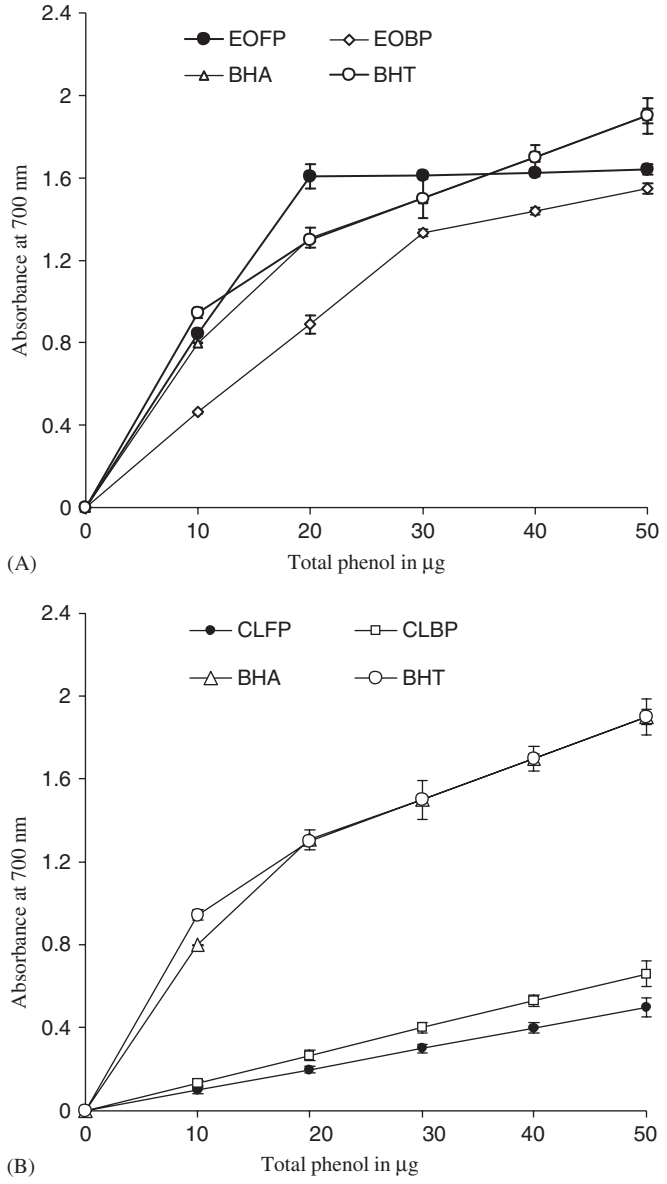

Fig. 1. Reducing power ability of free and bound phenolics of *Emblica officinalis* (A) and *Curcuma longa* (B).

Table 1
Comparative IC_{50} (concentration of sample or standard required to scavenge 50% of the DPPH free radicals) values of EOFP, EOBP, CLFP and CLBP compared with standard antioxidants (listed in ascending order)

Sample no.	Sample/ Standard	Standard	IC_{50} (in μg/mL)
1	Gallic acid	Std	0.45±0.01
2	EOFP	Samp	0.65±0.05
3	EOBP	Samp	0.85±0.04
4	Tannic acid	Std	0.99±0.04
5	Ascorbic acid	Std	1.05±0.05
6	Curcumin	Std	1.60±0.03
7	Ferulic acid	Std	2.40±0.07
8	CLBP	Samp	3.5±0.07
9	BHA	Std	3.9±0.05
10	CLFP	Samp	7.13±0.11
11	BHT	Std	8.5±0.18
12	Pro-coumaric acid	Std	31.25±0.31

Samp, sample; Std, standard.
Values are mean±standard deviation ($n = 3$).

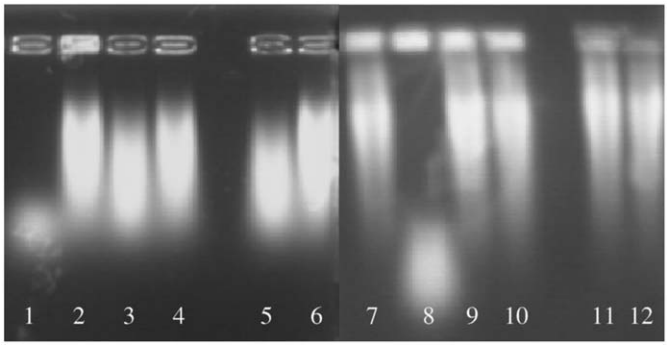

Fig. 2. DNA protection ability of free and bound phenolics of *Emblica officinalis* and *Curcuma longa*. Lanes 1 and 8—oxidized DNA, lanes 2 and 7—native DNA, Lanes 3 and 4—EOFP (1.5 and 3.0 μg), lanes 5 and 6—EOBP (1.5 and 3 μg), lanes 9 and 10—CLFP (1.5 and 3.0 μg), lanes 11 and 12—CLBP (1.5 and 3 μg).

E. officinalis phenolics are more potent than the standard antioxidants, while *C. longa* phenolic fractions showed activity comparable to that of BHA and BHT.

DNA protection activity of free and bound phenolics of E. officinalis and C. longa: Fig. 2 shows the DNA protective activity of *E. officinalis* and *C. longa*. The Fenton's reagent causing DNA fragmentation (as visualized by increased electrophoretic mobility of DNA) was recovered with the treatment of *E. officinalis* and *C. longa* phenolic extracts prior to oxidative stress. A dose-dependent protection was observed by both free and bound phenolics of *E. officinalis* and *C. longa* at 1.5 and 3.0 μg GAE. A significant (>80%, $P = 0.005$) protection to native DNA during oxidation in the presence of these fractions was observed. These results indicate that free and bound phenolics of *E. officinalis* and *C. longa* can quench the free radicals generated with the addition of Fenton's reagent, and they thereby protect the DNA against oxidative stress-induced damage.

Phenolic acids profile in free and bound phenolic fractions of E. officinalis and C. longa by HPLC analysis: HPLC protocol was standardized for the separation of phenolic acids, and the results indicated that—under isocratic elution condition—gallic acid, tannic acid and ascorbic acid could not be resolved; they yielded a peak at RT 2.36 min. To resolve these components, we optimized the condition of the run using the same C_{18} column but with the gradient run. In gradient run, ascorbic acid was separated with a wide range of retention time, i.e., 1.74 min relative to that of gallic acid and tannic acid, which eluted at 2.36 min. However, tannic acid and gallic

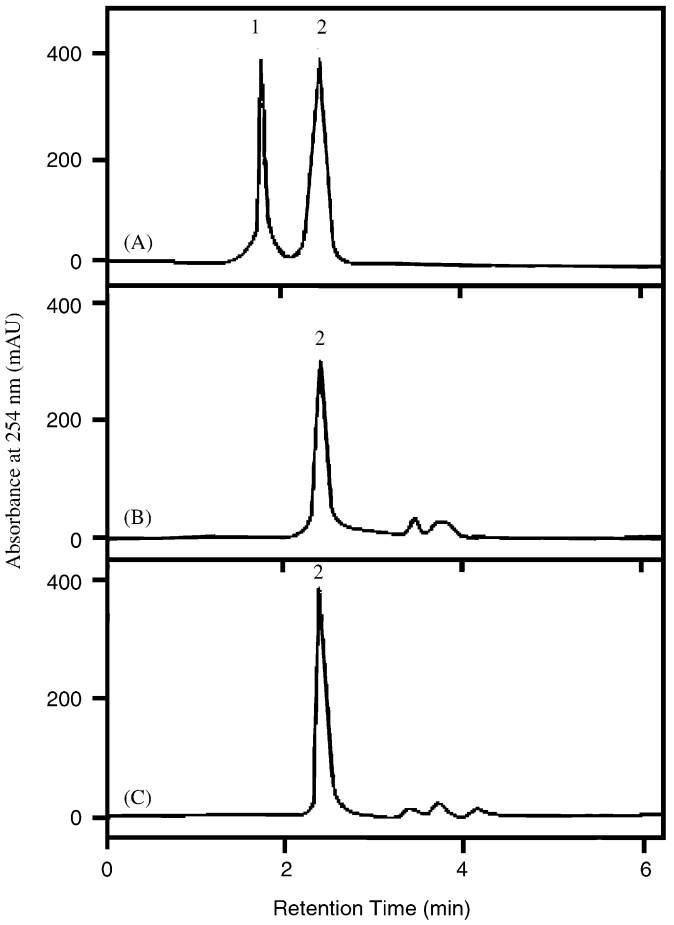

Fig. 3. HPLC profile of *Emblica officinalis* free and bound fractions compared with standards. (A) Standard ascorbic acid—1 (RT—1.74), tannic and gallic acid—2 (RT—2.360); (B) EOFP; (C) EOBP.

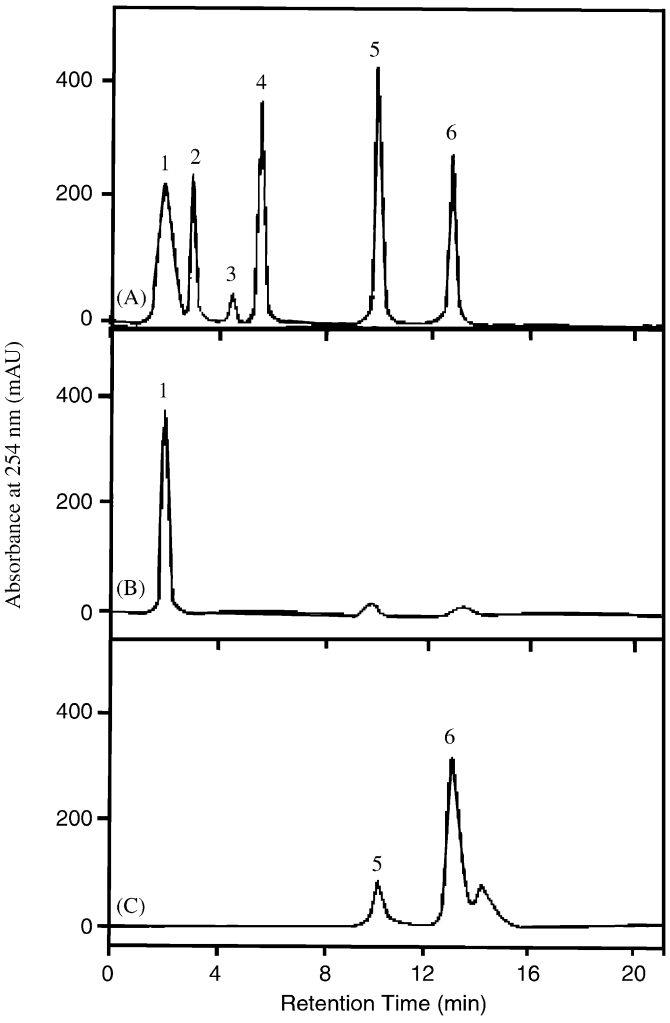

Fig. 4. HPLC profile of *Curcuma longa* free and bound fractions compared with standards. (A) Standard phenolic acids. 1—Standard curcumin (RT—1.92); 2—protocatechuic acid (RT—3.04); 3—gentesic acid (RT—4.49); 4—caffeic acid (RT—5.631); 5—*p*-coumaric acid (RT—10.024) and 6—ferulic acid (RT—12.874). (B) CLFP; (C) CLBP.

acid could not be separated with this condition. The elution profiles of both the phenolic fractions of *E. officinalis* showed a single peak, which coincided with the retention time of gallic and tannic acid peak indicating the presence of gallic acid and tannic acid as the major phenolic component. With this protocol it was evident that ascorbic acid was absent in *E. officinalis* phenolic fractions. Thus, antioxidant activity in *E. officinalis* in both EOFP (Fig. 3B) and EOBP (Fig. 3C) could be due to gallic and tannic acid only. This can also be substantiated by similar levels of free radical scavenging activity in EOFP and EOBP at GAE concentration of phenols. On the other hand, in the case of *C. longa*, at an equal concentration of phenol, CLBP showed at least a two-fold increase in activity over CLFP. This can be correlated to the presence of curcumin exclusively in CLFP (Fig. 4B), and the presence of *p*-coumaric acid (1.31 mg/g) and ferulic acid (0.29 mg/g) in CLBP (Fig. 4C). These results indicated the contribution of *p*-coumaric acid and ferulic acid in addition to curcumin for their antioxidant activity. These results were further confirmed (Table 1) by determining the free radical scavenging ability of standard curcumin, *p*-coumaric acid and ferulic acid where they exhibited an IC_{50} of 1.6, 31.25 and 2.4 μg/mL, respectively.

4. Discussion

In the present study, free and bound phenolic fractions of amla (*E. officinalis*) and turmeric (*C. longa*) have been investigated. Free and bound phenolics were isolated by differential extraction procedure, and antioxidant activity was evaluated by reducing power, DPPH scavenging ability and DNA protection against oxidative damage. Further, the active phenolic components present in each fraction were analysed to understand their contribution in addition to ascorbic acid and curcumin in *E. officinalis* and *C. longa*, respectively.

Determination of total phenol content as free and bound phenolics in *E. officinalis* and *C. longa* revealed that in *E. officinalis*, a major fraction (four-fold over bound form)

was contributed from free phenolic acids which is constituted by gallic and tannic acid, as revealed by HPLC (Fig. 3B). Further, the bound fraction also showed the same gallic and tannic acid (Fig. 3C) exhibiting equivalent activity at similar concentration of phenol. Hence, the results suggested that gallic and tannic acid may exist in free form, and that approximately 2% (dw) may be present in bound fraction and that this may be residual. However, the binding of the gallic and tannic acid to various other components of *E. officinalis* may not be ruled out, since these phenolic acids having higher amounts of hydroxyl group appear to bind to protein and carbohydrates as a result of their binding property (Fraizer et al., 2003; Wroblewski et al., 2001). Nevertheless, their bioavailability need not be questioned since the bound fraction can be released into circulation.

Our results indicated that gallic and tannic acid are the only phenolic acids present in both free and bound form in *E. officinalis* contributing to the antioxidant activity. Hence, it is possible that tannoid derivatives reported earlier (Bhattachary et al., 1999) may be constituted by enriched amount of gallic acid residues.

Further, it is very pertinent to address the question of the possibility of interference from ascorbic acid since only ascorbic acid has been reported to be the antioxidant component in *E. officinalis* (Khopde et al., 2001). In order to differentiate this, we prepared water extract of *E. officinalis* also in addition to free and bound phenolic extracts and analysed for the presence of ascorbic acid in each fraction by HPLC in addition to establishment of antioxidant activity. It was reported that both gallic acid and ascorbic acid were present in water extract (Duke, 1992). Ethyl acetate extracted EOFP contained a negligible amount of ascorbic acid and was totally absent in EOBP. These results indicate that the antioxidant activity of free and bound phenolic extracts of *E. officinalis* was contributed predominantly by phenolic acids. Further, *C. longa*, whose utility is well established in traditional medicine, was tested for the nature of phenolic acid content. Results indicated 4.4-fold higher antioxidant activity in *E. officinalis* compared to that of *C. longa*. Also, when phenolic acids were analysed in the free phenolic fraction only, curcumin was observed while proto-catechuic acid and ferulic acids were present in bound phenolic fraction. When antioxidant activity was measured at equal polyphenol concentrations, a different degree of antioxidant activity was found in *C. longa*. Results thus suggest that free phenolic fraction of *C. longa* contains only curcumin (3%, dw). Further differences in the antioxidant potential at equal concentration of phenolic content suggest that the two phenolic acids in the bound phenolic fraction might work at different potencies in the antioxidant assays. Evidently as indicated in Table 1, ferulic acid had a better antioxidant activity than *p*-coumaric acid. This warranted us to evaluate free radical scavenging activity of various phenolic acids and prior to this, their homogeneity was established by HPLC at equal phenol concentration. Therefore, in *C. longa* the predominant active phenolic antioxidant appears to be curcumin, which constitutes around 3% (dw), while in bound phenolics, *p*-coumaric and ferulic acid together constitutes to only 0.16% (dw).

Acknowledgements

The authors thank Dr V. Prakash, Director, CFTRI, for his keen interest in the work and encouragement. Authors are also thankful to Dr S.G. Bhat, Head, Department of Biochemistry and Nutrition, for his valuable suggestions. Mr. G. Suresh Kumar thanks University of Mysore for financial assistance.

References

Aggarwal, B.B., Kumar, A., Bharti, A.C., 2003. Anticancer potential of curcumin: preclinical and clinical studies. Anticancer Research 23, 363–398.

Ames, B.N., 1979. Identifying environmental chemicals causing mutations and cancer. Science 204, 587–593.

Bhattacharya, A.A., Chatterjee, A., Ghosal, S., Bhattacharya, S.K., 1999. Antioxidant activity of active tannoid principles of *Emblica officinalis* (amla). Indian Journal of Experimental Biology 37, 676–680.

Croft, K.D., 1998. The chemistry and biological effects of flavanoids and phenolic acids. Annals of the NewYork Academy of Sciences 854, 435–442.

Cuvrelier, M.E., Richard, H., Berset, C., 1992. Comparison of the antioxidant activity of some acid phenols, structure–activity relationship. Bioscience Biotehnology and Biochemistry 56, 324–325.

Doll, R., 1990. An overview of the epidemiological evidence linking diet and cancer. Proceedings of the Nutrition Society 49, 119–131.

Duke, J.A., 1992. Handbook of Phytochemical Constituents of GRAS Herbs and other Economic Plants. CRC Press, Boca Raton, FL.

Fraizer, R.A., Papadopoulou, A., Mueller-Harvey, I., Kisssoon, D., Green, R.J., 2003. Probing protein–tannin interactions by isothermal titration microcalorimetry. Journal of Agricultural and Food Chemistry 51, 5189–5195.

Jose, J.K., Kuttan, G., Kuttan, R., 2001. Antitumour activity of *Emblica officinalis*. Journal of Ethnopharmacology 75, 65–69.

Khopde, S.M., Indira Priyadarsini, K., Mohan, H., Gawandi, V.B., Satav, J.G., Yakhmi, J.V., Banavaliker, M.M., Biyani, M.K., Mittal, J.P., 2001. Characterization of the antioxidant activity of amla (*Phyllanthus emblica*) extract. Current Science 81, 185–191.

Lai, L.S., Chou, S.T., Chao, W.W., 2001. Studies on the antioxidant activities of Hsian-tsao (*Mesona procumbens* Hemsl) leaf gum. Journal of Agricultural and Food Chemistry 49, 963–968.

Nagabhushan, M., Bhide, S.V., 1986. Non-mutagenicity of curcumin and its antimutagenic action versus chili and capsaicin. Nutrition and Cancer—An International Journal 8, 201–210.

Nordkvist, E., Salomonsso, A., Aman, P., 1984. Distriubution of insoluble bound phenolic acids in barley grain. Journal of the Science of Food and Agriculture 35, 657–661.

Polasa, K., Sesikaran, B., Krishna, T.P., Krishnaswamy, K., 1991. Turmeric (*Curcuma longa*)-induced reduction in urinary mutagens. Food and Chemical Toxicology 29, 699–706.

Rodriguez, H., Akman, S., 1998. Mapping oxidative DNA damage at nucleotide level. Free Radical Research 29, 499–510.

Selvam, R., Subramanian, L., Gayathri, R., Angayarkanni, N., 1995. The anti-oxidant activity of turmeric (*Curcuma longa*). Journal of Ethnopharmacology 47, 59–67.

Sharma, N., Trikhaa, P., Athar, M., Raisuddin, S., 2000a. In vitro inhibition of carcinogen-induced mutagenecity by *Cassia occidentalis* and Emblica officinalis. Drug and Chemical Toxicology 23, 477–484.

Sharma, N., Trikha, P., Athar, M., Raisuddin, Aisuddin, S., 2000b. Inhibitory effect of *Emblica officinalis* on the in vivo clastogenicity of benzo[a]pyrene and cyclophosphamide in mice. Human & Experimental Toxicology 19, 377–384.

Subba Rao, M.V.S.S.T., Murlikrishna, G., 2002. Evaluation of the antioxidant properties of free and bound phenolic acid from native and malted finger millet (Ragi, Eleusine coracana Indaf-15). Journal of Agricultural and Food Chemistry 50, 889–892.

Temple, N.J., 2000. Antioxdants and disease. More questions than answers. Nutrition Research 20, 449–459.

Thakur, C.P., Thakur, B., Sinha, P.K., Sinha, S.K., 1988. The Ayurvedic medicines, Haritaki, Amla and Bahira reduce cholesterol induced atherosclerosis in rabbits. Indian Journal of Cardiology 21, 167–172.

Thompson, L.U., 1994. Antioxidant and hormone-mediated health benefits of whole grains. Critical Reviews in Food Science and Nutrition 34, 473–497.

Tripathi, S.N., Tiwari, C.M., Upadyay, B.N., Singh, R.S., 1979. Screening of hypoglycemic action in certain indigenous drugs. Journal of Research in Indian Medicine Yoga and Homoepathy 14, 159.

Willett, W.C., 2002. Balancing life-style and genomics research for disease prevention. Science 296, 695–698.

Wroblewski, K., Muhandiram, R., Chakrabartty, A., Bennick, A., 2001. The molecular interaction on human salivary histatins with polyphenolic compounds. European Journal of Biochemistry 268, 4384–4397.

Yen, G.C., Chen, H.Y., 1995. Antioxidant activity of various tea extracts in relation to their antimutagenicity. Journal of Agricultural and Food Chemistry 43, 27–32.

Yu, Z.F., Kong, L.D., Chen, Y., 2002. Antidepressant activity of aqueous extracts of *Curcuma longa* in mice. Journal of Ethnopharmacology 83, 161–165.