Reactivity of phenolic compounds towards free radicals under in vitro conditions

ARTICLE *in* JOURNAL OF FOOD SCIENCE AND TECHNOLOGY -MYSORE- · JANUARY 2015

Impact Factor: 2.2 · DOI: 10.1007/s13197-014-1704-0

3 AUTHORS, INCLUDING:

Sindhu Mathew
Lund University
17 PUBLICATIONS **775** CITATIONS
SEE PROFILE

Zainul Akmar Zakaria
Universiti Teknologi Malaysia
39 PUBLICATIONS **261** CITATIONS
SEE PROFILE

J Food Sci Technol
DOI 10.1007/s13197-014-1704-0

ORIGINAL ARTICLE

Reactivity of phenolic compounds towards free radicals under in vitro conditions

Sindhu Mathew · T. Emilia Abraham · Zainul Akmar Zakaria

Revised: 8 December 2014 / Accepted: 26 December 2014

Abstract The free radical scavenging activity and reducing power of 16 phenolic compounds including four hydroxycinnamic acid derivatives namely ferulic acid, caffeic acid, sinapic acid and p-coumaric acid, benzoic acid and its derivatives namely protocatechuic acid, gallic acid and vanillic acid, benzene derivatives namely vanillin, vanillyl alcohol, veratryl alcohol, veratraldehyde, pyrogallol, guaiacol and two synthetic antioxidants, butylated hydroxy anisole (BHA) and propyl gallate were evaluated using 1,1-Diphenyl-2-picrylhydrazyl radical ($DPPH^{\bullet}$), 2,2′-Azinobis-3-ethylbenzothiazoline-6-sulfonic acid radical ($ABTS^{+\bullet}$), Hydroxyl radical ($^{\bullet}OH$) and Superoxide radical ($O_2^{\bullet-}$) scavenging assays and reduction potential assay. By virtue of their hydrogen donating ability, phenolic compounds with multiple hydroxyl groups such as protocatechuic acid, pyrogallol, caffeic acid, gallic acid and propyl gallate exhibited higher free radical scavenging activity especially against $DPPH^{\bullet}$ and $O_2^{\bullet-}$. The hydroxylated cinnamates such as ferulic acid and caffeic acid were in general better scavengers than their benzoic acid counter parts such as vanillic acid and protocatechuic acid. All the phenolic compounds tested exhibited more than 85 % scavenging due to the high reactivity of the hydroxyl radical. Phenolic compounds with multiple hydroxyl groups also exhibited high redox potential. Exploring the radical scavenging and reducing properties of antioxidants especially those which are found naturally in plant sources are of great interest due to their protective roles in biological systems.

Keywords Free radical scavenging · Antioxidant activity · Phenolic compounds · Reducing power

Abbreviations

Prot	Protocatechuic acid
Guai	Guaiacol
Caff	Caffeic acid
Feru	Ferulic acid
Coum	p-Coumaric acid
Gall	Gallic acid
Vaci	Vanillic acid
Sina	Sinapinic acid
Prop	Propyl gallate
Veald	Veratraldehyde
BHA	Butylated hydroxy anisole
Veal	Veratryl alcohol
Valc	Vanillyl Alcohol
Pyro	Pyrogallol
Benz	Benzoic acid
Vani	Vanillin

Introduction

The protective effects of diets, rich in vegetables, fruits and cereals against various forms of cancer and cardiovascular diseases (De Ancos et al. 2000; Nicodemus et al. 2001) have been attributed, in large to the antioxidant nutrients and plant phenolics such as flavonoids, benzoic acid derivatives and

S. Mathew · T. E. Abraham
Chemical Science and Technology Division, National Institute of Interdisciplinary Science and Technology (NIIST, CSIR), Trivandrum 695019, Kerala, India

Z. A. Zakaria
Institute of Bioproduct Development, Universiti Teknologi Malaysia, 81310 Johor Bahru, Johor, Malaysia

Present Address:
S. Mathew (✉)
Institute of Bioproduct Development, Universiti Teknologi Malaysia, 81310 Johor Bahru, Johor, Malaysia
e-mail: sindhumathew@hotmail.com

Published online: 08 January 2015

phenylpropanoids (Lin et al. 2014; Pacifico et al. 2014; Hermann 1989). In addition to t2he free radical scavenging capacity (Lucini et al. 2015; Heo et al. 2014) plant phenolics have multiple biological activities including vasodilatory, anticarcinogenic, anti-inflammatory, antibacterial, immune stimulating, anti-allergic, antiviral and estrogenic effects (Heo et al. 2014; Crozier et al. 2009; Kumbulainen and Salonen 1999). There are different mechanisms of antioxidative action, for example, radical scavenging, recombination of radicals, chelation of transition metal ions, or electron transfer with formation of stable products (Haseloff et al. 1990).

The current tendency is to replace synthetic phenols like Butylated hydroxyanisole (BHA), Butylated hydroxyl toluene (BHT) etc. with phenolic compounds extracted from natural sources with comparable antioxidant power and better safety attributes (Moure et al. 2001). The antioxidant power of caffeic acid for example was well substantiated scientifically (Yanishlieva and Marinova 1995) and was found to have better stabilizing effect than BHA on the thermal oxidation of cod liver oil (Leonardis and Macciola 2003).

The antioxidant activity of phenolic compound is reasonably related to their structure, the substitutions on the aromatic ring and the structure of the side chain (Shahidi and Wanasundara 1992). The structure-antioxidant activity relationship (SAR) approach is considered to be useful for food, cosmetic and pharmaceutical applications as it provides evidence about the potency of natural phenolic constituents (Bountagkidou et al. 2010). Bilto et al. (2012), Sivakumar et al. (2011), Bountagkidou et al. (2010), Natella et al. (1999) and Torres de Pinedo et al. (2007) studied the SAR of flavonoids, chalcone derivatives, hydroxybenzaldehydes, benzoic and cinnamic acids and phenolic based antioxidants respectively employing different antioxidant assay methods. Cai et al. (2006) studied the SAR of phenolic antioxidants from Chinese medicinal plants using ABTS and DPPH radical scavenging assays. Kikuzaki et al. (2002) reported the scavenging ability of hydroxycinnamic acids towards DPPH radical. However, their studies were focused on ferulic acid related compounds, its alkyl esters and gallic acid esters. Zhang et al. (2006) compared the free radical scavenging activity of hydroxycinnamic acids namely caffeic, ferulic and sinapic acid. But they employed the oxygen radical absorbance capacity (ORAC) and lipid peroxide inhibition capacity (LPIC) assays. Pino et al. (2006) evaluated the capacity of hydoxycinnamic acids to trap peroxyl radicals by using oxygen radical absorbance capacity (ORAC) indexes. Haseloff et al. (1990) studied the in vitro hydroxyl radical scavenging activity of benzoic acid esters and propyl gallate. In some cases there has been discrepancy in the findings obtained from different studies due to the differences in the assay conditions and assay methods followed. Investigations on the SAR will improve the understanding of the relationship between antioxidant activity and chemical structure of many representative phenolic compounds found naturally in plant sources. In this study, we have attempted to evaluate the free radical quenching capacity using ABTS, DPPH, Hydroxyl and Superoxide radical as well as the reducing power of 16 phenolic compounds including the aldehyde and alcohol derivatives of benzene which are often reported to be co-extracted with the corresponding acids from natural sources (Robbins 2003), derivatives of benzoic acid, hydroxycinnamic acid and phenol and tried to compare them with synthetic antioxidants like BHA and propyl gallate.

Materials and methods

Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH$^{\bullet}$), 2,2′-Azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), Phenazine methosulphate (PMS), β-Nicotinamide adenine dinucleotide (NADH), 3,4-Dihydroxy cinnamic acid (Caffeic acid), 4-Hydroxy cinnamic acid (p-Coumaric acid), 3,5 Dimethoxy-4-hydroxy cinnamic acid (Sinapic acid), 4-Hydroxy-3-methoxy cinnamic acid (Ferulic acid), 3,4-Dimethoxy benzyl alcohol (Veratryl alcohol) and 3,4-Dihydroxy benzoic acid (Protocatechuic acid) were obtained from Sigma (MO,USA). 4-Hydroxy 3-methoxy benzaldehyde (Vanillin) was purchased from Sigma Aldrich Chemical Co. (Milwaukee, WI). 4-Hydroxy 3-methoxy benzyl alcohol (Vanillyl alcohol) was purchased from Lancaster, Morecambe, UK and 1, 2, 3-Trihydroxybenzene (Pyrogallol) from BDH, Lab Supplies, Poole, UK. Butylated hydroxyanisole, n-Propyl 3, 4, 5 trihydroxy benzoate (Propyl gallate) and Benzoic acid were purchased from SD Fine Chemicals (India). Thiobarbituric acid (TBA) and 3,4 Dimethoxy benzaldehyde (Veratraldehyde) were obtained from CDH (India), while Deoxyribose, Nitroblue tetrazolium (NBT), Trichloroacetic acid (TCA), Pottasium persulphate, 2-Methoxy phenol (Guaiacol), 4-hydroxy 3-methoxy benzoic acid (Vanillic acid) and Gallic acid were purchased from Sisco Research Lab (India). All the other chemicals used were of standard analytical grade.

Evaluation of free radical scavenging property and reducing power

DPPH free radical scavenging assay

The antioxidant activity of 16 phenolic compounds were assessed based on their ability to scavenge DPPH$^{\bullet}$ using the method of Brand-Williams et al. (1995). The method was slightly modified as reported by Mathew and Abraham (2006). Methanolic solutions (0.1 ml) of the standard

compounds were used at a concentration of 15 μM and added to 2.9 ml of 60 μM $DPPH^{\bullet}$. $DPPH^{\bullet}$ gets reduced and the resulting decrease in absorbance at 517 nm was recorded up to 30 min. The concentration of $DPPH^{\bullet}$ scavenged was determined using a calibration curve derived by linear regression. The percentage of $DPPH^{\bullet}$ scavenged was calculated using the formula % Scavenging=$(DPPH^{\bullet})_{T=0}/(DPPH^{\bullet})_T$ x100 where $(DPPH^{\bullet})_{T=0}$ is the concentration at zero time (initial concentration) and $(DPPH^{\bullet})_T$ refers to the concentration of the radical at respective time intervals.

ABTS radical cation decolorisation assay

Radical scavenging ability of the phenolic compounds were evaluated against $ABTS^{+\bullet}$ and measured spectrophotometrically at 734 nm (Re et al. 1999). The $ABTS^{+\bullet}$ solution was prepared by reaction of aqueous solution of ABTS (7 mM) with potassium persulfate as an oxidizing agent (final concentration of 2.45 mM). Methanolic solutions of the standard compounds were used at a concentration of 10 μM and added to $ABTS^{+\bullet}$ diluted to an absorbance of 0.7±0.05. The radical scavenging capacity was calculated using the formula

$$\% \text{ Scavenging} = [(AB_0 - AB_1)/AB_0 \text{x } 100]$$

where AB_0 is the absorbance of the control and AB_1 is the absorbance in the presence of the test sample. All assays were carried out in triplicate.

Hydroxyl ($OH^{\bullet}$) radical scavenging activity

The hydroxyl radicals were generated by the deoxyribose method (Halliwell et al. 1987) modified according to Mathew and Abraham (2006). The reaction mixture containing the standard compounds (25 μM), deoxyribose (3.75 mM), H_2O_2 (1 mM), Potassium phosphate buffer (20 mM, pH 7.4), $FeCl_3$ (0.1 mM), EDTA (0.1 mM) and Ascorbic acid (0.1 mM) were incubated in a water bath at 37±0.5 °C for 1 h. This was then followed by the addition of 1 ml of TBA (1 %*w*/*v*) and 1 ml of TCA (2.8 %*w*/*v*) and subjected to heating in a water bath at 100 °C for 20 min. The extent of deoxyribose degradation was determined from the absorbance of the resulting solution at 532 nm and measured using the formula

$$\% \text{ Inhibition} = [(AB_0 - AB_1)/AB_0 \text{x } 100]$$

where AB_0 is the absorbance of the control and AB_1 is the absorbance in the presence of the test sample. All assays were carried out in triplicate.

Superoxide anion scavenging activity

Superoxide radicals were generated by adding PMS (50 μM) to Tris–HCl buffer (pH 8.0) containing NBT (50 μM) and NADH (78 μM) and incubating at 25 °C for 5 min (Mathew and Abraham 2006). Standard compounds were added to the reaction mixture at a concentration of 15 μM. The absorbance recorded at 560 nm was a measure of the reduction of NBT and the radical scavenging activity was calculated as in the case of hydroxyl radical.

Reductive potential

The ability of the compounds to reduce ferricyanide complex to ferrous form were determined at a concentration of 500 μM according to the method reported by Mathew and Abraham (2006). The standard compounds in 1 ml of distilled water were mixed with phosphate buffer (pH 6.6) and potassium ferricyanide (1 %*w*/*v*) and incubated at 50 °C for 20 min. Trichloroacetic acid (10 %*w*/*v*) was added to the mixture, and centrifuged for 10 min at 1000*g*. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1 %*w*/*v*), and the absorbance was measured at 700 nm in a spectrophotometer. Higher absorbance of the reaction mixture indicated greater reductive potential. In the reductive potential assay, an easily reduced oxidant (Fe^{3+}) is used in stoichiometric excess and the phenolic compounds are supposed to act as reductants (Gul et al. 2011).

Statistical analyses

The statistical analyses of the data were performed using Microsoft excel 2003 and SPSS 16. The one way analysis of variance (ANOVA) and the significance of differences between sample means were calculated by Duncan's multiple range test using SPSS for Windows, IBM SPSS Statistics 16, Chicago, IL. *P* values≤0.05 were regarded as significant.

Results and discussion

The 16 compounds studied differ in the pattern of hydroxylation, methoxylation and substituents on their aromatic ring (Fig. 1). The phenolic compounds differed in their abilities to react with and quench $OH^{\bullet}$, $O_2^{\bullet+}$, $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ and reducing power. Monophenols were found to be less efficient than the polyphenols and the number and position of hydroxyl and methoxy groups in the phenolic rings determined the radical scavenging potential. The aldehyde and alcohol derivatives of benzene in general showed lower antioxidant activity than the benzoic acid derivatives which in turn exhibited lower activity than the hydroxycinnamic acid derivatives. In the

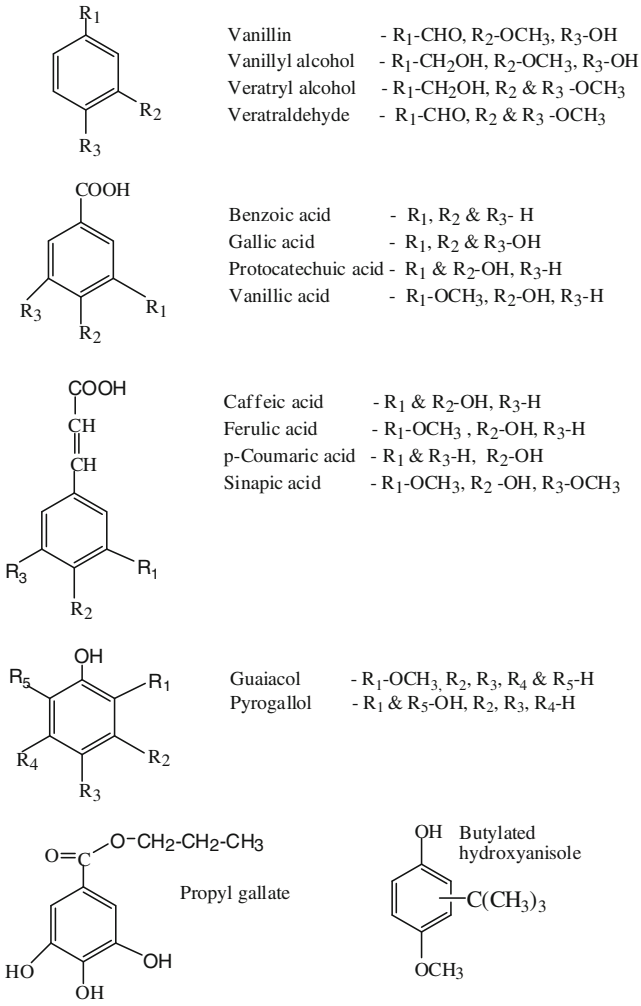

Fig. 1 Structure of the phenolic compounds assayed for free radical scavenging

DPPH radical scavenging assay, the phenolic compounds with multiple hydroxyl groups namely protocatechuic acid, pyrogallol, caffeic acid, gallic acid and propyl gallate exhibited higher scavenging capacity than the synthetic antioxidant BHA. A similar pattern was observed in the superoxide radical scavenging and redox potential capacity as well. However, BHA exhibited negligible superoxide radical scavenging capacity under the assay conditions.

DPPH$^{\bullet}$ scavenging activity

Phenolic compounds which possess antioxidant property can quench DPPH$^{\bullet}$ by providing hydrogen atoms or by electron donation, via a free-radical attack on the DPPH molecule and converting them to a colorless/bleached product (i.e., 2, 2-diphenyl-1-hydrazine, or a substituted analogous hydrazine) a stable diamagnetic molecule (Matthaus 2002) which result in decrease in the absorbance at 517 nm. The DPPH$^{\bullet}$ scavenging efficiency of the phenolic compounds were in the order: Caff > Prot > Gall > Prop > Pyro > BHA > Sina > Feru > Valc > Guai > Vaci > Coum > Benz > Veald > Vani > Veal (Fig. 2a) with negligible activity for vanillin and veratryl alcohol at the concentration studied. Vanillin was found to be a poor scavenger of DPPH$^{\bullet}$ and exhibited lower activity than its acid analogue, vanillic acid. The presence of –CHO moiety instead of a –COOH moiety was found to hinder the activity under the assay conditions (Bountagkidou et al. 2010). Caffeic acid scavenged 89.4 % of DPPH$^{\bullet}$ while veratryl alcohol scavenged only 0.38 % of the DPPH$^{\bullet}$ at 15 μM. From the above order of scavenging efficiency exhibited by the different compounds it can be concluded that the hydroxylated cinnamates in general are more effective than their benzoic acid counterparts (ferulic acid >vanillic acid; caffeic acid > protocatechuic acid). This can be explained in terms of the bulky -CH=CH-COOH group, which increases the activity by stabilizing the resultant phenoxy radicals (Yamagami et al. 2005). The double bond also participates in stabilizing the radicals of cinnamic acid derivatives by resonance (Cuvelier et al. 1992; Natella et al. 1999). Moreover, the electron withdrawing properties of the carboxylate group in benzoic acid has a negative influence on its H-donating ability and thereby on its scavenging ability. Vanillic acid therefore has a lower antioxidant activity than ferulic acid due to the adjacency of the carboxylate groups to the phenyl ring. The presence of –CH=CH-COOH groups in cinnamic acid ensures greater hydrogen donating ability and subsequent radical stabilization than the COO^{-} group in benzoic acid derivatives. Brand Williams et al. (1995) and von Gadow et al. (1997) had shown that p-coumaric acid and vanillic acid react poorly with DPPH$^{\bullet}$ and their hydrogen donating ability was reported to be lower than that of ferulic acid and BHA. Sinapic acid with methoxy groups at meta positions and hydroxyl group in para position had higher scavenging activity (50.4 %) than ferulic acid (39.5 %) with a single methoxy substitution at meta position. Electron delocalization and electron donation of an unshared pair of electrons from OCH_3 in the p-orbital stabilizes the phenoxyl radical (Velkov et al. 2007). More electron-donating substituents facilitate phenoxyl radical formation, and hydroxyl group substituents should be effective in stabilizing the resultant phenoxyl radicals.

Gallic acid and propyl gallate exhibited high DPPH$^{\bullet}$ scavenging activities (88.5 and 88.1 % respectively) which can be attributed to the multiple hydroxyl substitution pattern which is commonly regarded as important for the radical scavenging activities of phenolic acids (Cai et al. 2006). The presence of electron donating or high electron density hydroxyl group at meta and para positions of gallic acid attributes to the high scavenging ability of gallic acid (Jing et al. 2012). Vanillyl alcohol

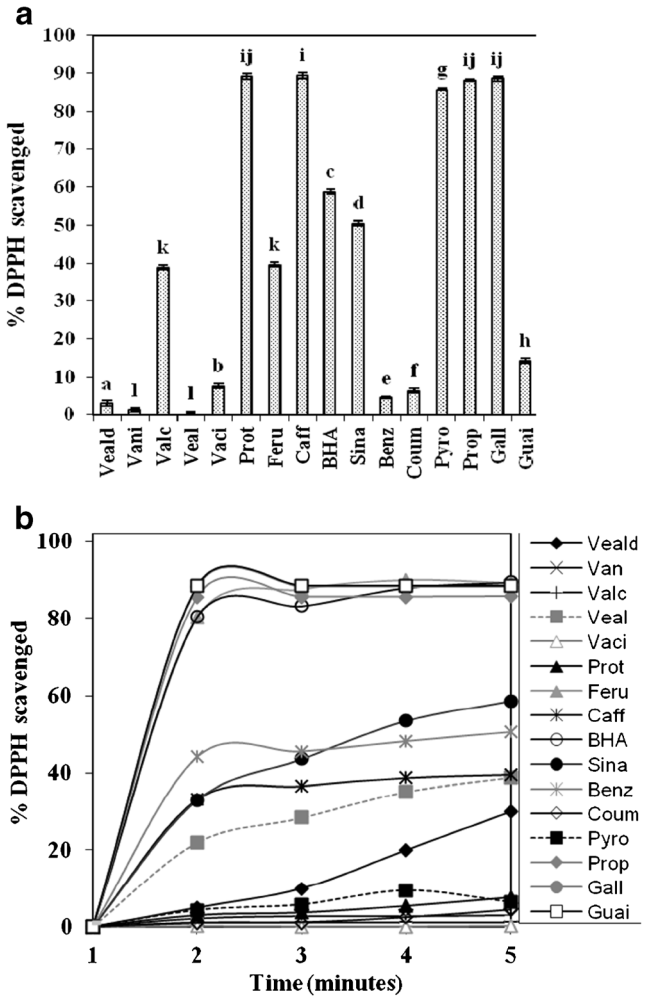

Fig. 2 **a** DPPH radical scavenging activity of the phenolic compounds. Bars with different letters are significantly different at $p \leq 0.05$. **b** Time profile studies of the DPPH radical scavenging activity of phenolic compounds

exhibited higher scavenging activity (38.9 %) than veratryl alcohol (0.38 %) due to para-hydroxy substitution.

Vanillin, at a concentration of 1 mM was shown to exhibit 22.9 % DPPH$^{\bullet}$ scavenging activity (Kumar et al. 2002) which is comparable to the results obtained in this study (Fig. 2a). The antioxidant activity of phenolic acids and their esters depend partly on the number of hydroxyl groups in the molecule (Dziedzic and Hudson 1983) which explains the high DPPH$^{\bullet}$ scavenging activity of caffeic acid, gallic acid, protocatechuic acid, propyl gallate and pyrogallol with two to three hydroxyl groups. Substitution with a hydroxyl group was found to be more effective (Marinova and Yanishlieva 1992) than methoxy group (Protocatechuic acid > vanillic acid; caffeic acid > ferulic acid). Kikuzaki et al. (2002) also have reported similar order of scavenging ability towards DPPH radicals: caffeic acid > sinapic acid > ferulic acid > p-coumaric acid.

Time profile studies (Fig. 2b) showed that propyl gallate, pyrogallol, gallic acid, caffeic acid and protocatechuic acid exhibit a faster reaction rate especially in the first 5 min compared to vanillyl alcohol, ferulic acid, BHA and sinapic acid with intermediate reaction rate. Veratryl alcohol, vanillin, veratralaldehyde, benzoic acid, guaiacol, vanillic acid and p-coumaric acid exhibited much slower radical quenching reaction. In most of the cases, the phenolic compounds exhibited two phases in their interaction with the radical, the first phase being rapid (first 5 min) followed by a slower phase in the next 25 min to approach steady state condition.

$ABTS^{+\bullet}$ scavenging activity

In the $ABTS^{+\bullet}$ scavenging assay, the efficiency of scavenging was in the order: Pyro > Gall > Feru > Prop > Caff > BHA > Guai > Prot > Valc > Sina > Coum > Vaci. Veald, Van, Benz and Veal exhibited low scavenging activity (Fig. 3a). Pyrogallol scavenged 99 % of $ABTS^{+\bullet}$ while veratraldehyde scavenged only 0.85 % of the radical at 10 μM. The trihydroxybenzoic acid derivatives, gallic acid and propyl gallate showed very good scavenging activity. For phenolic acids, it was demonstrated that compounds presenting a pyrogallol unit (three hydroxyl groups on the phenol ring) exhibited higher antioxidant properties than those presenting a catechol unit (two hydroxyl groups on the phenolic ring) (Siquet et al. 2006). Ferulic acid is more effective than p-coumaric acid due to the presence of the OCH_3 group in position ortho to the hydroxyl group. The electron donating methoxy group in ferulic acid allows increased stabilization of the resulting aryloxy radical through electron delocalization after hydrogen donation by the hydroxyl group (Pokorny 1987). However caffeic acid exhibited lower scavenging than ferulic acid. This result is consistent with the finding obtained in previous study where caffeic acid was reported to have lower activity than ferulic acid (Cai et al. 2006). Ortho-substitution with electron donating alkyl group in BHA and electron donating methoxy group in Guaiacol increases the stability of the aryloxy radical and in turn its antioxidant potential as well (Rice Evans et al. 1996).

The compounds exhibited a biphasic behavior with a rapid interactive phase with the radical in the first 1 min followed by a relatively slower phase (Fig. 3b). According to Nenadis et al. (2004) $ABTS^{+\bullet}$ assay may give an indication of the presence of antioxidants in a certain system but structure activity relationship (SARs) cannot be readily inferred.

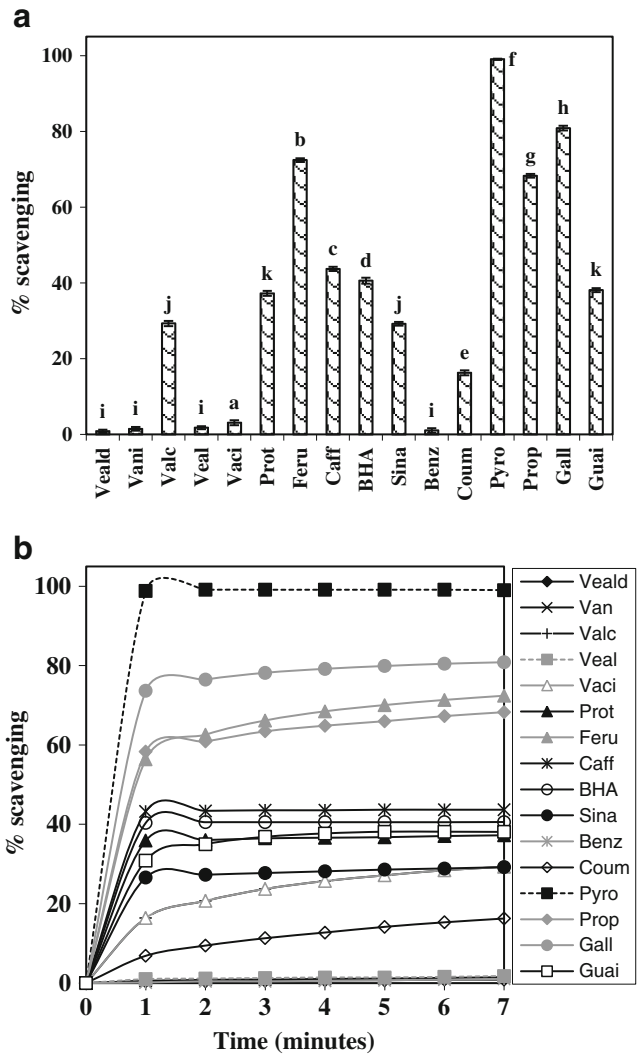

Fig. 3 **a** ABTS radical scavenging activity of the phenolic compounds. Bars with different letters are significantly different at $p \leq 0.05$. **b** Time profile studies of the ABTS radical scavenging activity of phenolic compounds

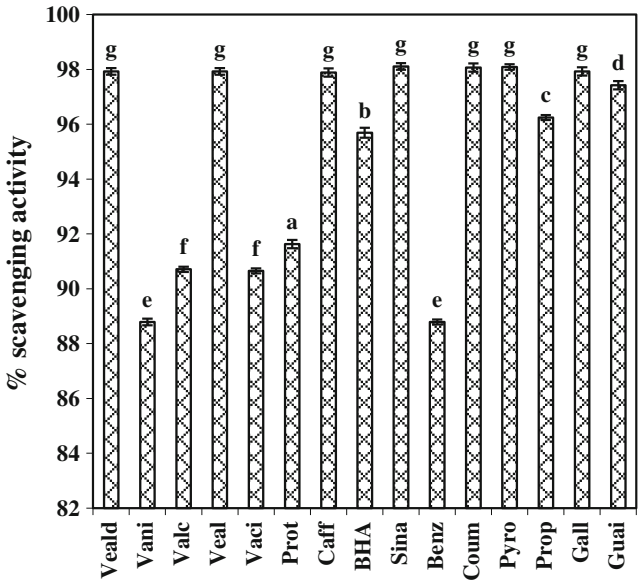

Fig. 4 Hydroxyl radical scavenging activity of the phenolic compounds. Bars with different letters are significantly different at $p \leq 0.05$

Hydroxyl radical scavenging

Hydroxyl radicals are the most destructive reactive oxygen species in food and biological systems. Due to their relatively short in vivo half-life, they can induce oxidative damage to almost all important biomolecules, including polyunsaturated fatty acids, nucleic acids, amino acids and proteins (Lipinski 2011).

The hydroxyl radical scavenging efficiency of the tested compounds were found to be in the order Sina > Pyro > Coum > Veald = Veal = Gall > Caff > Guai > Prop > BHA > Prot > Valc > Vaci > Vani = Benz (Fig. 4). Even the hydroxybenzoates are effective hydroxyl radical scavengers (Grootveld and Halliwell 1986) due to their propensity of hydroxylation and the high reactivity of the hydroxyl radical. The increase of methoxy groups substantially increased the antioxidant activity of the compounds by further stabilising the phenoxyl radical. Our studies confirmed that the antioxidant efficiency of monophenols are strongly enhanced by the introduction of a second hydroxy group and is increased by one or two methoxy substitutions in positions ortho to the OH group as in the case of sinapic acid. Liu and Mori (1993) showed that vanillic acid has superoxide and hydroxyl radical scavenging activities, which assist in terminating lipid peroxidation. Vanillyl alcohol has been shown to play a critical role in the free radical scavenging activities of *Gastrodia*, a traditional Chinese herb (Hsieh *et al*. 2000).

Superoxide radical scavenging

In this method, the superoxide anion reduces the yellow dye (NBT) to produce the blue formazan which is measured spectrophotometrically at 560 nm. The redox potentials of hydroxycinnamic acid derivatives are lower than that of oxy-radicals such as the hydroxyl radical $E_7 = 1.9$ V, superoxide radical anion $E_7 = 0.94$ V (Wardman 1989) which means that they are excellent scavengers of these oxy radicals. Compounds that possess more than one hydroxyl group in their aromatic ring like propyl gallate, gallic acid, caffeic acid, pyrogallol and protocatechuic acid exhibited stronger inhibitory potency than monohydroxyl substituents like p-coumaric acid and ferulic acid (Fig. 5). The O_2^- radical scavenging efficiency of the compounds was in the order: Prop >

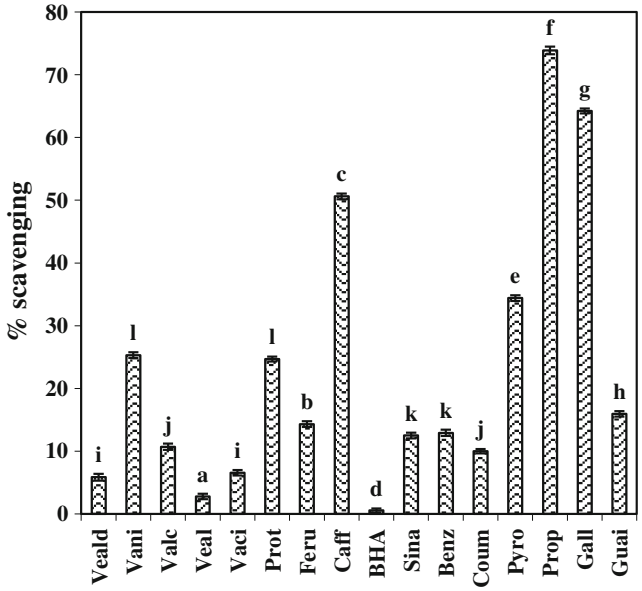

Fig. 5 Superoxide radical scavenging activity of the phenolic compounds. Bars with different letters are significantly different at $p \leq 0.05$

Gall > Caff > Pyro > Vani > Prot > Guai > Feru > Benz > Sina > Valc > Vaci > Veald > Veal. BHA exhibited negligible activity. Torres de Pinedo et al. (2007) reported that monophenolic compounds such as vanillyl alcohol exhibited better radical inhibition capacity than veratryl alcohol with both hydroxyl groups methoxylated. Protocatechuic acid and ferulic acid identified as active components in *Pleurotus tuber*, an edible mushroom has been reported to exhibit antioxidant and antiangiogenic properties (Lin et al. 2014).

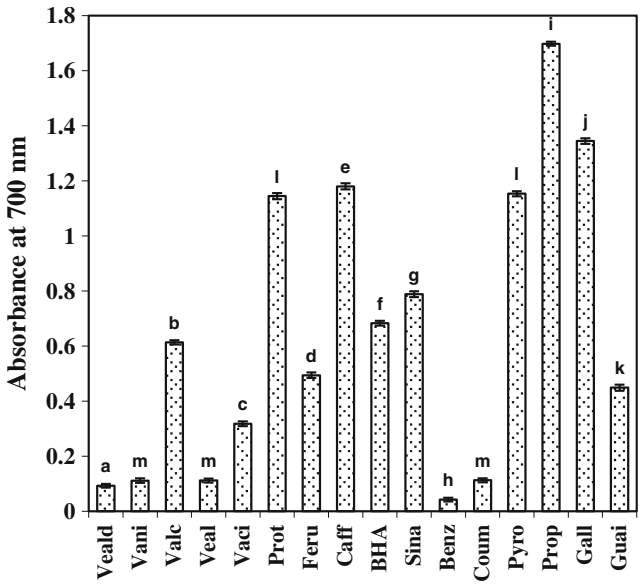

Fig. 6 Reducing power of the phenolic compounds. Bars with different letters are significantly different at $p \leq 0.05$

Reducing Power

In this assay, the yellow color of the test solution changes to various shades of green and blue, depending on the reducing power of each compound. The presence of reductants causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. Therefore, measuring the formation of Perl's Prussian blue at 700 nm helps to monitor the Fe^{2+}concentration. The reducing potential of the compounds was found to be in the order: Prop > Gall > Caff > Pyro > Prot > Sina > BHA > Valc > Feru > Guai > Vaci > Coum. Veal, Van, Veald and Benz exhibited very low reducing power (Fig. 6). The redox potentials of hydroxycinnamic acid derivatives are dependent on the electron donating property of the substituents in the benzene ring (Teixeira et al. 2013). Propyl gallate and gallic acid are very strong reducing agents, owing to the presence of three hydroxyl groups. The antioxidant efficiency of monophenols is increased substantially by one or two methoxy substitutions at the o-position relative to the hydroxyl (Vafiadis and Bakalbassis 2003; Cuvelier et al. 1992) which explains the reducing power in the order Sina > Feru > Coum.

Conclusion

In conclusion the results obtained in the present study have shown that out of the sixteen compounds studied except vanillin and benzoic acid most of the compounds can effectively scavenge free radicals of one type or the other including superoxide anion, hydroxyl radical and other free radicals under in vitro conditions. The basic structural organization of the phenolics determines the antioxidant activity. The substituents on the phenyl ring and the conjugated carbon skeleton play an important role in the antioxidant property of the phenolic compounds. Most of these phenolic compounds are active components in fruits, vegetables, medicinal plants and spices and serve as nutraceuticals and functional food components.

Acknowledgments The authors are thankful to the Director, NIIST (CSIR), India for providing the facilities for carrying out this research work. The first author acknowledges CSIR, India for the Research Fellowship and Universiti Teknologi Malaysia (UTM) for the Postdoctoral Fellowship.

References

Bilto YY, Suboh S, Aburjai T, Abdalla S (2012) Structure activity relationships regarding the antioxidant effects of the flavonoids on human erythrocytes. Natural Sci 4:740–747

Bountagkidou OG, Ordoudi SA, Tsimidou MZ (2010) Structure-antioxidant activity relationship study of natural

hydroxybenzaldehydes using in vitro assays. Food Res Int 43:2014–2019

Brand-Williams W, Cuvelier ME, Berset C (1995) Use of free radical method to evaluate antioxidant activity. Lebensm Wiss Technol 28:25–30

Cai YZ, Mei S, Jie X, Luo Q, Corke H (2006) Structure-radical scavenging activity relationships of phenolic compounds from traditional Chinese medicinal plants. Life Sci 78:2872–2888

Crozier A, Jaganath IB, Clifford MN (2009) Dietary phenolics: chemistry, bioavailability and effects on health. Nat Prod Rep 26:1001–1043

Cuvelier ME, Richard H, Berset C (1992) Comparison of the antioxidant activity of some acid phenols: structure-activity relationship. Biosci Biotechnol Biochem 56:324–325

De Ancos B, Gonzàlez EM, Cano MP (2000) Ellagic acid, vitamin C and total phenolic contents and radical scavenging capacity affected by freezing and frozen storage in raspberry fruit. J Agric Food Chem 48:4565–4570

Dziedzic SZ, Hudson BJF (1983) Polyhydroxychalcones and flavanones as antioxidants for edible foods. Food Chem 12:205–212

Grootveld M, Halliwell B (1986) Aromatic hydroxylation as a potential measure of hydroxyl radical formation in vivo. Biochem J 237:499–504

Gul MZ, Bhakshu LM, Ahmad F, Kondapi AK, Qureshi IA, Ghazi IA (2011) Evaluation of *Abelmoschus mosch*atus extracts for antioxidant, free radical scavenging, antimicrobial and antiproliferative activities using invitro assays. BMC Complement Altern Med 11:64–76

Halliwell B, Gutteridge JMC, Aruoma OI (1987) The deoxyribose method: a simple test tube assay for determination of rate constants for reactions of hydroxyl radicals. Anal Biochem 165:215–219

Haseloff RF, Blasig IE, Meffert H, Ebert B (1990) Hydroxyl radical scavenging and antipsoriatic activity of benzoic acid derivatives. Free Rad Biol Med 9:111–115

Heo BG, Park YJ, Park YS, Bae JH, Cho JY, Park K, Jastrzebski Z, Gorinstein S (2014) Anticancer and antioxidant effects of extracts from different parts of indigo plant. Ind Crop Prod 56:9–16

Hermann K (1989) Occurrence and content of hydroxycinnamic acid and hydroxybenzoic acid compounds in foods. Crit Rev Food Sci Nutr 28:315–347

Hsieh CL, Chang CH, Chiang SY, Li TC, Tang NY, Pon CZ, Hsieh CT, Lin JG (2000) Anticonvulsive and free radical scavenging activities of vanillyl alcohol in ferric chloride-induced epileptic seizures in Sprague–Dawley rats. Life Sci 67:1185–1195

Jing P, Zhao SJ, Jian WJ, Qian BJ, Dong Y, Pang J (2012) Quantitative studies on structure-DPPH scavenging activity relationships of food phenolic acids. Molecules 17:12910–12924

Kikuzaki H, Hisamoto M, Hirose K, Akiyama K, Taniguchi H (2002) Antioxidant properties of ferulic acid and its related compounds. J Agric Food Chem 50:2161–2168

Kumar SS, Priyadarsini KI, Sainis KB (2002) Free radical scavenging activity of vanillin and o-vanillin using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. Redox Rep 7:35–40

Kumbulainen JT, Salonen JT (1999) Natural antioxidants and anticarcinogens in nutrition: Health and diseases. Royal Society of Chemistry, Cambridge, p 12

Leonardis AD, Macciola V (2003) Effectiveness of caffeic acid as an antioxidant for cod liver oil. Inter J Food Sci Technol 38:475–480

Lin S, Lai TC, Chen L, Kwok HF, Lau CB, Cheung PCK (2014) Antioxidant and antiangiogenic properties of phenolic extract from *Pleurotus tuber-regium*. J Agric Food Chem 62:9488–9498

Lipinski B (2011) Hydroxyl radical and its scavengers in health and disease. Oxid Med Cell Longev 809696:1–9

Liu J, Mori A (1993) Antioxidant and pro-oxidant activities of p-hydroxybenzyl alcohol and vanillin: effects on free radicals, brain peroxidation and degradation of benzoate, deoxyribose, amino acids and DNA. Neuropharmacol 32:659–669

Lucini L, Pellizoni M, Pellegrino R, Molinari GP, Colla G (2015) Phytochemical constituents and in vitro radical scavenging activity of different *Aloe* species. Food Chem 170:501–507

Marinova EM, Yanishlieva NV (1992) Effect of temperature on the antioxidative action of inhibitors in lipid autoxidation. J Sci Food Agric 60:313–318

Mathew S, Abraham TE (2006) Invitro antioxidant activity and scavenging effects of *Cinnamomum verum* leaf extract assayed by different methodologies. Food Chem Toxicol 44:198–206

Matthaus B (2002) Antioxidant activity of extracts obtained from residues of different oilseeds. J Agric Food Chem 50:3444–3452

Moure A, Cruz JM, Franco D (2001) Natural antioxidants from residual sources. Food Chem 72:145–171

Natella F, Nardini M, di Felice M, Scaccini C (1999) Benzoic and cinnamic acid derivatives as antioxidants: Structure-activity relation. J Agric Food Chem 47:1453–1459

Nenadis N, Wang LF, Tsimidou M, Zhang HY (2004) Estimation of scavenging activity of phenolic compounds using the ABTS assay. J Agric Food Chem 52:4669–4674

Nicodemus KK, Jacobs DR, Folsom AR (2001) Whole and refined grain intake and risk of incident postmenopausal breast cancer. Cancer Cause Control 12:917–925

Pacifico S, Gallicchio M, Lorenz P, Duckstein SM, Potenza N, Galasso S, Marciano S, Fiorentino A, Stintzing FC, Monaco P (2014) Neuroprotective potential of *Laurus nobilis* antioxidant polyphenol enriched leaf extracts. Chem Res Toxicol 27:611–626

Pino E, Campos AM, Lopez-Alarcon C, Aspee A, Lissi E (2006) Free radical scavenging capacity of hydroxycinnamic acids and related compounds. J Phys Org Chem 19:759–764

Pokorny J (1987) Major factors affecting the autooxidation of lipids. In: Chan HWS (ed) Autooxidation of unsaturated lipids. Academic, London, pp 141–206

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999) Antioxidant activity applying an improved ABTS radical cation decolorisation assay. Free Radical Biol Med 26: 1231–1237

Rice Evans CA, Miller NJ, Paganga G (1996) Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Rad Biol Med 20:933–956

Robbins RJ (2003) Phenolic acids in foods: An overview of analytical methodology. J Agric Food Chem 51:2866–2887

Shahidi F, Wanasundara PKJ (1992) Phenolic antioxidants. Crit Rev Food Sci Nutr 32:67–103

Siquet C, Paiva-Martins F, Lima JL, Reis S, Borges F (2006) Antioxidant profile of dihydroxy- and trihydroxyphenolic acids–a structure activity relationship study. Free Radical Res 40:433–442

Sivakumar PM, Prabhakar PA, Doble M (2011) Synthesis, antioxidant evaluation and quantitative structure-activity relationship studies of chalcones. Med Chem Res 20:482–492

Teixeira J, Gaspar A, Garrido EM, Borges F (2013) Hydroxycinnamic acid antioxidants: An electrochemical overview. Biomed Res Int 2013, Article ID 251754, 11 pages

Torres de Pinedo A, Penalver P, Morales JC (2007) Synthesis and evaluation of new phenolic-based antioxidants: structure-activity relationship. Food Chem 103:55–61

Vafiadis AP, Bakalbassis EG (2003) A computational study of the structure–activity relationships of some *p*-hydroxybenzoic acid antioxidants. J Am Oil Chem Soc 80:1217–1223

Velkov ZA, Kolev MK, Tadjer AV (2007) Modeling and statistical analysis of DPPH scavenging activity of phenolics. Collec Czech Chem Commun 72:1461–1471

von Gadow A, Joubert E, Hansmann CF (1997) Comparison of the antioxidant activity of aspalathin with that of other plant phenols of

Rooibos tea (*Aspalathus linearis*), alpha-Tocopherol, BHT, and BHA. J Agric Food Chem 45:632–638

Wardman P (1989) Reduction potentials of one-electron couples involving free radicals in aqueous solutions. J Phys Chem Ref Data 18: 1637–1755

Yamagami C, Akamatsu M, Motohashi N, Hamada S, Tanahashi T (2005) Quantitative structure-activity relationship studies for antioxidant hydroxybenzalacetones by quantum chemical-and 3-D-QSAR(CoMFA) analyses. Bioorg Med Chem Lett 15: 2845–2850

Yanishlieva N, Marinova EM (1995) Effects of antioxidants on the stability of triacylglycerols and methyl esters of fatty acids of sunflower oil. Food Chem 54:377–382

Zhang J, Stanley RA, Adaim A, Melton LD, Skinner MA (2006) Free radical scavenging and cytoprotective activities of phenolic antioxidants. Mol Nutr Food Res 50:996–1005