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COMPARISON OF RADICAL-SCAVENGING ACTIVITIES FOR SELECTED PHENOLIC ACIDS

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Key words: phenolic acids, radical-scavenging activity, DPPH•

The capacity of selected phenolic acids (*i.e.* gallic, salicylic, *p*-hydroxybenzoic, gentisic, protocatechuic, vanillic, syringic, *o*-, *m*-, *p*-coumaric, caffeic, ferulic, isoferulic, and sinapic acids) to scavenge the "stable" free radical 2,2-diphenyl-1-picrylhydrazyl• (DPPH•) was evaluated using the classical assay. Compounds tested with this method demonstrated radical-scavenging activities to vary to a different extent, but in a concentration-dependent manner. Gallic and gentisic acids showed the strongest antiradical properties (EC_{50} 0.0237 and 0.0292 μmol/assay, respectively) whereas salicylic and *p*-hydroxybenzoic acids were the least active radical scavengers (EC_{50} > 800 μmol/assay).

INRTODUCTION

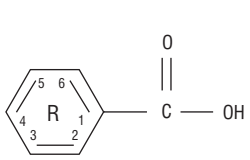
Benzoic and cinnamic acid derivatives are phenolic compounds endogenous to cereal grains (*e.g.* barley, buckwheat, oats, soybean, rye, wheat), oilseeds (*e.g.* rapeseed/canola, mustard), pulses/legumes (*e.g.* field pea, mung bean, chickpeas, lentils), vegetables (*e.g.* asparagus, carrots), and a myriad of other plant species [Weidner *et al.*, 1999; Weidner *et al.*, 2000; Amarowicz *et al.*, 1995; Naczk *et al.*, 1998; Shahidi *et al.*, 1994; Shahidi & Naczk, 2004]. The phenolic acids of plant material and those in food of plant origin exist in the free, esterified, glycosidic, and insoluble-bound forms [Sosulski *et al.*, 1982; Hermann, 1989]. In cereals, for example, much of the ferulic acid is present as ester derivatives of the stanol and sterol type [Herrmann, 1989]: the highest concentration of steroyl ferulates (*i.e.* γ-oryzanol) has been reported in rice bran oil [Xu & Godber, 1999].

Of the phenolic acids, ferulic acid has received much attention. For example, the antioxidant effect of ferulic acid on the peroxidation of ghee during storage for 30 days at 37°C was observed by Gupta *et al.* [1979]. Yagi and Ohishi [1979] reported the antioxidant activity of γ-oryzanol, a mixture of ferulic acid esters of triterpenoid alcohol. Toda *et al.* [1991] noted that ferulic acid scavenged the superoxide anion radical and inhibited lipid peroxidation induced by superoxide. A general review of the radical-scavenging activity of ferulates is presented by Graf [1992]. Brand-Williams *et al.* [1995] evaluated the antiradical activity of several phenolic acids. The antioxidant activity of prepared extracts from canola, rapeseed, mustard, cereals, and legumes, in which phenolic acids were the dominant phenolic constituent, has been reported in several studies [Amarowicz *et al.*, 1995; Amarowicz *et al.*, 1996; Amarowicz *et al.*, 2000; Amarowicz *et al.*, 2002; Karamać *et al.*, 2002]. More recently, Marinova and Yanishlieva [2003] reported on the antioxidant activity of some phenolic acids using bulk oil model systems.

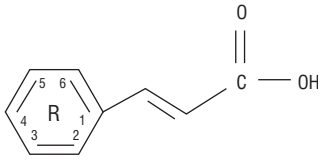
The aim of the present work was to compare the radical-scavenging activities of 14 phenolic acids using DPPH radical as a model system.

MATERIALS AND METHODS

Chemicals. Methanol of analytical grade was purchased from the P.O.Ch. Company (Gliwice, Poland). 2,2-diphenyl-1-picrylhydrazyl• (DPPH•), butylated hydroxyanisole and phenolic acid standards (*i.e.* gallic, salicylic, *p*-hydroxybenzoic, gentisic, protocatechuic, vanillic, syringic, *o*-, *m*-, *p*-coumaric, caffeic, ferulic, isoferulic, and sinapic) were obtained from the Sigma Chemical Co. Ltd. (Poznań, Poland). The chemical structures of these phenolic acids are presented in Figure 1.



Acids:	
Salicylic	R: 2-OH
p-Hydroxybenzoic	R: 4-OH
Gentistic	R: 2,5-di-OH
Protocatechuic	R: 3,4-di-OH
Gallic	R: 3,4,5-tri-OH
Vanillic	R: 3-OCH_3, 4-OH
Syringic	R: 3,5-di-OCH_3, 4-OH



Acids:	
o-Coumaric	R: 2-OH
m-Coumaric	R: 3-OH
p-Coumaric	R: 4-OH
Caffeic	R: 3,4-di-OH
Ferulic	R: 3-OCH_3, 4-OH
Isoferulic	R: 4-OCH_3, 3-OH
Sinapic	R: 3,5-OCH_3, 4-OH

FIGURE. 1. Chemical structures of selected phenolic acids.

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Scavenging of the DPPH radical. The scavenging capacity of phenolic acids for the "stable" free radical 2,2--diphenyl-1-picrylhydrazyl• (DPPH•) was monitored according to the method of Hatano *et al.* [1988] with slight modifications. In a series of test tubes, a 0.1-mL methanolic solution containing either 10 to 60 nmol of gallic, gentisic, caffeic, and syringic acids; or 20 to 100 nmol of sinapic, protocatechuic, and ferulic acids; or 4 to 20 μmol of vanillic, and isoferulic acids; or 40 to 200 μmol of *o*-, *m*-, and *p*-coumaric acids, was diluted with 2 mL of methanol into which 0.25 mL of a 1 mmol/L methanolic solution of DPPH• was pipetted. Each tube's contents were vortexed for 15 s and then left to stand at room temperature for 20 min, after which absorbance measurements of the solutions were taken at 517 nm. A methanolic solution of DPPH• that had decayed and hence no longer exhibited a purple colour (*i.e.* 1 mg of BHA dissolved in 2.1 mL of methanol with 0.25 mL of the DPPH• solution added) was chosen for background correction, instead of pure methanol.

The antiradical activity was defined as the amount of antioxidant necessary to decrease the initial DPPH• concentration by 50% (Efficient Concentration = EC_{50}). The results are also presented as 1/ EC_{50} (antiradical power – ARP), and as a relative ARP (*i.e.* activity of investigated compound in relation to the strongest scavenger in which the ARP was defined as 100%).

All radical-scavenging assays of DPPH• were repeated three times.

RESULTS AND DISCUSSION

The antiradical performance of the 14 phenolic acids investigated is depicted in Figures 2–6. Values for EC_{50}, ARP, and a relative ARP are presented in Table 1. The phenolic acids tested this way exhibited radical-scavenging activities to vary to a different extent degrees in a concentration-dependent fashion and were in the order of gallic > gentisic > syringic > caffeic > protocatechuic > sinapic > ferulic > isoferulic > vanillic > *p*-coumaric > *o*-coumaric > *m*-coumaric > salicylic ≈ *p*-hydroxybenzoic. The high values of scavenging efficiency for gallic, gentisic, caffeic,

TABLE 1. Antiradical activity of selected phenolic acids against DPPH•.

Phenolic acids	EC_{50} (μmol/assay)	ARP	Relative ARP (%)
Gallic	0.0237	42.19	100
Gentisic	0.0292	34.25	81.18
Syringic	0.0427	23.42	55.51
Caffeic	0.0478	20.92	49.58
Protocatechuic	0.0574	17.42	41.31
Sinapic	0.0724	13.81	32.73
Ferulic	0.0927	10.79	25.57
Isoferulic	5.68	0.176	0.42
Vanillic	14.37	0.069	0.16
p-Coumaric	66.29	0.015	0.04
o-Coumaric	130.05	0.008	0.02
m-Coumaric	>300	<0.003	<0.007
Salicylic	>800	<0.001	<0.002
p-Hydroxybenzoic	>800	<0.001	<0.002

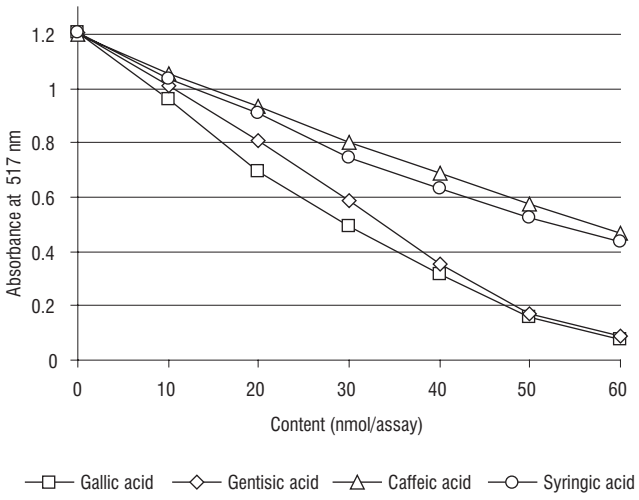

FIGURE 2. Radical-scavenging activities of gallic, gentisic, syringic, and caffeic acids on 2,2-diphenyl-1-picrylhydrazyl• (DPPH•), as measured by changes in absorbance at 517 nm.

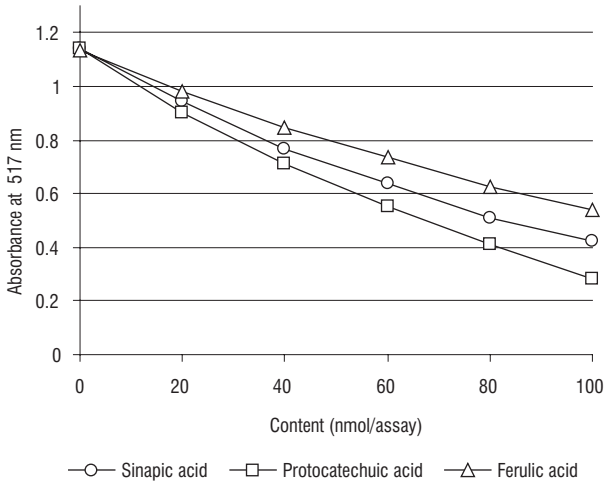

FIGURE 3. Radical-scavenging activities of protocatechuic, sinapic, and ferulic acids on 2,2-diphenyl-1-picrylhydrazyl• (DPPH•), as measured by changes in absorbance at 517 nm.

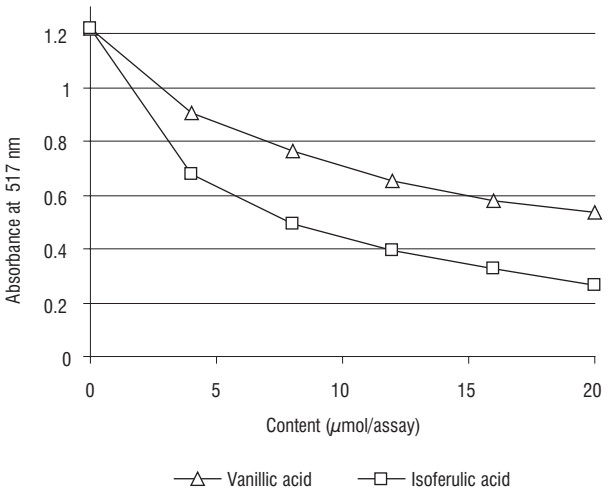

FIGURE 4. Radical-scavenging activities of isoferulic and vanillic acids on 2,2-diphenyl-1-picrylhydrazyl• (DPPH•), as measured by changes in absorbance at 517 nm.

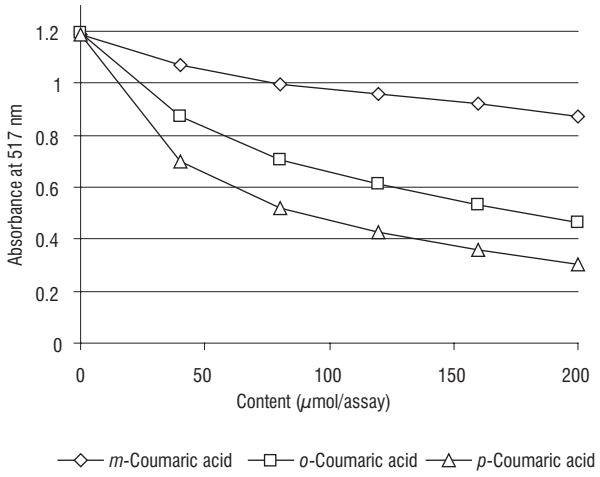

FIGURE 5. Radical-scavenging activities of *p*-, *o*-, and *m*-coumaric acids on 2,2-diphenyl-1-picrylhydrazyl• (DPPH•), as measured by changes in absorbance at 517 nm.

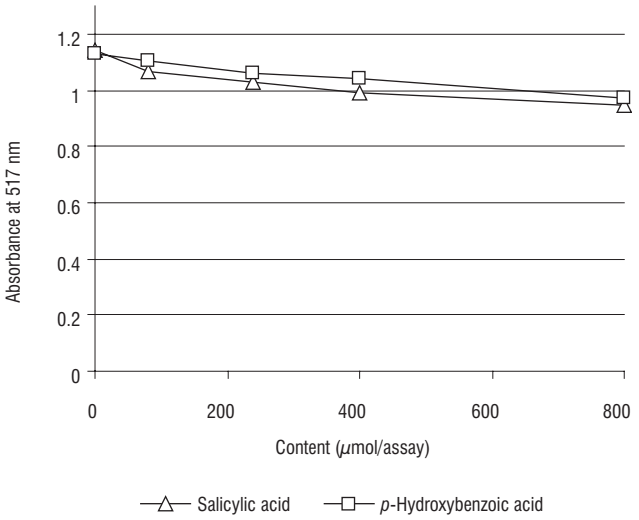

FIGURE 6. Radical-scavenging activities of salicylic and *p*-hydroxybenzoic acids on 2,2-diphenyl-1-picrylhydrazyl• (DPPH•), as measured by changes in absorbance at 517 nm.

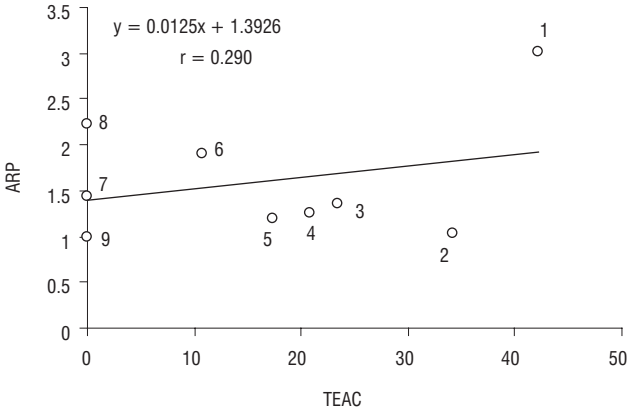

FIGURE 7. Relationship between the data acquired in the present study and the total antioxidant activity of several phenolic acids reported as TEAC by Rice-Evans *et al.* [1996]; 1 – gallic acid, 2 – gentisic acid, 3 – syringic acid, 4 – caffeic acid, 5 – protocatechuic acid, 6 - sinapic acid, 7 – vanillic acid, 8 – *p*-coumaric acid, 9 – *o*-coumaric acid.

and protocatechuic acids were reported by Brand-Williams *et al.* [1995]. The radical-scavenging activities of phenolic acids depend on the number of hydroxyl moieties attached to the aromatic ring of the benzoic or cinnamic acid molecule. Gallic acid, with three hydroxyl groups, was observed to be the most active phenolic acid. Dihydroxylation of the aromatic ring afforded a high ARP value for gentisic, caffeic, and protocatechuic acids. As expected, dihydroxy acids were more active than monohydroxy counterparts. Two methoxy moieties attached to the aromatic ring at positions 3 and 5 increased the radical-scavenging activity; that is, syringic acid was more active than *p*-hydroxybenzoic acid. The influence of –COOH and –CH=CH–COOH acid groups was not explicit. In the case of dihydroxy phenolic acids, gentisic acid (*i.e.* a benzoic acid derivative) was more active than sinapic acid (*i.e.* a cinnamic acid derivative). For phenolic acids with hydroxy and methoxy groups, vanillic acid (*i.e.* a benzoic acid derivative) was less active than ferulic acid (*i.e.* a cinnamic acid derivative), whereas syringic acid (*i.e.* a benzoic acid derivative) was more active than sinapic acid. The different radical-scavenging effects observed can be attributed to the varying abilities of the individual phenolic acids to react with DPPH• giving a stable non-radical product. A proposed chemical mechanism for the reaction between DPPH• and rosmaric acid was presented by Brand-Williams *et al.* [1995].

In the study of Pulido *et al.* [2000], the antioxidant activity of some phenolic acids, determined using a modified ferric reducing/antioxidant power (FRAP) assay was in the order of gallic acid > caffeic acid >ferulic acid. Ascorbate equivalents of gallic, caffeic, and ferulic acids determined using the FRAP method were 2.92, 1.68, 1.24, respectively [Hunter *et al.*, 2002]. Antioxidant activity of some phenolic acids in bulk sunflower oil model systems decreased in the following order: caffeic acid > sinapic acid > protocatechuic acid > syringic acid [Marinova & Yanishlieva, 2003]. Karamać *et al.* [2005] recently observed that ferulic acid exhibited a stronger antioxidant activity than isoferulic acid using a β-carotene-linoleate model system.

Rice-Evans *et al.* [1996] reported the total antioxidant activity, expressed as TEAC (*i.e.* Total Equivalent Antioxidant Capacity) values, of phenolic acids in the following order: gallic > *p*-coumaric > ferulic > vanillic > syringic > caffeic > *m*-coumaric > protocatechuic > gentisic > *o*-coumaric > salicylic > *p*-hydroxybenzoic. In Figure 7, the TEAC results of 9 phenolic acids from the aforementioned study are compared with the ARP values determined in the present work. A lack of correlation may suggest that phenolic acids have varying capabilities to scavenge free radicals of different types: in this case, namely DPPH• and $ABTS^{\bullet+}$. The greatest difference in the radical-scavenging activities against DPPH• and $ABTS^{\bullet+}$ were observed for vanillic, *p*-coumaric, and *o*-coumaric acids.

CONCLUSIONS

From the present study, it was observed that the number of hydroxyl moieties attached to phenolic acids of the benzoic and cinnamic acid families dictated their radical-scavenging activities. Of the 14 phenolic acids examined for their radical-scavenging activities, gallic acid was the strongest. Additionally, the nature of the radical seems to

influence the antiradical performance of the phenolic acid, as a lack of correlation existed between the radical-scavenging activities for $DPPH^{\bullet}$ and $ABTS^{\bullet+}$.

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PORÓWNANIE AKTYWNOŚCI PRZECIWRODNIKOWEJ WYBRANYCH FENOLOKWASÓW

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Aktwność przeciwrodnikową wybranych fenolokwasów (galusowego, *p*-hydroksybenzoesowego, gentyzowego, protokatechowego, wanilinowego, syryngowego, *o*-, *m*-, *p*-kumarowego, kawowego, ferulowego, izoferulowego i sinapowego) zanalizowano wobec wolnego rodnika 2,2-difenylo-1-pikrylhydrazylowego$^{\bullet}$ (DPPH$^{\bullet}$). Badane fenolokwasy wykazywały różną, zależną od stężenia, zdolność do zmiatania DPPH$^{\bullet}$. Najwyższą aktywność zanotowano dla kwasu galusowego i gentyzowego (EC_{50} 0.0237 i 0.0292 μmol/testowaną próbę), najniższą zaś dla kwasu salicylowego i *p*-hydroksybenzoesowego (EC_{50}>800 μmol/testowaną próbę).