Food and Chemical Toxicology 77 (2015) 12–21

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Antioxidant and DNA damage protection potentials of selected phenolic acids

Kemal Sevgi [a], Bektas Tepe [a,*], Cengiz Sarikurkcu [b]

[a] Department of Molecular Biology and Genetics, Faculty of Science and Literature, Kilis 7 Aralık University, TR-79000 Kilis, Turkey
[b] Department of Basic Pharmaceutical Sciences, Faculty of Pharmacy, Suleyman Demirel University, TR-32000 Isparta, Turkey

ARTICLE INFO

Article history:
Received 24 September 2014
Accepted 4 December 2014
Available online 24 December 2014

Keywords:
Rosmarinic acid
Ferulic acid
Antioxidant activity
DNA damage protection potential
pBR322 plasmid DNA

ABSTRACT

In this study, ten different phenolic acids (caffeic, chlorogenic, cinnamic, ferulic, gallic, p-hydroxybenzoic, protocatechuic, rosmarinic, syringic, and vanillic acids) were evaluated for their antioxidant and DNA damage protection potentials. Antioxidant activity was evaluated by using four different test systems named as β-carotene bleaching, DPPH free radical scavenging, reducing power and chelating effect. In all test systems, rosmarinic acid showed the maximum activity potential, while protocatechuic acid was determined as the weakest antioxidant in β-carotene bleaching, DPPH free radical scavenging, and chelating effect assays. Phenolic acids were also screened for their protective effects on pBR322 plasmid DNA against the mutagenic and toxic effects of UV and H_2O_2. Ferulic acid was found as the most active phytochemical among the others. Even at the lowest concentration value (0.002 mg/ml), ferulic acid protected all of the bands in the presence of H_2O_2 and UV. It is followed by caffeic, rosmarinic, and vanillic acids. On the other hand, cinnamic acid (at 0.002 mg/ml), gallic acid (at 0.002 mg/ml), p-hydroxybenzoic acid (at 0.002 and 0.004 mg/ml), and protocatechuic acid (at 0.002 and 0.004 mg/ml) could not protect plasmid DNA.

1. Introduction

Plants are known as the major sources of valuable bioactive substances (Tayel and El-Tras, 2012). Due to the excellent biological activity potentials, many plant species have scientifically been evaluated to improve the quality of human life. Natural antioxidants isolated from the plants have been investigated extensively to date. It is quite interesting to point out that fruits (grapes, pomegranate), vegetables (broccoli, potato, drumstick, pumpkin, curry, nettle), herbs, and spices (tea, rosemary, oregano, cinnamon, sage, thyme, mint, ginger, clove) are known as the important sources of phenolic acids (Shah et al., 2014). These plants also constitute an important part of our daily diet (Das et al., 2012; Devatkal et al., 2010; Huang et al., 2011; Kanatt et al., 2007; Rojas and Brewer, 2008; Shan et al., 2009; Wojciak et al., 2011).

Phenolic acids are known as strong antioxidant substances and can scavenge almost all oxidant molecules such as free radicals via their hydroxyl groups. Due to their highly hydroxylated molecular properties, each compound can scavenge one or two strong oxidant molecules (Sroka and Cisowski, 2003). However, mode of action of these substances also seems to be associated with their ability to modify cellular signaling processes that introduces a multiplier effect. Activation of Nrf2 pathway that results in the enhancement of multiple endogenous anti-oxidant mechanisms takes place among the numerous examples (Juurlink et al., 2014).

It is quite important to understand the formation of free radicals to establish and improve an efficient antioxidant defense system against toxic effect of oxidant substances. Hydroxyl radicals can be formed by Fenton reaction in the presence of transition metals (for example; Fe^{3+}) as well as H_2O_2. Hydrogen peroxide known as the most reactive reduced form of di-oxygen is capable of damaging almost every molecule of living cells (Rollet-Labelle et al., 1998; Singh et al., 2014). Hydroxyl radicals also tend to react with nucleotides and subsequently with whole DNA molecule. As a result of this process, DNA strand breakages occur and may lead to carcinogenesis, mutagenesis, cytotoxicity, and a number of genetic disorders (Moskovitz et al., 2002). Exposure of plasmid DNA to H_2O_2 results in the cleavage of the phosphodiester chains of supercoiled DNA and then a relaxed open-circular DNA form occurs. Subsequent cleavages occurring near the first breakage result in linear double-stranded DNA molecules. The formation of circular DNA form is accepted as the indicator of single-strand break. Similarly, formation of the linear forms of DNA is indicative of double-stranded breakage.

In this study, we aimed to evaluate the antioxidant and DNA damage protection potentials of several phenolic acids originated from the schikimic acid metabolic pathway: caffeic acid, chlorogenic acid, cinnamic acid, ferulic acid, gallic acid, *p*-hydroxybenzoic

* Corresponding author. Department of Molecular Biology and Genetics, Faculty of Science and Literature, Kilis 7 Aralık University, TR-79000 Kilis, Turkey. Tel.: +90 348 822 23 50; fax: +90 348 813 93 24.
E-mail address: bektastepe@yahoo.com (B. Tepe).

http://dx.doi.org/10.1016/j.fct.2014.12.006
0278-6915/

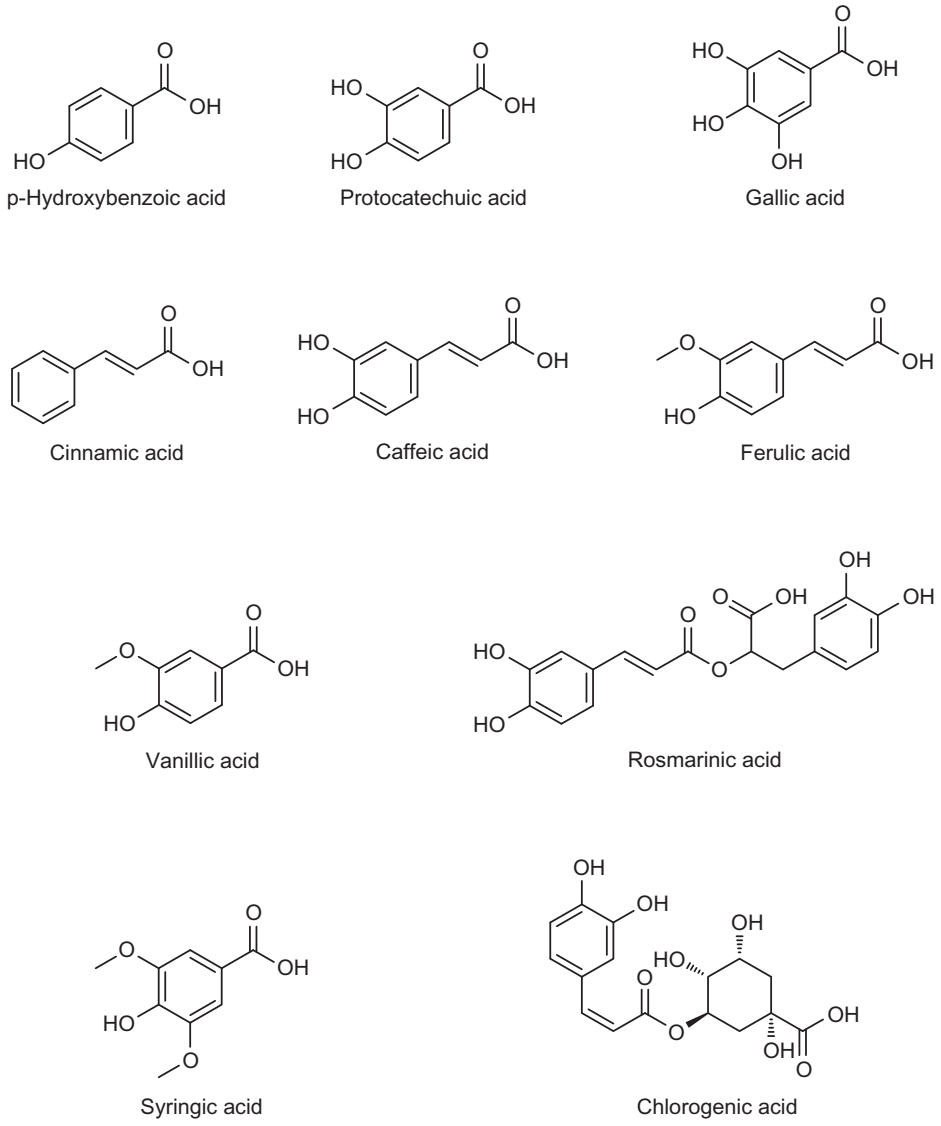

Fig. 1. Chemical structures of phenolic acids tested.

acid, protocatechuic acid, rosmarinic acid, syringic acid, and vanillic acid (Fig. 1). Antioxidant activities of the phenolic acids presented here were evaluated by using four different test systems named as β-carotene bleaching, DPPH free radical scavenging, reducing power and chelating effect. Phenolic acids were also screened for their protective effects on DNA against the mutagenic and toxic effects of UV and H_2O_2. As far as our literature survey could ascertain, antioxidant activity potentials of the phenolics presented here have previously been reported several times. On the other hand, the majority of data presented for these compounds are particularly based on the fractionation of the extracts of several plant species. There is a similar situation for DNA protective activity. In this study, we described the potential of these compounds by using the standard reference compounds. Additionally, DNA damage protection potentials of cinnamic, *p*-hydroxybenzoic, protocatechuic, syringic, and vanillic acids were reported for the first time in this study.

2. Materials and methods

2.1. Phytochemicals

All of the phenolic acids were purchased from Sigma-Aldrich.

2.2. Antioxidant activity

2.2.1. Total antioxidant activity by β-Carotene–linoleic acid method

In this assay antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation (Aktumsek et al., 2013). A stock solution of β-carotene–linoleic acid mixture was prepared as following: 0.5 mg β-carotene was dissolved in 1 ml of chloroform (HPLC grade). Twenty-five microliters of linoleic acid and 200 mg Tween 40 were added. Chloroform was completely evaporated using a vacuum evaporator. Then 100 ml of oxygenated distilled water was added with vigorous shaking; 2.5 ml of this reaction mixture was dispersed to test tubes and 0.5 ml of the extracts (2.0 mg/ml) in water were added and the emulsion system was incubated for up to 2 h at 50 °C. The same procedure was repeated with the positive control BHT, BHA and a blank. After this incubation period, absorbance of the mixtures was measured at 490 nm. Measurement of absorbance was continued until the color of β-carotene disappeared. The bleaching rate (R) of β-carotene was calculated according to Eq. (1).

$$R = \ln(a/b)/t \quad (1)$$

where, ln = natural log, a = absorbance at time 0, b = absorbance at time t (120 min) (Aktumsek et al., 2013). The antioxidant activity (AA, %) was calculated in terms of percent inhibition relative to the control using Eq. (2).

$$AA = [(Rcontrol - Rsample)/Rcontrol] \times 100 \quad (2)$$

Antioxidative activities of the extracts were compared with those of butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) at 2.0 mg/ml and blank consisting of only 0.5 ml water.

2.2.2. Scavenging effect on 1,1-Diphenyl-2-picrylhydrazyl (DPPH)

The hydrogen atoms or electrons donation abilities of the corresponding compounds were measured from the bleaching of purple colored methanol solution of DPPH (Aktumsek et al., 2013). One milliliter of various concentrations (0.2–1.0 mg/ml) of the extracts in water was added to a 1 ml of DPPH radical solution in methanol (final concentration of DPPH was 0.2 mM). The mixture was shaken vigorously and allowed to stand for 30 min; the absorbance of the resulting solution was measured at 517 nm with a spectrophotometer (Shimadzu UV-1601, Kyoto, Japan). Inhibition of free radical DPPH in percent (I %) was calculated in the following way:

$$I\% = 100 \times (A_{Control} - A_{Sample})/A_{Control}$$

where, $A_{Control}$ is the absorbance of the control reaction (containing all reagents except the test compound), and A_{Sample} is the absorbance of the test compound. BHT and BHA were used as a control.

2.2.3. Reducing power

The reducing power was determined according to the method of Aktumsek et al. (2013). Each extract (0.2–1.0 mg/ml) in water (2.5 ml) was mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide and the mixture was incubated at 50 °C for 20 min. Then, 2.5 ml of 10% trichloroacetic acid were added, and the mixture was centrifuged at 200 *g* (MSE Mistral 2000, London, UK) for 10 min. The upper layer (2.5 ml) was mixed with 2.5 ml of deionized water and 0.5 ml of 0.1% ferric chloride. Finally the absorbance was measured at 700 nm against a blank. BHT and BHA were used as a control.

2.2.4. Chelating effects on ferrous ions

The chelating effect was determined according to the method of Aktumsek et al. (2013). Briefly, 1 ml (2 mg/ml) of the extracts in water was added to 1 ml of water and a solution of 2 mM $FeCl_2$ (0.05 ml). The reaction was initiated by the addition of 5 mM ferrozine (0.2 ml). Then, the mixture was shaken vigorously and left at room temperature for 10 min. Absorbance of the solution was measured spectrophotometrically at 562 nm. The inhibition percentage of ferrozine–Fe^{2+} complex formation was calculated by using the formula given below:

$$\text{Metal chelating effect}\ (\%) = [(A_{Control} - A_{Sample})/A_{Control}] \times 100$$

where $A_{Control}$ is the absorbance of control (the control contains $FeCl_2$ and ferrozine, complex formation molecules) and A_{Sample} is the absorbance of the test compound. EDTA was used as a control.

2.3. DNA damage protection potential

DNA damage protection activities of the extracts were evaluated on pBR322 plasmid DNA (Vivantis). Plasmid DNA was oxidized with H_2O_2 + UV treatment in the presence of extracts and checked on 1% agarose gels according to Tepe et al. (2011). In brief, the experiments were performed in a volume of 10 µl in a microfuge tube containing 3 µl pBR322 plasmid DNA (172 ng/µl), 1 µl of 30% H_2O_2, and 5 µl of extract in the concentrations of 5, 10, 15 and 20 mg/ml, respectively. The reactions were initiated by UV irradiation and continued for 5 min on the surface of a UV transilluminator (DNR-IS) with an intensity of 8000 µW/cm^2 at 302 nm at room temperature. After irradiation, the reaction mixture (10 µl) along with gel loading dye (6×) was loaded on a 1% agarose gel for electrophoresis. Untreated pBR322 plasmid DNA was used as a control in each run of gel electrophoresis along with partially treated plasmid, i.e. only UV or only H_2O_2 treatment. Gels were stained with EtBr and photographed with the Gel documentation system (DNR-IS, MiniBIS Pro).

2.4. Statistical analyses

All assays were carried out in triplicate for all the experiments. The results are expressed as mean and standard deviation values (mean ± SD). Differences between means were determined by the analysis of variance (ANOVA) with Tukey's honestly significant difference *post hoc* test with $\alpha = 0.05$, which were analyzed with SPSS v. 14.0.

Table 1
Total antioxidant activities of phenolic acids by β-carotene bleaching method.[a]

Phenolic acids	Total antioxidant activity (%)[b]
Caffeic acid	96.20 ± 0.22*
Chlorogenic acid	85.73 ± 0.34**
Cinnamic acid	93.14 ± 0.06***
Ferulic acid	94.06 ± 0.44***
Gallic acid	82.30 ± 0.54****
p-Hydroxybenzoic acid	95.26 ± 0.40*
Protocatechuic acid	78.12 ± 0.07*****
Rosmarinic acid	98.92 ± 0.68******
Syringic acid	91.94 ± 0.24***
Vanillic acid	95.81 ± 0.17*
BHT	86.48 ± 1.93**
BHA	92.14 ± 0.15***

[a] Values expressed are means ± S.D. of three parallel measurements; in the same column, data marked with different numbers of superscript symbols indicate significant difference ($p < 0.05$).
[b] At 2.0 mg/ml concentration.

3. Results and discussion

Total antioxidant activities of the phenolic acids measured by β-carotene bleaching method are presented in Table 1. According to data presented, rosmarinic acid exhibited the highest activity (98.92%). It is followed by caffeic acid (96.20%). Activities of vanillic, *p*-hydroxybenzoic, ferulic, and cinnamic acids were found too close to each other. In this system, minimum activity was exhibited by protocatechuic acid (78.12%). As can be seen from the table, caffeic, cinnamic, ferulic, *p*-hydroxybenzoic, rosmarinic, and vanillic acids showed higher activity than those of positive controls, BHT and BHA.

Scavenging abilities of the phenolic acids on DPPH free radical were also tested (Table 2). None of the phenolic acids tested at 0.1 mg/ml concentration showed activity as high as positive controls, BHT and BHA. Additionally, at 0.1 mg/ml concentration, free radical scavenging ability of BHT was still higher than those of phenolic acids. On the other hand, caffeic, cinnamic, ferulic, *p*-hydroxybenzoic, rosmarinic, and vanillic acids showed higher

Table 2
Radical scavenging activities of phenolic acids.[a]

Phenolic acids	DPPH free radical scavenging activity (%)		
	0.1 mg/ml	0.3 mg/ml	0.5 mg/ml
Caffeic acid	59.12 ± 0.63*	83.35 ± 0.57*	88.13 ± 0.86*
Chlorogenic acid	50.07 ± 0.40**	71.75 ± 0.70**	76.96 ± 0.38**
Cinnamic acid	57.86 ± 0.24***	80.94 ± 0.19***	87.30 ± 0.48*
Ferulic acid	58.27 ± 0.32*	81.42 ± 0.51***	88.69 ± 0.27*
Gallic acid	47.19 ± 1.17****	68.24 ± 1.79****	73.62 ± 0.39***
p-Hydroxybenzoic acid	57.86 ± 1.29***	85.52 ± 0.32*****	84.73 ± 0.23****
Protocatechuic acid	47.44 ± 0.62****	65.37 ± 0.05******	70.12 ± 0.08*****
Rosmarinic acid	61.91 ± 1.39*****	85.31 ± 0.11*****	91.50 ± 0.64******
Syringic acid	55.83 ± 0.90******	76.93 ± 0.45*******	82.52 ± 0.68****
Vanillic acid	58.80 ± 0.69***	82.24 ± 0.27*****	87.39 ± 0.70*
BHT	87.14 ± 0.62*******	–	–
BHA	79.40 ± 0.13********	–	–

[a] Values expressed are means ± S.D. of three parallel measurements; in the same column, data marked with different numbers of superscript symbols indicate significant difference ($p < 0.05$).

Table 3
Reducing power potentials of phenolic acids.[a]

Phenolic acids	Reducing power potential (absorbance at 700 nm)		
	0.1 mg/ml	0.3 mg/ml	0.5 mg/ml
Caffeic acid	0.619 ± 0.012*	0.998 ± 0.041*	1.862 ± 0.018*
Chlorogenic acid	0.579 ± 0.090**	0.957 ± 0.034**	1.670 ± 0.074**
Cinnamic acid	0.598 ± 0.020**	0.962 ± 0.024**	1.716 ± 0.080***
Ferulic acid	0.602 ± 0.014***	0.984 ± 0.060***	1.798 ± 0.084****
Gallic acid	0.547 ± 0.022****	0.902 ± 0.011*****	1.407 ± 0.056*****
p-Hydroxybenzoic acid	0.611 ± 0.010*	1.130 ± 0.040*****	1.623 ± 0.018******
Protocatechuic acid	0.555 ± 0.020****	0.898 ± 0.060******	1.548 ± 0.086*******
Rosmarinic acid	0.632 ± 0.077*	1.081 ± 0.010*******	1.929 ± 0.061********
Syringic acid	0.600 ± 0.115***	1.004 ± 0.045********	1.769 ± 0.065****
Vanillic acid	0.602 ± 0.102***	0.986 ± 0.026***	1.799 ± 0.049****
BHT	2.012 ± 0.029****	–	–
BHA	1.114 ± 0.098*****	–	–

[a] Values expressed are means ± S.D. of three parallel measurements; in the same column, data marked with different numbers of superscript symbols indicate significant difference ($p < 0.05$).

activity than that of BHA at this concentration level. At 0.5 mg/ml concentration, majority of phenolic acids were found active on DPPH free radical except chlorogenic, protocatechuic, and gallic acids. As seen in the previous test system (β-carotene bleaching method), rosmarinic acid showed the highest radical scavenging ability (91.50%). It is followed by ferulic (88.69%), caffeic (88.13%), vanillic (87.39%), and cinnamic acids (87.30%). No statistical difference was determined between the results obtained from these phenolic acids. Protocatechuic acid exhibited the minimum scavenging activity potential in this test system (70.12%).

Phenolic acids were also tested for their reducing power potentials by the method of Aktumsek et al. (2013) at three different concentration levels (Table 3). At all concentration values, none of phenolic acids showed activity as high as BHT. At 0.3 mg/ml, only *p*-hydroxybenzoic acid showed higher activity than that of BHA. On the other hand, all of the phenolic acids exhibited higher activity than BHA. At this concentration value, maximum absorbance value was measured for rosmarinic acid (1.929). It is followed by caffeic acid (1.862). In this test system, gallic acid showed the weakest activity potential (1.407). Results obtained from this system were found to be statistically different except ferulic, syringic, and vanillic acids.

EDTA was used as positive control agent to determine the chelating effects of phenolic acids on ferrous ions. According to the results presented in Table 4, none of phytochemicals were active as EDTA. As seen in previous systems presented above, rosmarinic acid showed the highest activity (65.05%). It is followed by caffeic (62.14%) and vanillic acids (61.46%). Chelating effect of protocatechuic acid on ferrous ions was determined as 26.17%, which is the lowest value

Table 4
Chelating effects of phenolic acids.[a]

Phenolic acids	Chelating effect (%)
Caffeic acid	62.14 ± 0.52*
Chlorogenic acid	41.70 ± 0.38**
Cinnamic acid	59.44 ± 0.12*
Ferulic acid	60.71 ± 0.82*
Gallic acid	38.70 ± 0.72***
p-Hydroxybenzoic acid	39.73 ± 1.27***
Protocatechuic acid	26.17 ± 0.86****
Rosmarinic acid	65.05 ± 0.71*****
Syringic acid	50.71 ± 0.65******
Vanillic acid	61.46 ± 0.92*
EDTA	98.40 ± 0.35*******

[a] Values expressed are means ± S.D. of three parallel measurements; in the same column, data marked with different numbers of superscript symbols indicate significant difference ($p < 0.05$).

Table 5
A clear overview to the protected and non-protected bands of pBR322 plasmid DNA.

Phenolic acids	Concentration (mg/ml)	ocDNA[a]	lnDNA[b]	scDNA[c]
Caffeic acid	0.002	+	–	–
	0.004	+	+	+
	0.008	+	+	+
	0.016	+	+	+
	0.032	+	+	+
Chlorogenic acid	0.002	+	–	+
	0.004	+	–	+
	0.008	+	+	–
	0.016	+	+	–
	0.032	+	+	–
Cinnamic acid	0.002	–	–	–
	0.004	+	–	+
	0.008	+	+	+
	0.016	+	+	+
	0.032	+	+	+
Ferulic acid	0.002	+	+	+
	0.004	+	+	+
	0.008	+	+	+
	0.016	+	+	+
	0.032	+	+	+
Gallic acid	0.002	–	–	–
	0.004	+	–	–
	0.008	+	–	–
	0.016	+	+	–
	0.032	+	+	–
p-Hydroxybenzoic acid	0.002	–	–	–
	0.004	–	–	–
	0.008	+	–	+
	0.016	+	–	+
	0.032	+	–	+
Protocatechuic acid	0.002	–	–	–
	0.004	–	–	–
	0.008	+	+	–
	0.016	+	+	–
	0.032	+	+	–
Rosmarinic acid	0.002	+	–	+
	0.004	+	+	+
	0.008	+	+	+
	0.016	+	+	+
	0.032	+	+	+
Syringic acid	0.002	+	–	–
	0.004	+	+	–
	0.008	+	+	–
	0.016	+	+	–
	0.032	+	+	–
Vanillic acid	0.002	+	+	–
	0.004	+	+	+
	0.008	+	+	+
	0.016	+	+	+
	0.032	+	+	+

[a] ocDNA: Open circular DNA band.
[b] lnDNA: Linear DNA band.
[c] scDNA: Super coiled DNA band.

among compounds. Results obtained from caffeic, cinnamic, ferulic, and vanillic acids are found similar from the statistical point of view. A similar situation was observed for the results obtained for gallic and *p*-hydroxybenzoic acids.

Phenolic acids listed above were subjected to a test to determine their protective effect on pBR322 plasmid DNA against the toxic effects of UV and H_2O_2, which are highly mutagenic on DNA. Results obtained from this experiment are given in Table 5 and Figures 2, 3, 4, 5, and 6, where the electrophoretic pattern of pBR322 plasmid DNA after UV-photolysis of H_2O_2 in the presence and absence of phenolic acids (0.002–0.032 mg/ml) were presented.

As can be seen from the figures, DNA derived from pBR322 plasmid showed three bands on agarose gel electrophoresis; the faster moving band corresponded to the native form of supercoiled circular DNA (scDNA), the slower moving band was the open circular form (ocDNA), and linear DNA (lnDNA), which is the result of the cleavage of supercoiled circular DNA arisen from the UV photolysis of H_2O_2. OH produced from the UV photolysis of H_2O_2 forms DNA strand breakage and smears are seen on the gel.

As can be seen from Fig. 3, ferulic acid was found as the most active phytochemical among the others. Even at the lowest concentration value (0.002 mg/ml), ferulic acid protected all of the bands in the presence of H_2O_2 and UV. It is followed by caffeic, rosmarinic, and vanillic acids. These phytochemicals have protected the DNA bands at the concentrations ranging from 0.004 to 0.032 mg/ml. In addition to these findings, cinnamic acid was determined as another significant compound, which protected all the bands between 0.008 and 0.032 mg/ml concentration range. As can be seen from Table 5, all of the samples showing activity have protected the slower moving band (ocDNA) of plasmid DNA. Phenolic acids showed lower protective effect on scDNA when compared to the other bands.

Cinnamic acid (at 0.002 mg/ml), gallic acid (at 0.002 mg/ml), *p*-hydroxybenzoic acid (at 0.002 and 0.004 mg/ml), and protocatechuic acid (at 0.002 and 0.004 mg/ml) could not protect pBR322 plasmid DNA.

As far as our literature survey could ascertain, DNA damage protection potentials of cinnamic, *p*-hydroxybenzoic, protocatechuic, syringic, and vanillic acids have not previously been reported elsewhere. Therefore, data presented for these compounds could be assumed as the first record for the literature. However, caffeic, chlorogenic, ferulic, gallic, and rosmarinic acids have been evaluated by several authors via different test systems.

Ramos et al. (2010) have studied the protective effect of rosmarinic acid on cellular DNA in CaCo-2 and HeLa cells. According to this report, rosmarinic acid (at 50 µM concentration) after a 24 h incubation, had protective effect in CaCo-2 cells. The

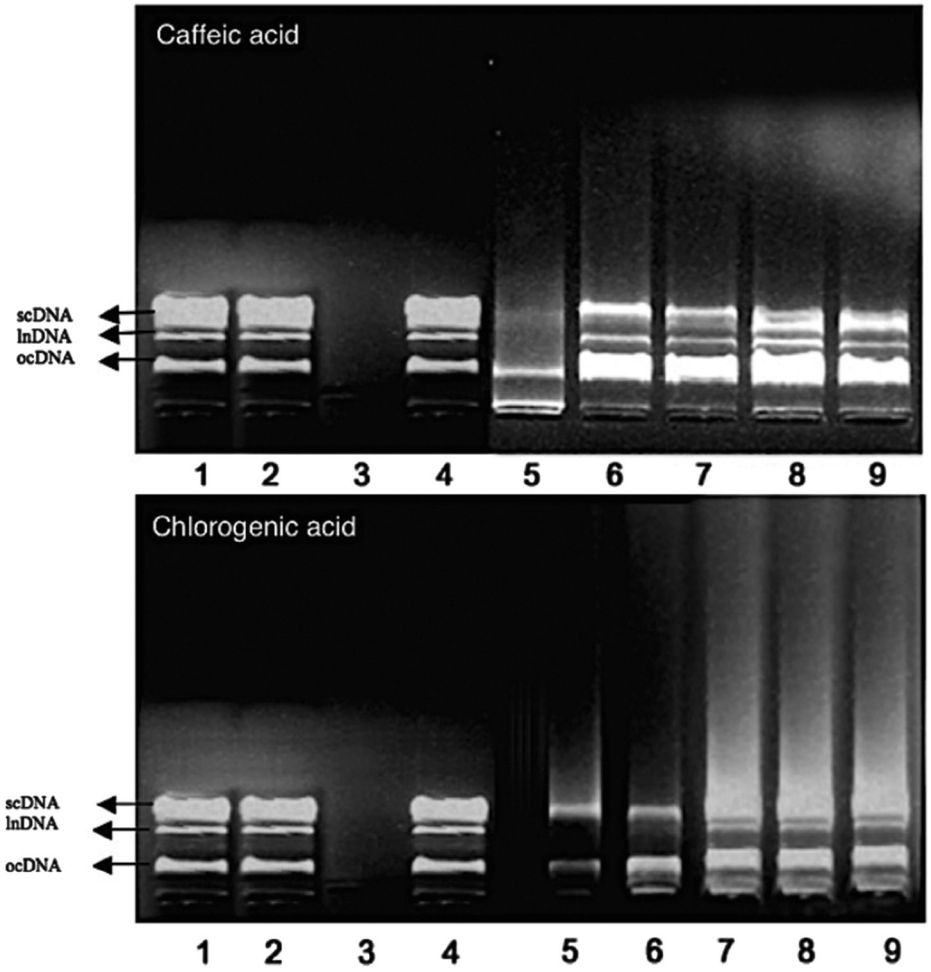

Fig. 2. DNA damage protection potentials of caffeic and chlorogenic acids. 1. Plasmid DNA (3.0 µl) + dH_2O (6.0 µl); 2. Plasmid DNA (3.0 µl) + dH_2O (6.0 µl) + UV; 3. Plasmid DNA (3.0 µl) + dH_2O (6.0 µl) + H_2O_2 (1.0 µl); 4. Plasmid DNA (3.0 µl) + dH_2O (6.0 µl) + UV + H_2O_2 (1.0 µl); 5. Plasmid DNA (3.0 µl) + Phenolic acid (0.002 mg/ml – 5 µl) + UV + H_2O_2 (1.0 µl); 6. Plasmid DNA (3.0 µl) + Phenolic acid (0.004 mg/ml – 5.0 µl) + UV + H_2O_2 (1.0 µl); 7. Plasmid DNA (3.0 µl) + Phenolic acid (0.008 mg/ml – 5.0 µl) + UV + H_2O_2 (1.0 µl); 8. Plasmid DNA (3.0 µl) + Phenolic acid (0.016 mg/ml – 5.0 µl) + UV + H_2O_2 (1.0 µl); 9. Plasmid DNA (3.0 µl) + Phenolic acid (0.032 mg/ml – 5.0 µl) + UV + H_2O_2 (1.0 µl).

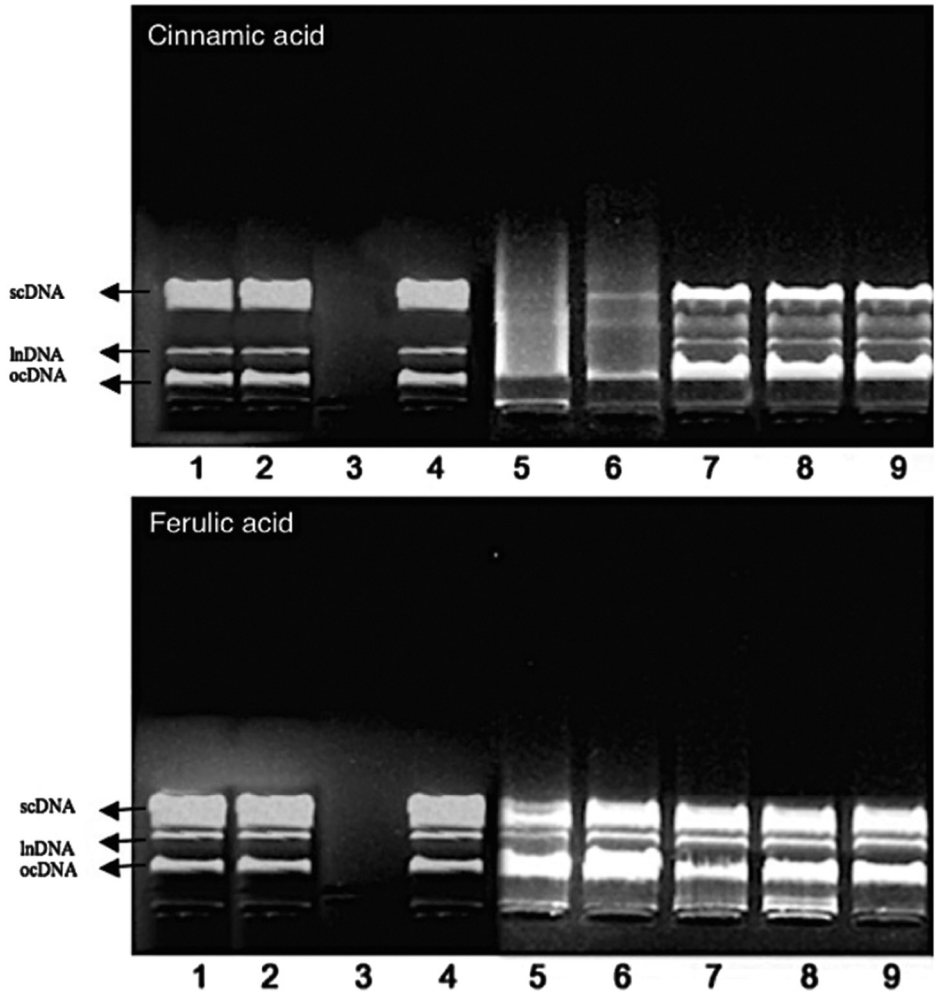

Fig. 3. DNA damage protection potentials of cinnamic and ferulic acids. 1. Plasmid DNA (3.0 μl) + dH_2O (6.0 μl); 2. Plasmid DNA (3.0 μl) + dH_2O (6.0 μl) + UV; 3. Plasmid DNA (3.0 μl) + dH_2O (6.0 μl) + H_2O_2 (1.0 μl); 4. Plasmid DNA (3.0 μl) + dH_2O (6.0 μl) + UV + H_2O_2 (1.0 μl); 5. Plasmid DNA (3.0 μl) + Phenolic acid (0.002 mg/ml – 5 μl) + UV + H_2O_2 (1.0 μl); 6. Plasmid DNA (3.0 μl) + Phenolic acid (0.004 mg/ml – 5.0 μl) + UV + H_2O_2 (1.0 μl); 7. Plasmid DNA (3.0 μl) + Phenolic acid (0.008 mg/ml – 5.0 μl) + UV + H_2O_2 (1.0 μl); 8. Plasmid DNA (3.0 μl) + Phenolic acid (0.016 mg/ml – 5.0 μl) + UV + H_2O_2 (1.0 μl); 9. Plasmid DNA (3.0 μl) + Phenolic acid (0.032 mg/ml – 5.0 μl) + UV + H_2O_2 (1.0 μl).

compound also protected genetic material against damage induced by H_2O_2. According to another study carried out by Vostalova et al. (2010), rosmarinic acid (at 0.18–1.8 mg/l concentration) reduced UV-B induced DNA breakage in HaCaT keratinocytes with the apoptotic process. Sanchez-Campillo et al. (2009) have studied the radioprotective-antimutagenic effects of rosmarinic acid by using micronucleus test. When orally administered, the compound inhibited cutaneous alterations caused by UV-A exposure and can be proposed as a photo protective agent. It is possible to increase the number of reports on this topic (Psotova et al., 2006; Silva et al., 2008; Vattem et al., 2006).

According to literature data, gallic acid has also DNA damage protection potential. Nair and Nair (2013) have studied the radioprotective effect of gallic acid in mice. According to this report, one hour prior to whole body gamma radiation exposure, the compound reduced the radiation-induced cellular damage in peripheral blood leukocytes, bone marrow cells, and splenocytes. According to another report, gallic acid consumption reduced the DNA damage caused by treatment of the cells with reactive oxygen species at a rate of 41% (Ferk et al., 2011). Although gallic acid has protective effect on DNA, some researchers showed the damage provoking effect of this compound on genetic material in several cancer cell lines such as human prostate cancer PC-3 and HeLa cells (Erol-Dayi et al., 2012; Liu et al., 2013). On the other hand, according to some reports, presence of gallic acid induces single and double strand breaks in plasmid DNA in free cell systems (Biso et al., 2010; Matsuda and Nakajima, 2012).

Ferulic acid is a monophenolic phenylpropanoid occurring in plant products such as rice bran, green tea, and coffee beans. Intraperitoneal administration of this compound at a dose of 50 mg/kg body weight resulted in significant decrease in the amount of DNA strand breaks in murine peripheral blood leukocytes and bone marrow cells of mice (Maurya and Devasagayam, 2013). Ferulic acid also showed anti-genotoxic effect on sepsis-induced DNA damage in the liver and kidney of Wistar albino rats (Bacanli et al., 2013). It has also been proven to have anticarcinogenic effect on UV-B irradiated human keratinocyte HaCaT cells (Lin et al., 2010). This compound has also been recorded for its protective effect on DNA strands as the main compound of sugarcane (*Saccharum officinarum* L.) (Abbas et al., 2014).

Chlorogenic acid has previously been evaluated for its DNA damage protection potential as well as the main component of several plants. According to Cinkilic et al. (2013), chlorogenic acid acted as a radio-protective agent on X-ray induced DNA damage in

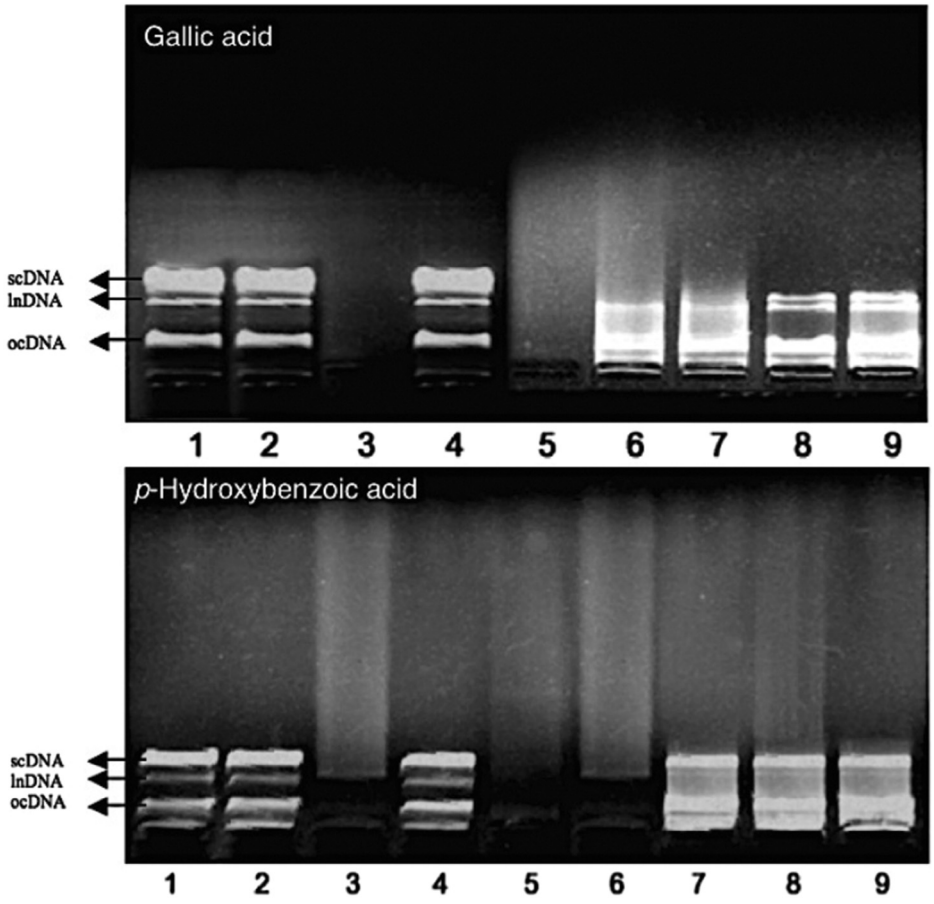

Fig. 4. DNA damage protection potentials of gallic and *p*-hydroxybenzoic acids. 1. Plasmid DNA (3.0 μl) + dH_2O (6.0 μl); 2. Plasmid DNA (3.0 μl) + dH_2O (6.0 μl) + UV; 3. Plasmid DNA (3.0 μl) + dH_2O (6.0 μl) + H_2O_2 (1.0 μl); 4. Plasmid DNA (3.0 μl) + dH_2O (6.0 μl) + UV + H_2O_2 (1.0 μl); 5. Plasmid DNA (3.0 μl) + Phenolic acid (0.002 mg/ml – 5 μl) + UV + H_2O_2 (1.0 μl); 6. Plasmid DNA (3.0 μl) + Phenolic acid (0.004 mg/ml – 5.0 μl) + UV + H_2O_2 (1.0 μl); 7. Plasmid DNA (3.0 μl) + Phenolic acid (0.008 mg/ml – 5.0 μl) + UV + H_2O_2 (1.0 μl); 8. Plasmid DNA (3.0 μl) + Phenolic acid (0.016 mg/ml – 5.0 μl) + UV + H_2O_2 (1.0 μl); 9. Plasmid DNA (3.0 μl) + Phenolic acid (0.032 mg/ml – 5.0 μl) + UV + H_2O_2 (1.0 μl).

human blood lymphocytes *in vitro*. In another study, chlorogenic acid isomers have been proven to have DNA damage protective effects (Xu et al., 2012). Chlorogenic acid as the main compound of *Sphallerocarpus gracilis* seeds and coffee has also been tested for its activity potential and showed significant protective effect (Gao et al., 2014; Rathod et al., 2013). Burgos-Moron et al. (2012) have reported chlorogenic acid induced cellular DNA damage and formation of topoisomerase I- and II–DNA complexes in cells.

Polylactide nanofibers loaded with caffeic acid were tested on the inhibition of oxidative DNA damage caused by free radicals initiated by 2,2′-azobis (2-amidinopropane hydrochloride) (Llorens et al., 2014). According to the results of this study, caffeic acid significantly inhibited oxidative DNA damage. Another evidence for the protective potential of this compound was reported by Kitsati et al. (2012). The researchers claimed that caffeic acid reaches the intracellular space and chelate intracellular labile iron.

As can be seen from the figures presented, protocatechuic acid showed quite weak protective effect on DNA. UV light (254 nm) induces the formation of 8-hydroxy-2′-deoxyguanosine (8-OHdG) in DNA via a singlet oxygen mechanism. Wei et al. (1998) have investigated several natural antioxidants including protocatechuic acid for their inhibition of UV-induced 8-OHdG. According to this report, protocatechuic acid did not fit into DNA and exhibited weak effect due to its weak intercalation potential.

These results suggest that the intercalation of phytochemical compounds into DNA may alter the DNA structural integrity, interrupt the production of oxidizing species, and subsequently reduce the formation of 8-OHdG by UV radiation.

4. Conclusions

The majority of studies carried out to determine the biological activity potentials of plant species are based on the crude extracts and/or essential oils. Recently, however, researchers have begun to focus on active phytochemicals to clarify the exact potentials of these substances particularly in food and pharmaceutical industries. Among these compounds, phenolic acids are especially important due to their wide distribution among the plant kingdom. Phenolic acids are found in most of the fruits and vegetables that form the significant portion of our diet. By this study, antioxidant and DNA damage protection potentials of 10 widely distributed phenolic acids were documented. In all test systems, rosmarinic acid was found as the most active compound, while protocatechuic acid exhibited weak activity. Especially in antioxidant test systems, in general, caffeic, cinnamic, ferulic, *p*-hydroxybenzoic, and vanillic acids also showed higher activity than those of the positive controls, BHT and BHA. Ferulic acid also showed significant protective activity

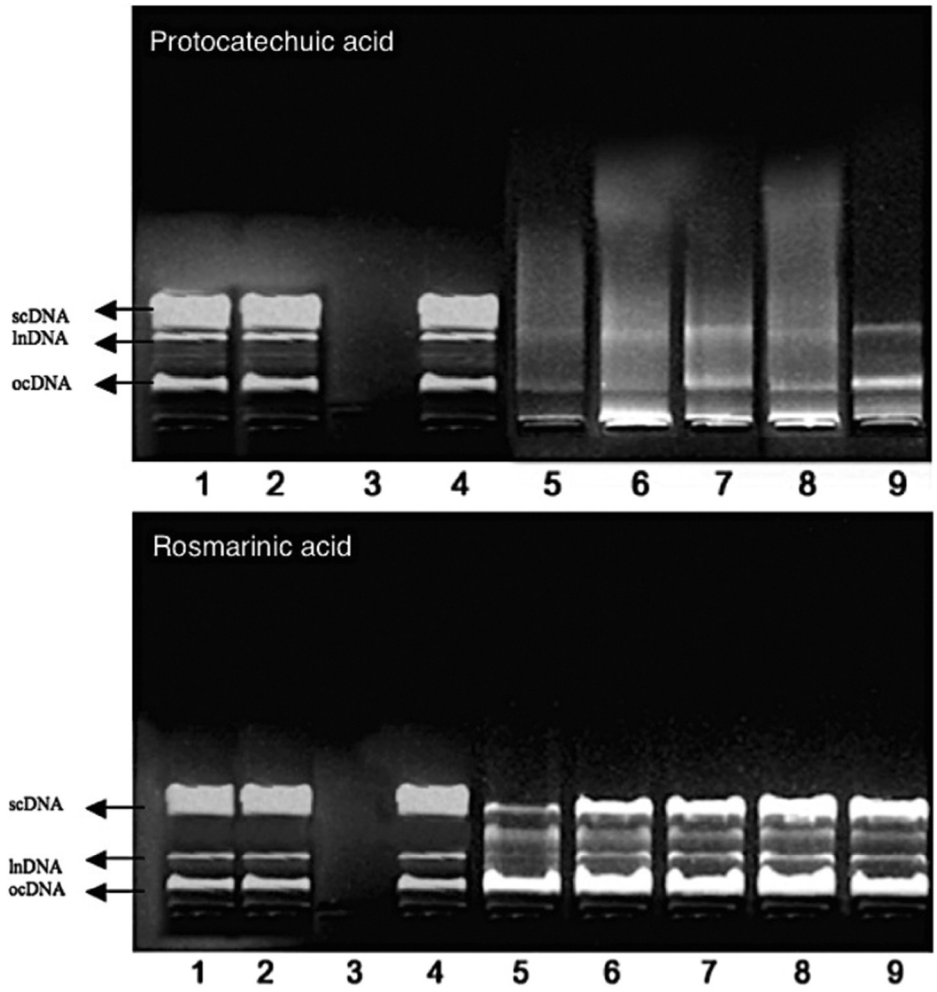

Fig. 5. DNA damage protection potentials of protocatechuic and rosmarinic acids. 1. Plasmid DNA (3.0 μl) + dH_2O (6.0 μl); 2. Plasmid DNA (3.0 μl) + dH_2O (6.0 μl) + UV; 3. Plasmid DNA (3.0 μl) + dH_2O (6.0 μl) + H_2O_2 (1.0 μl); 4. Plasmid DNA (3.0 μl) + dH_2O (6.0 μl) + UV + H_2O_2 (1.0 μl); 5. Plasmid DNA (3.0 μl) + Phenolic acid (0.002 mg/ml – 5 μl) + UV + H_2O_2 (1.0 μl); 6. Plasmid DNA (3.0 μl) + Phenolic acid (0.004 mg/ml – 5.0 μl) + UV + H_2O_2 (1.0 μl); 7. Plasmid DNA (3.0 μl) + Phenolic acid (0.008 mg/ml – 5.0 μl) + UV + H_2O_2 (1.0 μl); 8. Plasmid DNA (3.0 μl) + Phenolic acid (0.016 mg/ml – 5.0 μl) + UV + H_2O_2 (1.0 μl); 9. Plasmid DNA (3.0 μl) + Phenolic acid (0.032 mg/ml – 5.0 μl) + UV + H_2O_2 (1.0 μl).

against the hazardous effects of these substances as well as rosmarinic acid.

Antioxidant activity potentials of the phenolic acids presented here have previously been reported several times by different researchers. In general, results presented here are highly in agreement with these reports. In these studies, phenolic compounds as standards or as the main compounds of fractions obtained from the extracts of several plant species have been screened for their antioxidant activity potentials. Among the phenolic acids evaluated here, rosmarinic acid (Maheswarappa et al., 2014; Mushtaq et al., 2014; Sahin et al., 2014; Suwanchaikasem et al., 2014; Zhu et al., 2014), ferulic acid (Jimenez-Aspee et al., 2014; Konopka et al., 2014; Li et al., 2014; Ti et al., 2014), and caffeic acid (Kassim et al., 2014; Wan et al., 2014; Zhou et al., 2014) are the most frequently studied compounds due to their excellent activity potentials. However, the majority of data presented for these compounds are particularly based on the fractionation of the extracts of several plant species. Purity of the relevant compounds within the fractions is not clear and it is not possible to make a healthy evaluation using these samples. Therefore, the current study that was carried out by using standard reference compounds presents almost the exact potentials of them in terms of the test systems documented here. Chemical structure–activity relationship should be clarified by the further studies in addition to the effects of functional groups on activity potential.

Conflict of interest

The authors declare that there are no conflicts of interest.

Transparency document

The Transparency document associated with this article can be found in the online version.

Acknowledgement

Data concerning some phenolic acids presented here are basically originated from Mr. Kemal SEVGI's M. Sc. thesis. Some experiments available in the thesis were performed again to improve the quality of data obtained and in relation to this issue; some parts of data may vary from the thesis. The authors would like to thank the Research Council of Cumhuriyet University, Sivas-TURKEY for its financial support (CUBAP Project Number: F-337).

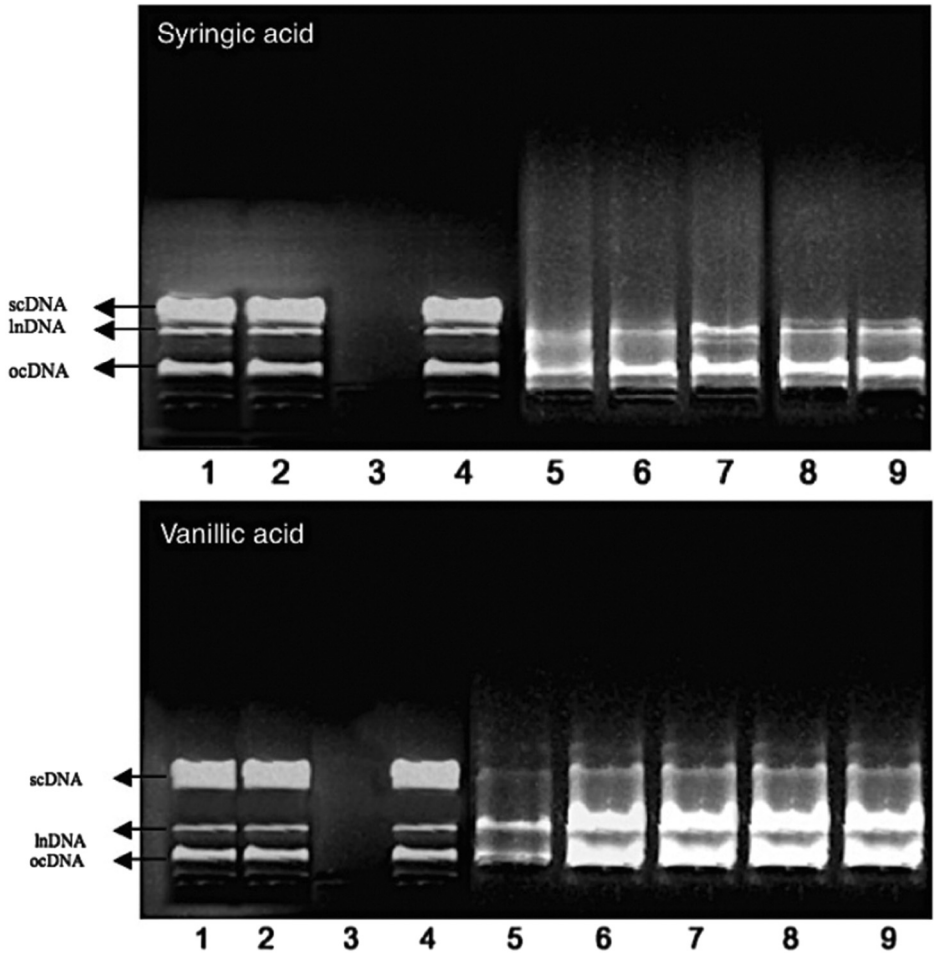

Fig. 6. DNA damage protection potentials of syringic and rosmarinic acids. 1. Plasmid DNA (3.0 μl) + dH_2O (6.0 μl); 2. Plasmid DNA (3.0 μl) + dH_2O (6.0 μl) + UV; 3. Plasmid DNA (3.0 μl) + dH_2O (6.0 μl) + H_2O_2 (1.0 μl); 4. Plasmid DNA (3.0 μl) + dH_2O (6.0 μl) + UV + H_2O_2 (1.0 μl); 5. Plasmid DNA (3.0 μl) + Phenolic acid (0.002 mg/ml – 5 μl) + UV + H_2O_2 (1.0 μl); 6. Plasmid DNA (3.0 μl) + Phenolic acid (0.004 mg/ml – 5.0 μl) + UV + H_2O_2 (1.0 μl); 7. Plasmid DNA (3.0 μl) + Phenolic acid (0.008 mg/ml – 5.0 μl) + UV + H_2O_2 (1.0 μl); 8. Plasmid DNA (3.0 μl) + Phenolic acid (0.016 mg/ml – 5.0 μl) + UV + H_2O_2 (1.0 μl); 9. Plasmid DNA (3.0 μl) + Phenolic acid (0.032 mg/ml – 5.0 μl) + UV + H_2O_2 (1.0 μl).

References

Abbas, S.R., Sabir, S.M., Ahmad, S.D., Boligon, A.A., Athayde, M.L., 2014. Phenolic profile, antioxidant potential and DNA damage protecting activity of sugarcane (*Saccharum officinarum*). Food Chem. 147, 10–16.

Aktumsek, A., Zengin, G., Guler, G.O., Cakmak, Y.S., Duran, A., 2013. Antioxidant potentials and anticholinesterase activities of methanolic and aqueous extracts of three endemic *Centaurea* L. species. Food Chem. Toxicol. 55, 290–296.

Bacanli, M., Aydin, S., Taner, G., Sahin, T., Basaran, A.A., Basaran, N., 2013. Antigenotoxic effects of ferulic acid on sepsis-induced DNA damage in the liver and kidney of Wistar albino rats. Toxicol. Lett. 221, S125.

Biso, F.I., Rodrigues, C.M., Rinaldo, D., dos Reis, M.B., Bernardi, C.C., de Mattos, J.C.P., et al., 2010. Assessment of DNA damage induced by extracts, fractions and isolated compounds of *Davilla nitida* and *Davilla elliptica* (Dilleniaceae). Mutat. Res. 702, 92–99.

Burgos-Moron, E., Calderon-Montano, J.M., Orta, M.L., Pastor, N., Perez-Guerrero, C., Austin, C., et al., 2012. The coffee constituent chlorogenic acid induces cellular DNA damage and formation of topoisomerase I- and II-DNA complexes in cells. J. Agric. Food Chem. 60, 7384–7391.

Cinkilic, N., Cetintas, S.K., Zorlu, T., Vatan, O., Yilmaz, D., Cavas, T., et al., 2013. Radioprotection by two phenolic compounds: chlorogenic and quinic acid, on X-ray induced DNA damage in human blood lymphocytes *in vitro*. Food Chem. Toxicol. 53, 359–363.

Das, A.K., Rajkumar, V., Verma, A.K., Swarup, D., 2012. *Moringa oleifera* leaves extract: a natural antioxidant for retarding lipid peroxidation in cooked goat meat patties. Int. J. Food Sci. Technol. 47, 585–591.

Devatkal, S.K., Narsaiah, K., Borah, A., 2010. Anti-oxidant effect of extracts of kinnow rind, pomegranate rind and seed powder in cooked goat meat patties. Meat Sci. 85, 155–159.

Erol-Dayi, O., Arda, N., Erdem, G., 2012. Protective effects of olive oil phenolics and gallic acid on hydrogen peroxide-induced apoptosis. Eur. J. Nutr. 51, 955–960.

Ferk, F., Chakraborty, A., Jager, W., Kundi, M., Bichler, J., Misik, M., et al., 2011. Potent protection of gallic acid against DNA oxidation: results of human and animal experiments. Mutat. Res. 715, 61–71.

Gao, C.Y., Tian, C.R., Zhou, R., Zhang, R.G., Lu, Y.H., 2014. Phenolic composition, DNA damage protective activity and hepatoprotective effect of free phenolic extract from *Sphallerocarpus gracilis* seeds. Int. Immunopharmacol. 20, 238–247.

Huang, B., He, J., Ban, X., Zeng, H., Yao, X., Wang, Y., 2011. Antioxidant activity of bovine and porcine meat treated with extracts from edible lotus (*Nelumbo nucifera*) rhizome knot and leaf. Meat Sci. 87, 46–53.

Jimenez-Aspee, F., Quispe, C., Soriano, M.D.P.C., Gonzalez, J.F., Haneke, E., Theoduloz, C., et al., 2014. Antioxidant activity and characterization of constituents in copao fruits (*Eulychnia acida* Phil., Cactaceae) by HPLC-DAD-MS/MSn. Food Res. Int. 62, 286–298.

Juurlink, B.H.J., Azouz, H.J., Aldalati, A.M.Z., AlTinawi, B.M.H., Ganguly, P., 2014. Hydroxybenzoic acid isomers and the cardiovascular system. Nutr. J. 13, 63.

Kanatt, S.R., Chander, R., Sharma, A., 2007. Antioxidant potential of mint (Mentha spicata L.) in radiation-processed lamb meat. Food Chem. 100, 451–458.

Kassim, M., Mansor, M., Kamalden, T.A., Shariffuddin, I.I., Hasan, M.S., Ong, G., et al., 2014. Caffeic acid Phenethyl ester (cape): scavenger of peroxynitrite *in vitro* and in sepsis models. Shock 42, 154–160.

Kitsati, N., Fokas, D., Ouzouni, M.D., Mantzaris, M.D., Barbouti, A., Galaris, D., 2012. Lipophilic caffeic acid derivatives protect cells against H_2O_2-induced DNA damage by chelating intracellular labile iron. J. Agric. Food Chem. 60, 7873–7879.

Konopka, I., Tanska, M., Faron, A., Czaplicki, S., 2014. Release of free ferulic acid and changes in antioxidant properties during the wheat and rye bread making process. Food Sci. Biotechnol. 23, 831–840.

Li, T., Shen, P.Y., Liu, W., Liu, C.M., Liang, R.H., Yan, N., et al., 2014. Major polyphenolics in pineapple peels and their antioxidant interactions. Int. J. Food Prop. 17, 1805–1817.

Lin, X.F., Min, W., Luo, D., 2010. Anticarcinogenic effect of ferulic acid on ultraviolet-B irradiated human keratinocyte HaCaT cells. J. Med. Plant Res. 4, 1686–1694.

Liu, K.C., Ho, H.C., Huang, A.C., Ji, B.C., Lin, H.Y., Chueh, F.S., et al., 2013. Gallic acid provokes DNA damage and suppresses DNA repair gene expression in human prostate cancer PC-3 cells. Environ. Toxicol. 28, 579–587.
Llorens, E., del Valle, L.J., Puiggali, J., 2014. Inhibition of radical-induced oxidative DNA damage by antioxidants loaded in electrospun polylactide nanofibers. Macromol. Res. 22, 388–396.
Maheswarappa, N.B., Subbaiah, V., Muthupalani, M., Yamagani, P.K., Mohan, K., Keshapaga, U.R., et al., 2014. Antioxidant activity of carnosic acid and rosmarinic acid in raw and cooked ground chicken patties. J. Sci. Food Agric. 94, 273–279.
Matsuda, E., Nakajima, A., 2012. Effect of catechins and tannins on depleted uranium-induced DNA strand breaks. J. Radioanal. Nucl. Chem. 293, 711–714.
Maurya, D.K., Devasagayam, T.P.A., 2013. Ferulic acid inhibits gamma radiation-induced DNA strand breaks and enhances the survival of mice. Cancer Biother. Radiopharm. 28, 51–57.
Moskovitz, J., Yim, K.A., Choke, P.B., 2002. Free radicals and disease. Arch. Biochem. Biophys. 397, 354–359.
Mushtaq, N., Schmatz, R., Pereira, L.B., Ahmad, M., Stefanello, N., Vieira, J.M., et al., 2014. Rosmarinic acid prevents lipid peroxidation and increase in acetylcholinesterase activity in brain of streptozotocin-induced diabetic rats. Cell Biochem. Funct. 32, 287–293.
Nair, G.G., Nair, C.K.K., 2013. Radioprotective effects of gallic acid in mice. Biomed. Res. Int. 953079.
Psotova, J., Svobodova, A., Kolarova, H., Walterova, D., 2006. Photoprotective properties of Prunella vulgaris and rosmarinic acid on human keratinocytes. J. Photochem. Photobiol. B, Biol. 84, 167–174.
Ramos, A.A., Azqueta, A., Pereira-Wilson, C., Collins, A.R., 2010. Polyphenolic compounds from *Salvia* species protect cellular DNA from oxidation and stimulate DNA repair in cultured human cells. J. Agric. Food Chem. 58, 7465–7471.
Rathod, M.A., Patel, D., Das, A., Tipparaju, S.R., Shinde, S.S., Anderson, R.F., 2013. Inhibition of radical-induced DNA strand breaks by water-soluble constituents of coffee: phenolics and caffeine metabolites. Free Radic. Res. 47, 480–487.
Rojas, M.C., Brewer, M.S., 2008. Effect of natural antioxidants on oxidative stability of frozen, vacuum-packaged beef and pork. J. Food Qual. 31, 173–188.
Rollet-Labelle, E., Grange, M.J., Elbim, C., Marquetty, C., Gougerot-Pocidalo, M.A., Pasquier, C., 1998. Hydroxyl radical as a potential intracellular mediator of polymorphonuclear neutrophil apoptosis. Free Radic. Biol. Med. 24, 563–572.
Sahin, S., Ari, F., Demir, C., Ulukaya, E., 2014. Isolation of major phenolic compounds from the extracts of Prunella L. species grown in Turkey and their antioxidant and cytotoxic activities. J. Food Biochem. 38, 248–257.
Sanchez-Campillo, M., Gabaldon, J.A., Castillo, J., Benavente-Garcia, O., Del Bano, M.J., Alcaraz, M., et al., 2009. Rosmarinic acid, a photo-protective agent against UV and other ionizing radiations. Food Chem. Toxicol. 47, 386–392.
Shah, M.A., Bosco, S.J.D., Mir, S.A., 2014. Plant extracts as natural antioxidants in meat and meat products. Meat Sci. 98, 21–33.
Shan, B., Cai, Y.Z., Brooks, J.D., Corke, H., 2009. Antibacterial and antioxidant effects of five spices and herb extracts as natural preservatives of raw pork. J. Sci. Food Agric. 89, 1879–1885.
Silva, J.P., Gomes, A.C., Coutinho, O.P., 2008. Oxidative DNA damage protection and repair by polyphenolic compounds in PC12 cells. Eur. J. Pharmacol. 601, 50–60.
Singh, P., Vishwakarma, S.P., Singh, R.L., 2014. Antioxidant, oxidative DNA damage protective and antimicrobial activities of the plant *Trigonella foenum-graecum*. J. Sci. Food Agric. 94, 2497–2504.
Sroka, Z., Cisowski, W., 2003. Hydrogen peroxide scavenging, antioxidant and anti-radical activity of some phenolic acids. Food Chem. Toxicol. 41, 753–758.
Suwanchaikasem, P., Chaichantipyuth, C., Sukrong, S., 2014. Antioxidant-guided isolation of rosmarinic acid, a major constituent from *Thunbergia laurifolia*, and its use as a bioactive marker for standardization. Chiang Mai J. Sci. 41, 117–127.
Tayel, A.A., El-Tras, W.F., 2012. Plant extracts as potent biopreservatives for *Salmonella typhimurium* control and quality enhancement in ground beef. J. Food Saf. 32, 115–121.
Tepe, B., Degerli, S., Arslan, S., Malatyali, E., Sarikurkcu, C., 2011. Determination of chemical profile, antioxidant, DNA damage protection and antiamoebic activities of *Teucrium polium* and *Stachys iberica*. Fitoterapia 82, 237–246.
Ti, H.H., Li, Q., Zhang, R.F., Zhang, M.W., Deng, Y.Y., Wei, Z.C., et al., 2014. Free and bound phenolic profiles and antioxidant activity of milled fractions of different indica rice varieties cultivated in southern China. Food Chem. 159, 166–174.
Vattem, D.A., Jang, H.D., Levin, R., Shetty, K., 2006. Synergism of cranberry phenolics with ellagic acid and rosmarinic acid for antimutagenic and DNA protection functions. J. Food Biochem. 30, 98–116.
Vostalova, J., Zdarilova, A., Svobodova, A., 2010. *Prunella vulgaris* extract and rosmarinic acid prevent UVB-induced DNA damage and oxidative stress in HaCaT keratinocytes. Arch. Dermatol. Res. 302, 171–181.
Wan, Y.Y., Chen, S.Y., Huang, Y.W., Li, X., Zhang, Y., Wang, X.J., et al., 2014. Caffeic acid pretreatment enhances dehydration tolerance in cucumber seedlings by increasing antioxidant enzyme activity and proline and soluble sugar contents. Sci. Hortic. (Amsterdam) 173, 54–64.
Wei, H.C., Ca, Q.Y., Rahn, R., Zhang, X.S., Wang, Y., Lebwohl, M., 1998. DNA structural integrity and base composition affect ultraviolet light-induced oxidative DNA damage. Biochemistry 37, 6485–6490.
Wojciak, K.M., Dolatowski, Z.J., Okon, A., 2011. The effect of water plant extracts addition on the oxidative stability of meat products. Acta Sci. Pol. Technol. Aliment. 10, 175–188.
Xu, J.G., Hu, Q.P., Liu, Y., 2012. Antioxidant and DNA-protective activities of chlorogenic acid isomers. J. Agric. Food Chem. 60, 11625–11630.
Zhou, X.J., Yan, L.L., Yin, P.P., Shi, L.L., Zhang, J.H., Liu, Y.J., et al., 2014. Structural characterisation and antioxidant activity evaluation of phenolic compounds from cold-pressed *Perilla frutescens* var. *arguta* seed flour. Food Chem. 164, 150–157.
Zhu, F.X., Asada, T., Sato, A., Koi, Y., Nishiwaki, H., Tamura, H., 2014. Rosmarinic acid extract for antioxidant, antiallergic, and alpha-glucosidase inhibitory activities, isolated by supramolecular technique and solvent extraction from Perilla leaves. J. Agric. Food Chem. 62, 885–892.