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ANTIOXIDANT AND FREE RADICAL SCAVENGING ACTIVITIES OF *RHUS CORIARIA* AND *CINNAMOMUM CASSIA* EXTRACTS

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Antioxidant and free radical scavenging activities of the extracts of sumac (*Rhus coriaria*) fruits and cassia (*Cinnamomum cassia*) cortex were studied. Plant samples were extracted with methanol:water (80:20) and an aliquot of each extract was fractionated using *n*-hexane and ethyl acetate. Antioxidant activities of *n*-hexane, ethyl acetate and water fractions were measured using Fe^{+2} induced linoleic acid-TBA-peroxidation reaction and the Rancimat methods. Free radical scavenging activities of the fractions were determined on 2,2-diphenyl-1-picrylhydrazyl radical (DPPH). Results were compared with those for butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT). The ethyl acetate fraction of plant materials exhibited a marked antiradical activity on $DPPH^{\bullet}$, higher than those of BHT and BHA; however, their antioxidant activity on the linoleic acid peroxidation was less than those of BHA and BHT.

Keywords: antioxidant activity, *Cinnamomum cassia*, *Rhus coriaria*

Lipid oxidation is not only the cause of various disorders such as aging, heart disease, stroke, emphysema, mutagenesis and carcinogenesis, but also lowers the quality of food products due to the deterioration of flavour (YAGI, 1987; JACOB, 1994; HALLIWELL, 1997). Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propylgallate (PG) are widely used, however, the use of synthetic antioxidants in food products is being questioned (BRANEN, 1975; TAKAJASHI & HIRAGA, 1978). Consumers have also become more cautious about the nutritional quality and safety of food additives. In response to the growing consumer demand, investigations on antioxidants from natural sources have gained interest (POKORNY, 1991; PRAT, 1992; LARSON, 1997).

Spices have received particular attention as sources of antioxidants (FISHER, 1991; NAKATANI, 1994; MADSON et al., 1997). CHIPAULT and co-workers (1956) examined more than 70 spices for antioxidant activity in edible oils. SAITO and co-workers (1976) concluded that the most pronounced effects were found for rosemary and sage, however, thyme, marjoram and oregano among the herbs and clove, ginger, nutmeg and mace among the spices had strong activity.

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Sumac (*Rhus coriaria*, Anacardiaceae) and cassia (*Cinnamomum cassia*, Lauraceae) spices have been widely used as flavouring ingredients in food recipes for many years. Sumac is native to the Mediterranean and Southeastern Anatolian regions of Turkey. The spice, produced by grinding the dried fruit with salt, is used as a condiment (WETHERILT & PALA, 1994). Cassia, indigenous to the Orient, is the oldest spice known to man and stem barks of cassia are used mostly in baking in its ground form (VERNIN et al., 1994). Only a limited number of studies concerning antioxidant activity of sumac and cassia have been reported. ÖZCAN and AKGÜL (1995) observed that sumac was effective in stabilizing sunflower oil. DHULEY (1999) reported that *Cinnamomum zeylanicum* significantly enhanced the antioxidative enzyme activities. YAKOZAVA and co-workers (1998) concluded that among the 79 traditional Chinese prescriptions and 28 crude drugs, *Cinnamomum cassia* and *Rhus coriaria* showed free radical scavenging activity. No researcher, however, has compared the activities of the extracts of sumac and cassia in different antioxidant activity test systems.

The objective of our work was to evaluate the antioxidant activity of different fractions of aqueous methanol extracts from sumac and cassia in oil and oil-in-water-emulsion systems, as well as free radical scavenging activity on DPPH radical.

1. Materials and methods

1.1. Materials and reagents

Sumac (*Rhus coriaria* L.) and cassia (*Cinnamomum cassia* L.) were purchased from a local market in Eskisehir, Turkey. BHA, BHT, thiobarbituric acid and linoleic acid were purchased from Sigma Chemical Co. (St. Louis, MO), methanol, *n*-hexane and ethyl acetate were from E. Merck Co. (Darmstadt, Germany) and 2,2-diphenyl-1-picrylhydrazyl radical (DPPH$^{\bullet}$) was from Aldrich Chemical Co. (Milwaukee, WI). Crude sunflower oil was kindly provided by Demircanlar Co., Eskisehir, Turkey.

1.2. Preparation of the extracts

Figure 1 shows the scheme for the preparation of extracts from the plant samples. Powdered plant samples (40 g each) were extracted with petroleum ether to remove fats. Fat-free material was air dried and continuously extracted for 8 h in a Soxhlet extractor with aqueous methanol (80%). Methanol was evaporated under vacuum at 40 °C and the remaining aqueous extract was partitioned first with *n*-hexane and then with ethyl acetate. Organic solvents were evaporated to dryness in vacuo from the first two fractions and the remaining aqueous fraction was freeze dried and weighed to determine the yields of soluble constituents, total phenolic content and their antioxidant activity.

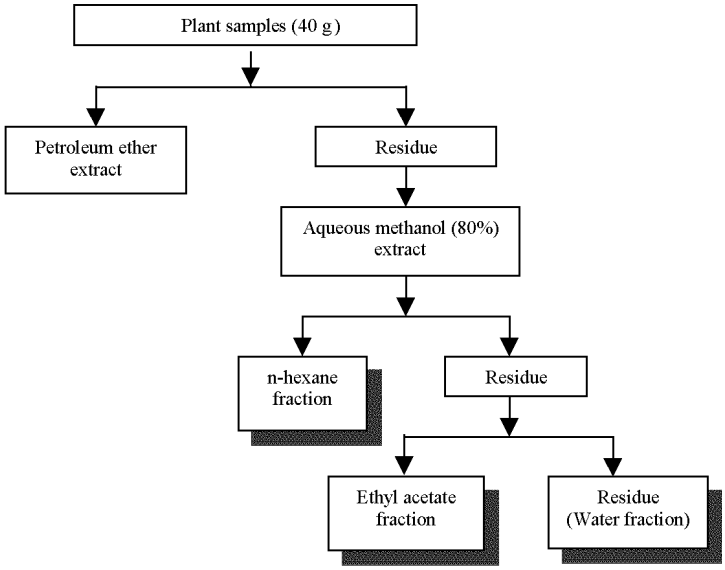

Fig. 1. Fractionation scheme of sumac and cassia

1.3. Determination of total phenolics

The total phenolics were determined colourimetrically as modified by HOFF and SINGLETON (1977) as follows. Samples (0.5 ml) were introduced into test tubes; 2.5 ml of Folin Ciocalteu (10% in water) reagent solution and 7.5 ml of Na_2CO_3 (20% in water) solution were added. The tubes were shaken and allowed to stand at room temperature in the dark for 2 h. Absorption at 750 nm was measured. Total phenolic content was expressed as gallic acid equivalents (GAE) in mg/g dry material. The results are expressed as the average of four measurements.

1.4. Free radical scavenging activity on DPPH

Free radical scavenging effects of the fractions on DPPH were estimated according to the method of SANCHEZ-MORENO and co-workers (1998) with some modification. An aliquot of methanol (0.1 ml) solution containing different sample concentrations (see Table 2) was added to 3 ml of 0.05 mM 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\bullet}$) in methanol, prepared daily. The concentrations of the samples in the reaction solutions were expressed as $\mu g\ ml^{-1}$. The mixture was shaken vigorously and left standing at

room temperature for 30 min; absorbance of the resulting solution was then measured spectrophotometrically at 517 nm. The radical scavenging activity of the tested samples, expressed as % inhibition against $DPPH^{\bullet}$, was calculated as follows:

$$\text{Inhibition (\%)} = [(A_{control} - A_{sample})/A_{control}] \times 100.$$

EC_{50} values were obtained from the inhibition curve. Free radical scavenging activity determination was repeated four times for each sample and the means are reported.

1.5. Measurement of antioxidant activity by rancimat method

A 743 Rancimat apparatus (Metrohm AG, Switzerland) was used with the air supply at 20 l h^{-1} and the heating temperature was 100 °C. Sunflower oil without added antioxidant were run in duplicate. Each sample was dispersed in 3 g of sunflower oil rich in linoleic acid (65% of fatty acids) at concentrations of 0.02%, 0.5% and 1%. The test was run in triplicate.

1.6. Antioxidant activity in a linoleic acid system

Antioxidant activity of the fractions was determined using Fe^{+2} induced-linoleic acid-TBA peroxidation method (OHKAWA et al., 1976). Each sample, dissolved in methanol (0.1 ml), was mixed with linoleic acid emulsion (0.25 ml, 0.1 M) at the level of 1% and 0.02% (w/w) of linoleic acid. Linoleic acid emulsion was prepared in a mixture of phosphate buffer (0.064 mM, pH 7.4) and ethanol (1:1). Following the addition of 0.1 ml $FeCl_2$ solution (1 mM), the mixture was incubated at 40 °C for 24 h. Then, 0.5 ml of HCl (0.1 M), 0.2 ml of sodium dodecyl sulphate (9.8%), 0.9 ml of distilled water and 2 ml of TBA (0.6%) were added to the mixture which was heated at 80 °C in a water bath for 30 min. After cooling for 10 min in an ice-bath, 5.0 ml of *n*-butanol was added, and the mixture was centrifuged at 2000×g to recover the supernatant. Absorbance of the supernatant was measured spectrophotometrically at 532 nm. Antioxidant activity determination was repeated six times for each sample and the means are reported. A low absorbance value indicated a high antioxidant activity. Linoleic acid with no antioxidant addition was used as control. Percent inhibition of linoleic acid peroxidation was calculated from the following equation:

$$\text{Inhibition (\%)} = (A_{control} - A_{sample})/A_{control} \times 100$$

1.7. Statistical analysis

Variance analysis of the results was performed using General Linear Model procedure of SAS Statistical Software, Version 6 (SAS INSTITUTE, 1989). Multiple comparison of the means was performed by least significant difference (LSD) test at α=0.05 level.

2. Results and discussions

2.1. Amount of total phenolics

Table 1 shows the yields of fractions of sumac and cassia expressed as percentage of dry matter, and the total extractable phenols determined with Folin Ciocalteu reagent, using gallic acid as standard for calibration curve. Results of triplicate analyses are expressed as milligram of gallic acid equivalents (GAE) per gram of extract. Total extractable material with methanol:water (80:20) from defatted sumac and cassia was found to be 23.0% and 18.1%, respectively. For both plant materials, the highest fraction yield was obtained with water fraction, followed by ethyl acetate; however, the higher phenolic acid content was in the ethyl acetate fractions. The total phenolic contents in the ethyl acetate fractions of sumac and cassia were 454.4 mg GAE g^{-1} extract and 489.4 mg GAE g^{-1} extract, respectively. Although extraction yield of the water fraction of sumac was the highest among the fractions obtained from sumac, the total phenolics amount was the lowest.

Table 1. Yields and abbreviations of fractions obtained from aqueous methanol (80%) extracts of sumac and cassia

Fractions	Abbreviation	Extraction yields (%) (w/w on dry weight basis)	Total phenolic content (mg GAE[a]/g extract)[b]
Sumac – *n*-hexane	SH	0.5	105.3±0.53
Sumac – ethyl acetate	SE	9.7	454.4±0.79
Sumac – water	SW	12.8	37.4±0.06
Cassia – *n*-hexane	CH	0.48	n.d.
Cassia – ethyl acetate	CE	3.75	489.4±0.97
Cassia – water	CW	13.9	348.5±0.08

[a] Total phenolic content is expressed as gallic acid equivalents (GAE)
[b] Results are represented as means±standard deviation (n=4)
n.d.: not detected

2.2. Antioxidant activity

$DPPH^{\bullet}$ free radical scavenging, inhibition of sunflower peroxidation in a bulk-oil system with Rancimat test and inhibition of linoleic acid peroxidation in an oil-water emulsion, using Fe^{+2} induced linoleic acid-TBA system were used to measure the antioxidant activity of fractions of the polar extracts of sumac and cassia.

The $DPPH^{\bullet}$ free radical method determined the antiradical power of antioxidants. $DPPH^{\bullet}$ is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule (SANCHEZ-MORENO et al., 1999). Free radical scavenging activities of the fractions of sumac and cassia are given in Table 2.

Table 2. Free radical scavenging effects of *n*-hexane, ethyl acetate and water fractions of sumac and cassia on DPPH[a]

Sample[c]	Inhibition (%)			EC_{50}[b] (μg ml^{-1})
	Concentrations (μg ml^{-1})			
	3	6	12	
SH	7.64±0.40^{E}	13.56±0.67^{E}	27.20±0.40^{E}	22.48^{D}
SE	32.93±2.50^{A}	58.44±1.18^{A}	93.44±0.12^{A}	5.24^{A}
SW	2.25±0.45^{F}	6.40±0.20^{G}	11.46±0.70^{F}	50.40^{E}
CH	2.20±0.26^{F}	2.28±0.28^{F}	4.26±0.58^{G}	210.48^{F}
CE	27.40±3.06^{B}	52.20±1.65^{B}	92.32±0.56^{A}	5.98^{A}
CW	20.67±1.31^{C}	41.07±2.50^{C}	73.99±1.03^{B}	7.81^{B}
BHT	13.52±0.52^{D}	25.56±0.88^{D}	48.01±0.81^{D}	12.49^{C}
BHA	28.08±0.70^{B}	41.37±2.50^{C}	68.48±0.55^{C}	7.89^{B}

[a] Results are represented as means±standard deviation (n=4). Values in a column with different superscripts (A–G) are significantly different (P<0.05)
[b] The lower EC_{50} indicates higher antiradical activity
[c] For sample code see Table 1

Ethyl acetate fractions of sumac and cassia (SE and CE) and water fraction (CW) of cassia exhibited free radical scavenging activity. Their activities were stronger (P<0.05) than those of BHT and BHA at all the concentrations tested. SW and CH, however, showed no activity.

Free radical scavenging activities of ethyl acetate fractions of sumac and cassia increased from 32.93% to 93.44% and 27.40% to 92.32%, respectively, as concentration of total phenolics of SE and CE increased from 1×10^{-3} to 5×10^{-3} μg total phenolics per ml and 1.5×10^{-3} to 6×10^{-3} μg total phenolics per ml, respectively. The correlation between the total phenolics amount and free radical scavenging effect for ethyl acetate fractions of sumac and cassia were 0.98–0.99 (expressed as linear correlation coefficient, r). The concentration of antioxidant needed to decrease the initial sample concentration by 50% (EC_{50}) is a parameter widely used to measure the antioxidant power (SANCHEZ-MORENO et al., 1998). The lower the EC_{50} the higher the antiradical activity.

The antioxidant activity of the fractions on the peroxidation of sunflower oil, as determined by the Rancimat method, is shown in Table 3. The data obtained from three replicates of each concentration of 0.02%, 0.5% and 1% were averaged and given as Induction Index (induction time of sunflower oil+sample/induction time of sunflower oil).

Table 3. Induction index of sumac fractions added to sunflower oil at three concentrations by Rancimat

Sample[c]	Induction index[a,b]		
	0.02%	0.5%	1%
SE	1.21±0.05^{A}	2.39±0.10^{B}	3.69±0.11^{A}
SH	1.08±0.06^{A}	1.83±0.31^{C}	2.23±0.08^{B}
BHT	1.15±0.03^{A}	2.04±0.08^{C}	2.30±0.12^{B}
BHA	1.19±0.05^{A}	2.60±0.01^{A}	2.40±0.05^{B}

[a] Induction index: Induction time of sunflower oil+sample/Induction time of sunflower oil
[b] Results are represented as means±standard deviation (n=3). Values in a column with different superscripts (A–C) are significantly different (P<0.05)
[c] For sample code see Table 1

Table 4. Inhibitory (%) effects of *n*-hexane, ethyl acetate and water fractions on TBARS formation in Fe^{+2} induced linoleic acid peroxidation

Samples[c]	Inhibition (%)[a,b]	
	0.02%	1%
SH	17.70±3.30^{D}	52.49±1.40^{B}
SE	47.55±0.90^{C}	41.30±5.50^{B}
SW	15.40±0.98^{D}	32.90±4.70^{C}
CH	0.00±0.00^{E}	44.4±2.80^{B}
CE	50.65±1.75^{C}	44.38±3.45^{B}
CW	46.74±1.78^{C}	33.27± 6.5^{C}
BHT	56.24±1.75^{B}	88.26±1.80^{A}
BHA	61.84±1.05^{A}	87.31±1.10^{A}

[a] Inhibition % (capacity to inhibit the peroxide formation in linoleic acid)
[b] Results are represented as means±standard deviation (n=6). Values in a column with different superscripts (A–E) are significantly different (P<0.05)
[c] For sample code see Table 1

Higher induction index indicates higher antioxidant activity. Only the SE and SH showed antioxidant activity on the peroxidation of sunflower oil. There was no significant difference (P>0.05) between the activities of the fractions and the synthetic antioxidants tested at the level of 0.02%. SE exhibited higher (P<0.05) antioxidant power than BHT and BHA at 1%. Activity of SH was also similar (P>0.05) to that of BHT, BHA at the level of 1%. None of the fractions of cassia showed activity in sunflower oil. Induction period of sunflower oil measured on the Rancimat increased with an increasing concentration of ethyl acetate and hexane fraction of polar extract from sumac.

The antioxidant activities of *n*-hexane, ethyl acetate and water fractions from sumac and cassia at concentrations of 0.02% and 1% on linoleic acid peroxidation with Fe^{+2} induced linoleic acid peroxidation-TBA reactive substances were investigated and the results are shown in Table 4.

Although fractions of SE, CE and CW showed antioxidant activity comparable with that of BHT at the concentration of 0.02%, their effect was decreased at 1%. Antioxidant activities of SH, SW and CH were increased with increasing concentration, however, none of the fractions showed stronger activity than BHA and BHT at all the concentrations tested ($P<0.05$).

3. Conclusion

Results in this study clearly indicate that ethyl acetate fraction of methanolic extract of sumac, SE exhibited significant antioxidant activity in both oil and oil-in-water emulsion system. Free radical scavenging activity of SE was also higher than those of synthetic antioxidants at all concentrations tested. Ethyl acetate fraction of cassia, CE also retarded the linoleic acid peroxidation in oil-in-water emulsion and showed a remarkable free radical scavenger activity, however, it did not exhibit any activity in the sunflower oil.

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