Antioxidant and Antimutagenic Activities of Turkish Pistachio (*Pistacia vera*) Rosy Hull Extract

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In the present study, the ethanolic extract of the rosy hull of *P. vera* was isolated, and their antioxidant and antimutagenic properties were investigated. The antioxidant activity was determined by inhibition of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, total antioxidant activity and phenolic compounds. The antimutagenic activity was investigated by Ames *Salmonella*/microsome mutagenicity test. The bacterial mutant strains, *Salmonella typhimurium* TA98 and TA100 was used to determine antigenotoxic potentials of the test compounds. The IC_{50} value for DPPH radicals was 5.57 mg/ml. The total antioxidant activity increased with the increasing concentration of the extracts (1, 2.5 and 5 mg/ml), which contained linoleic acid emulsion. The total phenolic content was 17.86± 0.45 mg gallic acid equivalent/g extract. The results showed that the ethanolic extract of *P. vera* showed antimutagenic effects at 1, 0.1, and 0.01 mg/plate concentrations. To our knowledge, this is the first study of the antioxidant and antimutagenic activities of the ethanolic extract of the rosy hulls of Turkish Pistachio. Our results indicate that the consumption of the rosy hull of *P.vera* would exert several beneficial effects by virtue of their antioxidant and antimutagenic activities. These activities are an important topic in the medical field as well as in the food industry.

Key words: *Pistacia vera*, rosy hull, AMES, antioxidant.

Among the aromatic plants belonging to the family of Anacardiaceae, the genus *Pistacia* is noteworthy for its numerous species and varieties of wild-growing plants. Anatolia is surely one of the main origins of pistachio species[1]. Pistachio nut trees (*P. vera* L.) are cultivated mainly in the southeastern region of Turkey[2]. *Pistacia* species have caught up the interest of researchers due to the study on different part of this plant such as leaves, kernels, hulls and gum demonstrate various biological activities such as antioxidant[3-5], antimicrobial[5], anti-inflammatory[6,7], hypoglycemic[8] and anti-insect activities[9].

Exposure to genotoxic chemicals present in food, in the environment, and used in medical treatment can alter the genetic material permanently, and thus may lead cancer[10]. Antigenotoxic plants can counter or prevent the adverse effect caused by DNA damaging chemicals[11].

Investigation on pistachio green hull has showed antioxidant, antimicrobial and antimutagenic activity[5]. However, as far as we know, no literatures on antioxidant and antimutagenic activities of the ethanolic extract of the rosy hulls of Turkish Pistachio (*P. vera*) have

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been published. Thus, the objective of the present work was to determine the antioxidant and antimutagenic activities of the ethanolic extract of rosy hull of *P. vera*.

MATERIALS AND METHODS

Plant material

The rosy hulls of *P. vera* naturally growing plants were collected from Sanliurfa, Turkey. The rosy hulls were air-dried at room temperature for later analysis.

Preparation of ethanolic extract

The air dried and powdered rosy hulls were extracted with ethanol (Merck) using the Soxhlet apparatus. The extract was evaporated and then extracted in ethanol/water (1:1, v/v), and then kept in small sterile opac bottles under refrigerated conditions until used.

Bacterial strains

S. typhimurium TA98 and *S. typhimurium* TA100 strains were used for the antimutagenity test. The strains were analyzed for their histidine requirement, biotin requirement, the combination of both, rfa mutation, excision repair capability, the presence of the plasmid pKM101, and spontaneous mutation rate according to Mortelmans and Zeiger[12]. Working cultures were prepared by inoculating nutrient broth with the frozen cultures, followed by an overnight incubation at 37°C with gentle agitation[13].

Antioxidant activity

Determination of DPPH radical scavenging activity

Antioxidant activity of the extract was determined based on its ability to react with the stable 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical[14]. Fifty µl of the extract (2.5, 5 and 10 mg/ml in ethanol/water (1:1, v/v)) was added to 5 ml DPPH solution (% 0.004) in ethanol. After incubation at room temperature for 30 min, the absorbance of each solution was determined at 517 nm. Percentage of inhibition and the concentration of sample required for 50% scavenging of the DPPH free radical (IC_{50}) were determined. BHT and ascorbic acid were used as a control.

Total antioxidant activity by the β- carotene-linoleic acid method

The total antioxidant activity of the ethanolic extract of the rosy hull of *P. vera* was evaluated by the β- carotene- linoleic acid model[15]. 0.5 mg of the β-carotene in 1 ml of chloroform, 25 µl of linoleic acid and 200 mg of Tween-40 (polyoxyethylene sorbitan monopalmitate) were mixed together. The chloroform was completely evaporated using a vacuum evaporator and the resulting solution was diluted with 100 ml of oxygenated water. 2.5 ml aliquots of this mixture were transferred into different tubes containing 0.5 ml of samples at 1, 2.5 and 5 mg/ml concentrations in ethanol/water (1:1, v/v). The same procedure was repeated with the positive control BHT, ascorbic acid, and a blank. The emulsion system was incubated for up to 2 h at 50°C. Measurement of absorbance was continued until the color of β-carotene disappeared in the control. After this incubation period, absorbance of the mixtures was measured at 490 nm. All determinations were performed in triplicate.

The bleaching rate (R) of β-carotene was calculated using the following formula. R=ln (a/b)/ t where, ln= natural log, a= absorbance at time 0, b= absorbance at time t (120 min). The antioxidant activity (AA) was calculated in terms of percent inhibition relative to the control using the formula $AA= [(R_{Control}- R_{Sample})/R_{Control}]x100$. Antioxidative activities of the extracts were compared with those of BHT and ascorbic acid at 0.5 mg ml^{-1}.

Determination of total phenolic compounds

The phenolic constituent of the extract was determined by the method involving the Folin-Ciocalteu reagent and gallic acid as standard[16,17]. Two hundred microliter of extract solution containing 10 mg extract was added to a test tube. Then, 100 µl Folin-Ciocalteu reagent was added and tube was shaken vigorously. After 3 min, a 2 ml solution of Na_2CO_3 (0.5%) was added and the mixture was allowed to stand for 2 h with intermittent shaking. Absorbance was measured at 760 nm.

Content of phenolic compounds was determined as mg gallic acid equivalents per gram of mg/g GAE extract using the following linear equation based on the calibration curve: A=0.025C-0.0218, R^2= 0.994 where A is the absorbance and C gallic acid equivalents.

Antimutagenic activity

In our previous study, the mutagenity of the ethanolic extract of the rosy hull of *P. vera* was studied and the ethanolic extract of the rosy hull,

which was tested at three different concentrations including 1, 0.1, and 0.01 mg/plate, did not show any mutagenic effect in the mutagenicity assays performed with *S. typhimurium* TA98 and TA100[18]. In this study, the plate incorporation method was used to assess the results of antimutagenicity assays[19]. The known mutagens 4-nitro-o-phenylenediamine (4-NPD) 3 µg/plate) *S. typhimurium* TA98 and sodium azide (NaN_3) (8 µg/plate) for *S. typhimurium* TA100 were used as positive controls and ethanol/ water (1:1, v/v) was used as negative control in antimutagenicity tests. In the antimutagenicity test performed with these strains, 100 µl of the overnight bacterial culture, 100 µl mutagen, 100 µl test compounds at different concentrations (1, 0.1, and 0.01 mg/plate), and 500 µl phosphate buffer were added to 2 ml of the top agar containing 0.5 mM histidine/biotin. The mixture was poured onto minimal glucose plates. Histidine independent revertant colonies and viable cells were scored on plates after incubation at 37°C for 48 or 72 h.

For the antimutagenicity assays, the % inhibition was calculated according the formula given below.

[% Inhibition = (1- T/M) x 100]

Where T is the number of revertants per plate in the presence of mutagen and the test sample and M is the number of revertants per plate in the positive control. The antimutagenic effect (inhibition%) between 25-40% was defined as moderate antimutagenicity, 40% or more as strong antimutagenicity and 25% or less inhibition as no antimutagenicity[20-22].

RESULTS AND DISCUSSION

Free radical-scavenging capacity of the corresponding extract, measured by DPPH assay, and the IC_{50} values of the extract, BHT and ascorbic acid are shown in Table 1. Lower IC_{50} value indicates higher antioxidant activity.

The results indicate that the radical scavenging activity of BHT and ascorbic acid were higher than that of the extract. The DPPH scavenging capacity of the extract may be mostly related to their phenolic content. The DPPH radical is a widely used model to evaluate the antioxidant property of plant extracts[23].

Table 1. Free radical scavenging capasities of the ethanolic extract of *P. vera* rosy hull and standarts measured in DPPH assay

Sample	IC_{50} value (mg/ml)
P. vera rosy hull	5.57
BHT	0.95
Ascorbic acid	0.48

Table 2. Antioxidant activity (%) of the ethanolic extract of *P. vera* rosy hull in β-carotene- linoleic acid test system

Sample	Concentration (mg/ml)			
	0.5	1	2.5	5
P. vera rosy hull	-	83.46	87.97	93.26
BHT	98.68	-	-	-
Ascorbic acid	51.16	-	-	-

Total antioxidant activities of the ethanolic extract of *P. vera* rosy hull, according to β- carotene-linoleic acid method, are shown in Table 2. When screened for its radical scavenging and total antioxidant properties, the ethanolic extract of *P. vera* rosy hull provided dose-dependent results on different assays.

Phenolic compounds in plant extracts contribute significantly to their antioxidant potential because of their unique structure. Phenolics are composed of one (or more) aromatic rings bearing single or multiple hydroxyl groups and are therefore potentially able to quench free radicals by forming resonance stabilized phenoxyl radical[24,25].

In this study the phenolic content of the ethanolic extract of *P. vera* rosy hull was found as 17.86±0.45 GAE/g extract. The data obtained from

this part is especially shows a correlation with those obtained from the β- carotene- linoleic acid test system.

The possible antimutagenic potential of the ethanolic extract of the rosy hull of *P. vera* was examined against 4-NPD and NaN_3 in *S. typhimurium* TA 98 and TA 100, respectively. The results were evaluated by using standard plate incorporation method and summarized in Table 3.

Table 3. The antimutagenicity assay results of the ethanolic extract of the rosy hull of *P. vera* for *S. typhimurium* TA98 and TA 100 bacterial strains

Test items	Concentration (mg/plate)	Number of revertants			
		TA98		TA100	
		Mean± S. error	Inhibition%	Mean± S. error	Inhibition%
Negative control	-	7± 2.91		37.4± 6.02	
4-NPD*	3	405.6± 47.67		-	
NaN_3*	8	-		451± 58.15	
Extract	1	81.2± 33.23	80	261.2± 15.78	42.08
	0.1	209.8± 22.98	48.27	314.6± 7.79	30.24
	0.01	250.33± 11.37	38.28	350± 7.25	22.39

*4-NPD and NaN_3 were used as positive controls for S. *typhimurium* TA98 and TA100 strains, respectively

In the antimutagenicity assays performed with TA98 and TA100 strains, the ethanolic extract of the rosy hull of *P. vera* showed antimutagenic effects at 1, 0.1, and 0.01 mg/plate concentrations. The strongest antimutagenic activity was observed at 1 mg/ plate concentration against *S. typhimurium* TA 98 strain. As the results only one concentration (0.01 mg/plate) did not any antimutagenic effect against *S. typhimurium* TA 100. The data suggested that the ethanolic extract of the rosy hull of *P. vera* has better antimutagenic effect (38.28 - 80%) on the TA98 strain. The antimutagenic activity of the ethanolic extract of the rosy hull of *P. vera* was determined as dose dependent.

Rajaei *et al.*[5] showed that the crude and purified water extract of *P. vera* green hull were neither toxic nor mutagenic to the bacteria at the tested concentrations, and the extracts of *P. vera* green hull was showed antimutagenic and antioxidant activities.

The occurrence rate of cancer is increasing worldwide and the determination of chemopreventive or chemoprophylaxis compound is important in the effort to reduce the risk of cancer. A plant extract indicating antimutagenicity is not necessarily an anticarcinogen; however, it is an indication of possible candidates for such purposes[26].

CONCLUSION

The results of this study indicate that, the ethanolic extract of the rosy hull of *P. vera*, which were investigated in the present study, can be considered genotoxically safe at the tested concentrations and most of them provided important antimutagenic properties.

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