Assessment of Antioxidant Activity of Extracts from Unique Greek Varieties of *Leguminosae* Plants Using *In vitro* Assays

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Abstract. *Background: It is believed that legumes are a very good source of micronutrients and phytochemicals that present chemopreventive activity against diseases such as diabetes, coronary heart disease and colon cancer. Methanolic and aqueous extracts from 11 unique varieties of Leguminosae family plants cultured in Greece were tested using three different in vitro assays in order to investigate the mechanisms by which phytochemicals present in these legumes exert their chemoprevention. Materials and Methods: The extracts were tested by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay, the hydroxyl radical- and the peroxyl radical-induced DNA strand scission assays. Hydroxyl ($OH^{\bullet}$) and peroxyl ($ROO^{\bullet}$) radicals were generated from ultraviolet (UV) photolysis of hydrogen peroxide (H_2O_2) and thermal decomposition of 2,2'-azobis-(2-amidinopropane hydrochloride) (AAPH) respectively. Results: In the DPPH assay, all the tested extracts displayed potent radical scavenging efficiency. Furthermore, most of the Leguminosae family plant extracts exerted significant protective activity against DNA damage induced by both reactive oxygen species, although they were more effective in inhibiting $ROO^{\bullet}$-induced rather than $OH^{\bullet}$-induced DNA strand scission. Conclusion: The results suggest that the free radical scavenging activity of Leguminosae plants may be one of the mechanisms accounting for their chemoprevention.*

Abbreviations: Radical scavenging capacity, RSC; 2,2'-azo*bis*(2-amidinopropane hydrochloride), AAPH; 1,1-diphenyl-2-picrylhydrazyl, DPPH$^{\bullet}$; reactive oxygen species, ROS; hydrogen peroxide, H_2O_2; hydroxyl radical, $OH^{\bullet}$; peroxyl radical, $ROO^{\bullet}$.

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Nowadays, chemoprevention is considered as a very promising approach to the major problem of cancer. Food-derived products have been reported as an excellent source for the development of chemopreventive agents (1, 2). For example, several epidemiological and *in vivo* studies have shown that the consumption of foods such as fruits, vegetables and legumes are associated with reduced incidence of several cancer types (*e.g.* breast, prostate, colon and lung cancer) (2-4).

Legumes consumed worldwide are considered to have beneficial health implications in diseases such as diabetes, obesity (5), cardiovascular diseases (6) and various cancer types (7). Beans and other legumes have long been recognized for their high protein content but they also constitute a good source of starch, dietary fibers, folate, minerals such as Ca, Fe, K, Mg and Zn, and have a low fat content (6, 8). In addition to their nutritive value, legumes contain significant quantities of polyphenolic compounds such as flavonoids (*e.g.* kaempherol, quercetin, anthocyanins and tannins), isoflavones (*e.g.* genistein and daidzein), phenolic acids (*e.g. p*-hydroxybenzoic acid, vanillic acid, *p*-coumaric and ferulic acid) and lignans (9-13). Flavonoids and phenolic acids derived from many edible plant sources have also long been recognized for their significant antioxidant and chemopreventive activities (14, 15). Furthermore, the cancer chemopreventive and cardioprotective activity of isoflavones and lignans have been widely studied, as they make up an important part of soybean micronutrients (16-18).

However, only a few studies have been carried out so far for the mechanisms by which legumes (apart from soybeans) exert their chemopreventive activity. Therefore, the aim of the present study was to investigate extracts derived from unique varieties of *Leguminosae* plants cultured in Greece for their possible antioxidant and antimutagenic activity against reactive oxygen species (ROS)-induced DNA damage using three *in vitro* assays. Firstly, the antioxidant capacity of the extracts was assessed

using the DPPH radical scavenging assay. Afterwards, in order to examine the ability of extracts to inhibit DNA strand cleavage induced by hydroxyl and peroxyl radicals, two different ROS-induced DNA strand scission assays were used. Hydroxyl and peroxyl radicals were generated from UV photolysis of hydrogen peroxide (H_2O_2) and from thermal decomposition of 2,2'-azo*bis*-(2-amidinopropane hydrochloride) (AAPH) respectively. DNA strand breakage induced by ROS is considered to be involved in severe pathologies such as mutagenesis and carcinogenesis (19). In previous studies, we have shown that extracts derived from plants such as grapes were effective inhibitors of ROS-induced mutagenesis (20-21).

Materials and Methods

Chemicals. Hydrogen peroxide (H_2O_2) was purchased from Merck (Darmstandt, Germany). 2,2'-Azo*bis*(2-amidinopropane hydrochloride) (AAPH) and 1,1-diphenyl-2-picrylhydrazyl (DPPH$^\bullet$) were obtained from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals and solvents used in this study were of the highest purity commercially available.

Preparation of extracts. The air-dried aerial parts, seeds, seed coat and fructus of the tested plants were pulverized and extracted with methanol (2/1) and water (2/1) at room temperature. Each solvent extraction was repeated three times for 48 h per extraction. The solvents were evaporated under reduced pressure and the residues were diluted in methanol or water.

DPPH assay. The radical scavenging efficiency of the extracts was determined on the basis of the scavenging activity of DPPH$^\bullet$. The method of Brand-Williams *et al.* (22) was used with slight modifications. Briefly, the reaction was carried out in 1 ml of methanol containing 100 μM freshly made DPPH$^\bullet$ in methanol and the tested extracts at different concentrations (0.1, 0.5, 1, 5 and 10 mg/ml). The contents were vigorously mixed, incubated at room temperature in the dark for 20 minutes and the absorbance was read at 517 nm using a Hitachi U-1500 Spectrophotometer (San Jose, USA). In each experiment, the tested extract alone in methanol was used as blank and DPPH$^\bullet$ alone in methanol was used as control. All experiments were carried out in triplicate and on at least two separate occasions. The radical scavenging capacity (RSC) of the tested extracts was expressed as a percentage of DPPH$^\bullet$ elimination calculated according to the following equation:

$$\% \text{ RSC} = \frac{\text{absorbance of control} - \text{absorbance of tested extract}}{\text{absorbance of control}} \times 100$$

Moreover, IC_{50} showing the concentration of the tested extract scavenging DPPH$^\bullet$ radical by 50% was evaluated.

Hydroxyl radical-induced DNA strand scission assay. DNA strand breakage was measured by the conversion of supercoiled Bluescript-SK+ plasmid double-stranded DNA to open circular and linear forms. Hydroxyl radical-induced DNA relaxation assays were performed according to the method of Keum *et al.* (23) with some modifications. The reaction mixture (10 μl) consisted of 1 μg Bluescript-SK+ plasmid DNA isolated from a large scale bacterial culture, 10 mM Tris-HCl – 1 mM EDTA, the tested extract at different concentrations (100, 200, 400, 800, 1600 μg/ml) and 40 mM H_2O_2. Hydroxyl radicals (OH$^\bullet$) were generated from UV photolysis of hydrogen peroxide (H_2O_2) after irradiation of the reaction mixture with a 300 W UV lamp (OSRAM) for 3 min at a distance of 50 cm. The reaction was terminated by the addition of 3 μl loading buffer (0.25% bromophenol blue and 30% glycerol) and analyzed in 0.8% agarose gel electrophoresis at 70 V for 1 h. The gels were stained with ethidium bromide 0.5 μg/ml, destained with water, photographed by UV translumination using the Vilber Lourmat photodocumentation system (DP-001.FDC; Torcy, France) and analyzed with Gel-Pro Analyzer version 3.0 (MediaCybernetics, Silver Spring, USA). In addition, Bluescript-SK+ plasmid DNA was treated with each extract alone at the highest concentration used in the assay in order to test the effects of extracts on plasmid DNA conformation. Each experiment was repeated three times.

Peroxyl radical-induced DNA strand scission assay. The assay was performed using the procedure of Chang *et al.* (24). Peroxyl radicals were generated from thermal decomposition of 2,2'-azo*bis*(2-amidinopropane hydrochloride) (AAPH). The reaction mixture (10 μl) containing 1 μg Bluescript-SK+ plasmid DNA, 2.5 mM AAPH in phosphate-buffered saline (PBS) and the tested extract at different concentrations (5, 10, 20, 50, 100, 200 μg/ml) was incubated in the dark for 45 min at 37°C. After incubation, the reaction was terminated by the addition of 3 μl loading buffer (0.25% bromophenol blue and 30% glycerol) and analyzed in gel electrophoresis as described previously. Each experiment was repeated three times. In all three assays, oleuropein was used as a positive control since it is known for its potent antioxidant activity (25-26).

Percentage inhibition of radical-induced DNA strand scission. The percentage inhibition of radical-induced DNA strand cleavage by the extracts was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{(S_o - S)}{S_{control} - S_o} \times 100$$

Where $S_{control}$ is the percentage of supercoiled DNA in the negative control sample (without tested extracts and AAPH or H_2O_2+UV), S_o is the percentage of supercoiled plasmid DNA in the control sample (without tested extracts but in the presence of the radical initiating factor AAPH or H_2O_2+UV) and S is the percentage of supercoiled plasmid DNA in the sample with the tested extracts and AAPH or H_2O_2+UV. It should be noted that isolated Bluescript-SK+ plasmid DNA contained approximately 10-20% open circular DNA prior to treatment.

Statistical analysis. For statistical analysis, one-way ANOVA was applied followed by Dunnett's test for multiple pair-wise comparisons. Dose-response relationships were examined by Spearman's correlation analysis. Differences were considered significant at $p<0.05$.

Table I. *Antioxidant activity (IC_{50}) of the Leguminosae plant extracts. DPPH assay.*

	Plant part	Methanolic extract IC_{50} (mg/ml)	Aqueous extract IC_{50} (mg/ml)
Phaseolus vulgaris	Seeds	4.7±0.3*	7.6±0.3*
	Aerial	0.7±0.2*	0.25±0.05*
Vicia faba	Aerial	0.25±0.05*	0.17±0.01*
Vicia tenuifolia subsp. *stenophylla*	Fructus	2.7±0.1*	1.9±0.2*
	Aerial	3.1±0.1*	1±0.2*
Lens culinaris	Aerial	1.25±0.05*	0.4±0.05*
	Seeds	6.8±0.3*	2.3±0.2*
Lupinus albus	Seeds	3.7±0.1*	4.4±0.2*
	Aerial	0.7±0.1*	0.4±0.05*
	Seed coat	1.6±0.1*	0.6±0.1*
Lotus edulis	Aerial	2.4±0.1*	2.1±0.3*
	Fructus	2.7±0.1*	1.5±0.1*
Lotus longisiliquosus	Aerial	2.1±0.1*	0.4±0.02*
Tetragonolobus purpureus	Fructus	5.5±0.2*	0.8±0.04*
	Aerial	1.7±0.1*	0.6±0.05*
Lathyrus sativus	Aerial	0.6±0.1*	0.27±0.05*
Lathyrus clymenum	Aerial	-	6.5±0.2*
Lathyrus laxiflorus subsp. *laxiflorus*	Aerial part	0.056±0.005*	0.08±0.006*
Oleuropein[a]	-	0.065±0.002*	

[a]Oleuropein: was used as a positive control. Values are the mean±SD from two independent experiments carried out in triplicate. *$p<0.05$ when compared with control (DPPH plus methanol).

Results

Antioxidant activity of Leguminosae extracts. The free radical scavenging activity of *Leguminosae* extracts was assessed by DPPH assay. All of the tested extracts, both methanolic and aqueous, were effective scavengers of the $DPPH^{\bullet}$ radical. Table I lists the IC_{50} values which indicate the concentration of extracts necessary to reduce 100 μM of $DPPH^{\bullet}$ radical by 50%; the lower the IC_{50} value, the higher the antioxidant activity of the extract. IC_{50} data range from 0.056 to 7.6 mg/ml. According to the IC_{50} values, the most potent scavengers of the $DPPH^{\bullet}$ radical were the extracts of *Lathyrus laxiflorus* subsp. *laxiflorus*. The IC_{50} values of its aqueous and methanolic extracts were 0.08 mg/ml and 0.056 mg/ml respectively.

Protective effects of Leguminosae plant extracts against hydroxyl radical-induced DNA strand scission. The UV photolysis of hydrogen peroxide (H_2O_2) produces hydroxyl radicals ($OH^{\bullet}$) which cause DNA single-strand breaks resulting in the conversion of the supercoiled form to open circular form. Twelve methanolic and ten aqueous extracts derived from *Leguminosae* plants exhibited inhibitory activity particularly at high concentrations (Table II). Among them, methanolic extracts of aerial parts of *Lotus edulis* and *Lathyrus laxiflorus* subsp. *laxiflorus* and aqueous extracts of aerial parts of *Phaseolus vulgaris*, *Tetragonolobus purpureus* and *Lathyrus laxiflorus* subsp. *laxiflorus* showed concentration-dependent inhibition of hydroxyl radical-induced plasmid relaxation (Table II). Methanolic and aqueous *Lathyrus laxiflorus* subsp. *laxiflorus* extracts exerted the most potent inhibition. The methanolic extract showed 52%, 62% and 69% inhibitory activity at concentrations of 400 μg/ml, 800 μg/ml and 1600 μg/ml respectively (Figure 1, 2A; Table II), while the aqueous extract showed 36%, 44% and 55% inhibitory activity at 400 μg/ml, 800 μg/ml and 1600 μg/ml respectively (Figure 1, 2B; Table II). As mentioned above, these extracts were also the most potent DPPH radical scavengers (Table I).

In addition to these two very potent extracts, aqueous extract of aerial parts of *Tetragonolobus purpureus* was also an efficient extract exhibiting 18%, 29%, 38% and 47% inhibitory activity at 200 μg/ml, 400 μg/ml, 800 μg/ml and 1600 μg/ml respectively (Figure 1, 2C, Table II). The other methanolic and aqueous extracts inhibited plasmid relaxation to a lesser extent reaching 20-42% inhibition at the highest tested concentration of 1600 μg/ml (Table II). For example, the aqueous extract of aerial parts of *Phaseolus vulgaris* showed 34% inhibition at 1600 μg/ml (Figure 1, 2D; Table II).

Protective effects of Leguminosae plant extracts against peroxyl radical-induced DNA strand scission. In this assay, peroxyl free radicals ($ROO^{\bullet}$) were produced from thermal decomposition of AAPH 2,2'-azo*bis*(2-amidinopropane hydrochloride). When Bluescript-SK+ plasmid DNA was incubated for 45 minutes in the dark in the presence of peroxyl radicals, the supercoiled conformation converted into the open circular form.

As shown by the results in Table III, most of both methanolic and aqueous extracts exerted concentration-dependent inhibition of peroxyl radical-induced DNA damage (Figure 3). Only three aqueous extracts, *P. vulgaris* seed extract, *Lupinus albus* seed coat extract and *Lathyrus clymenum* aerial part extract showed no statistically significant inhibitory activity at any of the tested concentrations (5 μg/ml-200 μg/ml) (Table III). From the other extracts, thirteen methanolic and thirteen aqueous extracts inhibited peroxyl radical-induced DNA damage by 40-100% at the highest tested concentration (200 μg/ml). Only methanolic extract of seeds and extract of aqueous aerial parts of *L. albus* showed 16% and 24% inhibition respectively at 200 μg/ml. Similar to the results obtained from both DPPH assay and hydroxyl radical-induced DNA strand scission (Tables I, II), the most potent extracts against peroxyl radical-induced DNA strand breakage were methanolic and aqueous extracts of aerial parts of *L.*

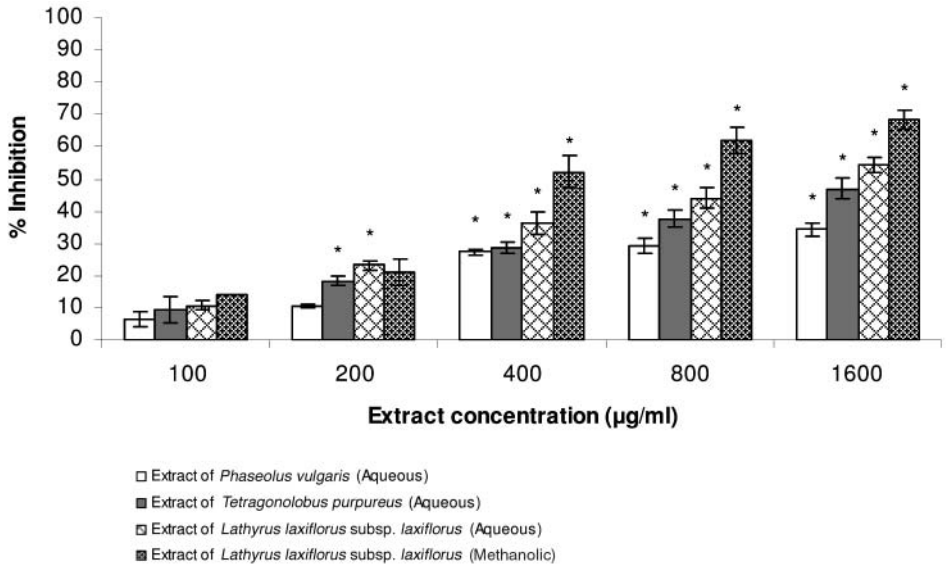

Figure 1. *Inhibitory activity of four Leguminosae plant extracts against hydroxyl radical-induced DNA strand scission. Values are the mean of three independent experiments and error bars denote standard mean error. *p<0.05 when compared with control.*

laxiflorus subsp. *laxiflorus* which showed 40% and 51% inhibition respectively at 5 μg/ml and reached 100% inhibition at 200 μg/ml (Table III).

It should be noted that methanol used as a solvent inhibited hydroxyl radical and peroxyl radical-induced DNA strand scission. Thus, methanolic extracts of *P. vulgaris* and *Lens culinaris* seed extracts as well as *L. albus* aerial part extract dissolved in methanol were not tested with the above assays.

Discussion

Legumes are a worldwide consumed functional food and are gaining a lot of interest for the beneficial health effects attributed to their phytochemical compounds (7-9, 16). Since the mechanisms by which legumes exert their chemopreventive activity have not been elucidated, the aim of the present study was to examine the possible antioxidant and protective activity against ROS-induced DNA damage of methanolic and aqueous extracts derived from unique plant varieties cultured in Greece by using different *in vitro* assays.

According to the IC_{50} values (Table I), the significant $DPPH^{\bullet}$ radical scavenging capacity of all the tested *Leguminosae* plant extracts indicates that they contain compounds capable of scavenging free radicals. Antioxidant activity of different *P. vulgaris* extracts has been previously assessed using a DPPH assay and a fluorescence-based lipid oxidation bioassay (13, 27, 28). The polyphenolic compounds isolated from them, such as flavonols, phenolic acids, proanthocyanidins and condensed tannins were considered to be responsible for the observed antioxidant activity. A DPPH assay was also used in order to assess the antioxidant activity of extracts of *Lens culinaris* seed coat and cotyledon (29, 30). The antioxidant activity of these extracts was also attributed to the high concentrations of phenolic compounds, especially flavonoids, present in the seed coat. Because the extracts tested in the present study displayed strong antioxidant activity, two different *in vitro* assays were used in order to test if the extracts could prevent ROS-induced DNA strand breakage.

All the extracts (apart from three), both methanolic and aqueous, showed significant inhibition of $OH^{\bullet}$-induced DNA strand breakage. Among the extracts, those derived from *L. laxiflorus* subsp. *laxiflorus* were the most potent. The difference in antioxidant efficiency observed between the different extracts may be attributed to their different chemical composition. The protective activity of *Leguminosae* plant extracts against hydroxyl radical-induced DNA damage may be due to their ability to prevent the reaction of $OH^{\bullet}$ with hydrogen atoms at C3', C4' and C5' sites of the sugar moiety of DNA, since that reaction leads to DNA strand breaks (31). Moreover, it has been proposed that UV radiation induces the production of ROS such as $OH^{\bullet}$ radicals which damage macromolecules such as lipids, proteins and DNA resulting in the onset of several skin diseases including skin cancer (32, 33). Thus, the present results suggest that these extracts could be used as botanical supplements in skin care products such as sunscreens, skin care lotions and moisturizing creams to prevent the detrimental effects of solar UV radiation.

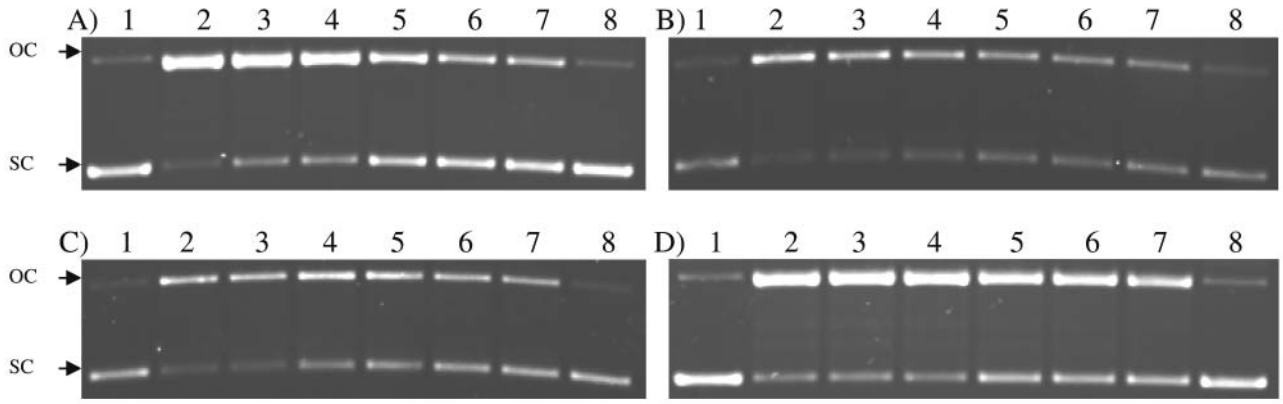

Figure 2. *Effects of Leguminosae plant extracts on DNA strand scission induced by hydroxyl radicals generated from UV photolysis of H_2O_2. A, Methanolic extract of the aerial part of Lathyrus laxiflorus subsp. laxiflorus; B, Aqueous extract of the aerial part of Lathyrus laxiflorus subsp. laxiflorus; C, Aqueous extract of the aerial part of Tetragonolobus purpureus; D, Aqueous extract of the aerial part of Phaseolus vulgaris. Lane 1: negative control; lane 2: H_2O_2+UV; lanes 3-7: H_2O_2+UV+100 μg/ml, 200 μg/ml, 400 μg/ml, 800 μg/ml and 1600 μg/ml respectively; lane 8: 1600 μg/ml extract alone. OC: Open circular DNA; SC: Supercoiled plasmid DNA.*

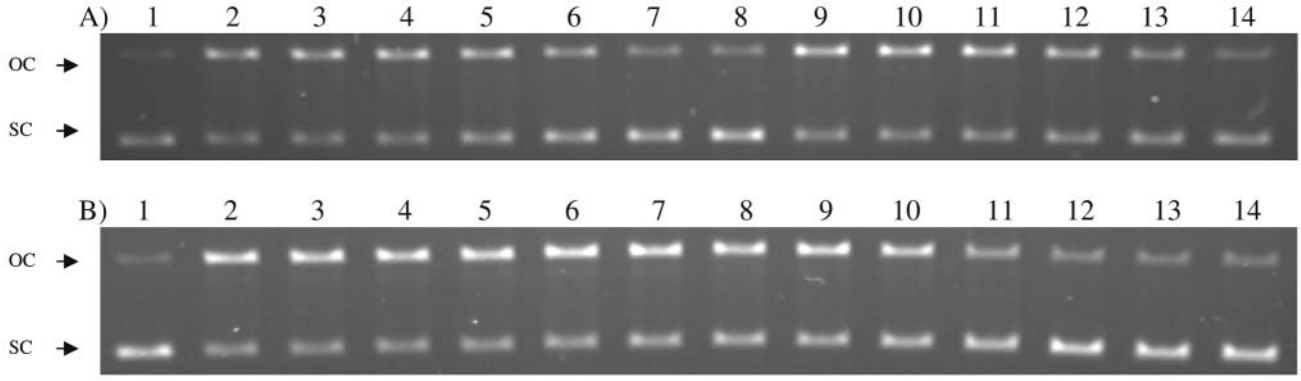

Figure 3. *Effect of Leguminosae plant extracts on peroxyl radical-induced plasmid DNA stand scission. A, Methanolic extract of fructus and aerial parts of Lotus edulis. Lane 1: negative control; lane 2: 2.5 mM AAPH; lanes 3-8: AAPH plus 5 μg/ml, 10 μg/ml, 20 μg/ml, 50 μg/ml, 100 μg/ml and 200 μg/ml fructus extract respectively; lanes 9-14: AAPH plus 5 μg/ml, 10 μg/ml, 20 μg/ml, 50 μg/ml, 100 μg/ml and 200 μg/ml aerial part extract respectively. B, Aqueous extract of seeds and aerial parts of Phaseolus vulgaris. Lane 1: negative control; lane 2: 2.5 mM AAPH; lanes 3-8: AAPH plus 5 μg/ml, 10 μg/ml, 20 μg/ml, 50 μg/ml, 100 μg/ml and 200 μg/ml seed extract respectively; lanes 9-14: AAPH plus 5 μg/ml, 10 μg/ml, 20 μg/ml, 50 μg/ml, 100 μg/ml and 200 μg/ml aerial part extract respectively.OC: Open circular DNA; SC: Supercoiled plasmid DNA.*

Most of the tested extracts, both methanolic and aqueous, were more potent inhibitors of DNA strand breakage activity induced by AAPH than by UV. The extracts exerted significant inhibitory activity against AAPH-induced conversion of supercoiled plasmid DNA conformation at concentrations ranging from 5 to 200 μg/ml, while they had no effect on UV-induced DNA strand breakage at these concentrations. This result could be attributed to the different radical species produced by AAPH and UV. Thermal decomposition of AAPH generates peroxyl radicals, while UV photolysis of H_2O_2 produces $OH^{\bullet}$. Furthermore, peroxyl radicals comprise a major initiating factor of lipid peroxidation chain reactions (34). Thus, the ability of the extracts to protect DNA strand breakage by scavenging peroxyl radicals could suggest that these extracts may also prevent lipid peroxidation. For example, isoflavones present in soybeans have been shown to inhibit low density lipoprotein oxidation (35, 36).

In conclusion, the results of the present study suggest that the tested *Leguminosae* plant extracts had high antioxidant activity by scavenging $DPPH^{\bullet}$ radicals. Moreover, most of the tested extracts inhibited both hydroxyl and peroxyl radical-induced DNA strand scission. To the best of our knowledge, this is the first report regarding the antioxidant activity of the tested *Leguminosae* family varieties apart from *P. vulgaris* and L. culinaris. The latter have been

Table II. *Inhibition of hydroxyl radical-induced DNA strand scission by Leguminosae plant extracts.*

Dose (μg/ml)		Methanolic extract						Aqueous extract					
	Plant part	100	200	400	800	1600	r	100	200	400	800	1600	r
		% Inhibition						% Inhibition					
Phaseolus vulgaris[a]	Aerial	10±3	10±3	31±4*	37±4*	38±2*	0.881	NI	11±2	27±1*	29±3*	34±2*	0.934
	Seeds	NT	NT	NT	NT	NT	-	NI	NI	23±2*	19±1*	18±1*	0.681
Vicia faba	Aerial	NI	NI	27±2*	29±3*	32±3*	0.774	NI	NI	28±3*	31±2*	37±3*	0.960
Vicia tenuifolia subsp.	Fructus	NI	NI	NI	NI	NI	-	NI	NI	NI	NI	NI	-
stenophylla	Aerial	NI	NI	19±2	19±1	23±1*	0.776	NI	NI	NI	NI	16±3	-
Lens culinaris	Aerial	NI	NI	31±3*	32±2*	33±3*	0.848	NI	NI	19±1*	18±1*	27±3*	0.758
	Seeds	NT	NT	NT	NT	NT	-	NI	NI	11±3	13±2	16±3	0.734
Lupinus albus	Seeds	NI	NI	NI	NI	NI	-	NI	NI	NI	NI	NI	-
	Aerial	NT	NT	NT	NT	NT	-	NI	NI	17±2*	16±1*	20±1*	0.859
	Seed coat	NI	NI	NI	NI	NI	-	NI	NI	NI	NI	NI	-
Lotus edulis	Aerial	NI	8±2	28±2*	35±1*	42±2*	0.974	NI	NI	21±2*	22±2*	30±3*	0.931
	Fructus	NI	NI	32±1*	34±2*	37±3*	0.840	NI	NI	NI	NI	NI	-
Lotus longisiliquosus	Aerial	NI	NI	30±2*	33±1*	33±1*	0.905	NI	NI	13±5	14±4	20±3	0.805
Tetragonolobus purpureus	Fructus	NI	NI	18±4	21±2*	23±3*	0.729	NI	12±3	15±2	21±5	26±3*	0.925
	Aerial	NI	NI	26±2*	27±2*	32±2*	0.768	9±4	18±1*	29±2*	38±3*	47±3*	0.975
Lathyrus sativus	Aerial	NI	NI	28±3	24±1*	31±2*	0.851	NI	NI	NI	NI	13±2	-
Lathyrus clymenum	Aerial	-	-	-	-	-	-	NI	NI	NI	NI	NI	-
Lathyrus laxiflorus subsp.	Aerial	14±3	21±4	52±5*	62±4*	69±3*	0.966	11±1	23±1*	36±3*	44±3*	55±2*	0.981
laxiflorus													
Oleuropein[b]	-	NI	11±2	23±2*	33±3*	42±3*	0.986						

Values are the means±SE of % inhibition from three independent experiments. [a]*Phaseolus vulgaris* was cultivated in Vrondou, Serres, Greece. [b]Oleuropein was used as positive control. r: represents the dose-response correlation coefficient. NI: no significant inhibition (less than 8%). NT: not tested. *$p<0.05$ when compared with control (plasmid DNA plus H_2O_2+UV).

Table III. *Inhibition of peroxyl radical-induced DNA strand scission by Leguminosae plant extracts.*

Dose (μg/ml)		Methanolic extract							Aqueous extract						
	Plant part	5	10	20	50	100	200	r	5	10	20	50	100	200	r
		% Inhibition							% Inhibition						
Phaseolus vulgaris[a]	Aerial	NI	14±4	24±1*	56±2*	81±6*	93±4*	0.968	16±5	26±4	57±4*	84±1*	87±1*	91±1*	0.987
	Seeds	NT	NT	NT	NT	NT	NT	-	NI	NI	NI	NI	10±2	18±4	0.904
Vicia faba	Aerial	NI	13±2	39±4*	69±3*	78±4*	84±5*	0.979	10±1*	26±1*	58±1*	87±1*	93±1*	96±1*	0.991
Vicia tenuifolia	Fructus	NI	NI	NI	19±1	37±4*	65±2*	0.985	NI	NI	14±3	38±3*	63±3*	80±3*	0.958
subsp. *stenophylla*	Aerial	NI	NI	NI	17±5	36±5	67±6*	0.972	NI	NI	15±4	43±3*	67±5*	84±6*	0.950
Lens culinaris	Aerial	NI	NI	20±2*	44±4*	61±3*	78±1*	0.971	NI	14±2*	32±2*	67±3*	73±1*	84±3*	0.984
	Seeds	NT	NT	NT	NT	NT	NT	-	NI	NI	NI	31±4	49±2*	73±1*	0.897
Lupinus albus	Seeds	NI	NI	NI	10±3	13±2*	16±1*	0.968	NI	NI	NI	NI	NI	9±3	-
	Aerial	NT	NT	NT	NT	NT	NT	-	NI	NI	NI	14±6	17±4	26±4*	0.757
	Seed coat	NI	NI	28±3*	49±4*	64±2*	67±3*	0.975	NI	NI	NI	14±4	17±2	20±3	0.732
Lotus edulis	Aerial	NI	NI	24±3*	50±3*	79±1*	90±4*	0.983	NI	NI	15±5	41±3*	67±4*	76±3*	0.919
	Fructus	NI	NI	NI	21±2*	40±3*	74±4*	0.947	NI	NI	10±3	49±5*	71±2*	81±2*	0.977
Lotus longisiliquosus	Aerial	NI	NI	12±2	34±1*	72±2*	92±2*	0.943	NI	12±1	33±2*	74±2*	84±3*	84±3*	0.976
Tetragonolobus	Fructus	NI	NI	NI	8±2	24±1*	48±4*	0.841	NI	NI	NI	16±2	39±3*	67±3*	0.976
purpureus	Aerial	NI	NI	9±1	30±2*	58±5*	88±2*	0.953	NI	11±4	34±2*	73±3*	94±3*	99±1*	0.976
Lathyrus sativus	Aerial	NI	20±1*	38±3*	87±4*	96±2*	93±4*	0.985	NI	12±2	45±3*	73±4*	75±4*	78±3*	0.920
Lathyrus clymenum	Aerial	-	-	-	-	-	-	-	NI	NI	NI	NI	NI	NI	-
Lathyrus laxiflorus	Aerial	40±2*	73±5*	89±6*	89±6*	93±7*	98±3*	0.851	51±6*	83±4*	84±2*	97±2*	99±1*	99±1*	0.852
subsp. *laxiflorus*															
Oleuropein[b]	-	36±3*	57±4*	75±1*	93±1*	95±1*	95±1*	0.947							

[a]*Phaseolus vulgaris* was cultivated in Vrondou, Serres, Greece. [b]Oleuropein was used as positive control. Values are the means±SE of % inhibition from three independent experiments. r: represents the dose-response correlation coefficient. NI: no significant inhibition (less than 8%). NT: not tested. *$p<0.05$ when compared with control (plasmid DNA plus AAPH).

previously tested for their antioxidant (13, 27-29) and antimutagenic ativity (37, 38). The present results indicate that the antioxidant activity of *Leguminosae* plant extracts may be one of the mechanisms accounting for their possible chemopreventive abilities.

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