

Biological Activity of Some Naturally Occurring Resins, Gums and Pigments Against *In Vitro* LDL Oxidation

Nikolaos K. Andrikopoulos^{1*}, Andriana C. Kaliora¹, Andreana N. Assimopoulou² and Vassilios P. Papapeorgiou²

¹Food Chemistry Laboratory, Science of Dietetics-Nutrition Department, Harokopio University, 70 El. Venizelou Ave., 176 71 Kallithea, Athens, Greece

²Organic Chemistry Laboratory, Chemical Engineering Department, Faculty of Engineering, Aristotle University of Thessaloniki, 540 06 Thessaloniki, Greece

Naturally occurring gums and resins with beneficial pharmaceutical and nutraceutical properties were tested for their possible protective effect against copper-induced LDL oxidation *in vitro*. Chios mastic gum (CMG) (*Pistacia lentiscus* var. *Chia* resin) was the most effective in protecting human LDL from oxidation. The minimum and maximum doses for the saturation phenomena of inhibition of LDL oxidation were 2.5 mg and 50 mg CMG (75.3% and 99.9%, respectively). The methanol/water extract of CMG was the most effective compared with other solvent combinations. CMG when fractionated in order to determine a structure-activity relationship showed that the total mastic essential oil, collofonium-like residue and acidic fractions of CMG exhibited a high protective activity ranging from 65.0% to 77.8%. The other natural gums and resins (CMG resin 'liquid collection', *P. terebinthus* var. *Chia* resin, dammar resin, acacia gum, tragacanth gum, storax gum) also tested as above, showed 27.0%–78.8% of the maximum LDL protection. The other naturally occurring substances, i.e. triterpenes (amyrin, oleanolic acid, ursolic acid, lupeol, 18- α -glycyrrhetic acid) and hydroxynaphthoquinones (naphthazarin, shikonin and alkannin) showed 53.5%–78.8% and 27.0%–64.1% LDL protective activity, respectively. The combination effects (68.7%–76.2% LDL protection) of ursolic-, oleanolic- and ursodeoxycholic- acids were almost equal to the effect (75.3%) of the CMG extract in comparable doses. Copyright © 2003 John Wiley & Sons, Ltd.

Keywords: *Pistacia* spp.; hydroxynaphthoquinones; pentacyclic triterpenes; acacia gum; tragacanth gum.

INTRODUCTION

The naturally occurring resins, gums and pure substances (triterpenes and hydroxynaphthoquinones), examined in the present study, have been previously recognized for their beneficial pharmaceutical and/or nutraceutical properties, but the biological effect on the *in vitro* or *in vivo* oxidation of human low-density lipoproteins (LDL) has not been investigated yet.

LDL is the main carrier of cholesterol to tissues and is implicated in the deposition of cholesterol to endothelial tissue, leading to the formation of atherosclerotic plaque, which is one of the main factors for cardiovascular heart disease (CHD) (Goldstein and Brown, 1997). In recent investigations (Steinbrecher *et al.*, 1990) the oxidatively modified LDL, probably resulting from an inadequate intake of antioxidants, has been shown to enhance the formation of foam cells in the atherosclerotic plaque, consequently increasing the risk of CHD. Therefore, a dietary intake of antioxidants or absorption of drugs resulting in the transportation of LDL protecting substances to the blood circulation may play an important role in decreasing the risk of CHD.

Pistacia lentiscus var. *Chia* cv. Anacardiaceae, which is grown almost exclusively on Chios Island, Greece, gives a resinous exudate, the crude Chios mastic gum (CMG) or simply mastic gum, after longitudinal incisions at close intervals from the base of the trunk up to the thicker branches of the tree, characterized as 'normal collection'. Recently, a new type of resin collection has been developed. This includes either the addition or injection of phytohormones to the tree and so that the resin is collected in a less viscous form, the so-called 'liquid collection'. In the 'liquid type' collection, resin production occurs without cutting the tree, as happens in the 'normal collection'. CMG is basically consumed as chewing gum and also in other culinary uses, especially in the Greek, Turkish and Arabic kitchen, i.e. in powder form as a food additive, in the form of sugar containing gel as a table-sweetener and as mastic oil as a drink and sweetener additive. The treatment of various gastric malfunctions, such as gastralgia, dyspepsia, gastric ulcer (Al Said *et al.*, 1986) and recently ulcer therapy through the encapsulation of the bacteria *Helicobacter pylori* (Huwez *et al.*, 1998) have been well documented, while other uses as a food preservative (Willblock, 1999) or antioxidant (Abdel-Rahman and Soad, 1975) have also been reported. Mastic gum mainly consists of triterpenes of the oleanane, euphane and lupane type.

The resin of *Pistacia terebinthus* var. *Chia*, a very similar species to *P. lentiscus*, is the air-dried resinous

* Correspondence to: Dr N. K. Andrikopoulos, Food Chemistry Laboratory, Science of Dietetics-Nutrition Department, Harokopio University, 70 El. Venizelou Ave., 176 71 Kallithea, Athens, Greece.
E-mail: nandrikop@hua.gr

exudate, obtained exactly as the CMG resin by 'normal collection'. *P. terebinthus* resin has been used extensively for the treatment of cancer as reported in the Bible. Dioscorides suggested that terebinth was antidotal, aphrodisiac and expectorant and suitable for the treatment of leprosy. It also has the stimulant and diuretic properties of the coniferous turpentine. It is said to be used in Greece for mixing in small proportions with wine, in order to preserve it (Duke, 1983). Dammar resin is extracted from plants belonging to the family *Dipterocarpaceae* and consists mainly of tetracyclic dammarane type terpenes (Ioakeimoglou, 1993).

Acacia gum or gum arabic, which is the dried exudate from *Acacia* species, is mainly used in oral and topical pharmaceutical formulations as a suspending and emulsifying agent, often in combinations with tragacanth gum. It is also used in the preparation of pastilles and lozenges and as a tablet binder. Acacia gum is also used in cosmetics and foods. Tragacanth gum, which is the plant exudate of the genus *Astragalus*, is used as an emulsifying and suspending agent in a variety of pharmaceutical formulations and also in creams, gels and emulsions, in tablet formulations and food products (Wade and Weller, 1994). Storax gum, which is the oleoresinous substance derived from the trunk of *Liquidambar orientalis*, is used in perfumes, externally for various skin diseases and internally as an expectorant (Fokas, 1984).

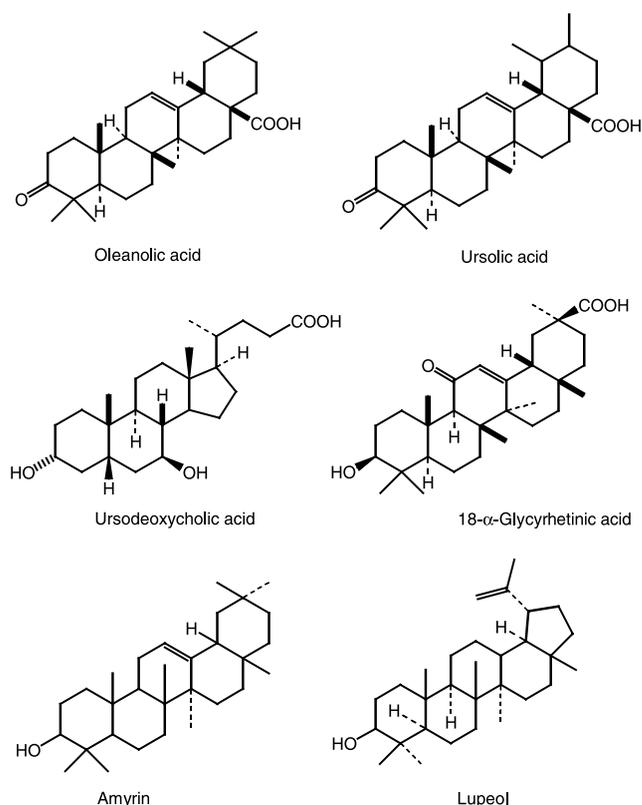
Several triterpenes and naphthoquinones have been identified as the constituents of gum/resin of *Boraginaceae* root respectively, with well-established beneficial properties. The chiral hydroxynaphthoquinones alkannin and shikonin, which are pigments, have been proven to possess strong wound healing, antibacterial, antiinflammatory and anticancer activities (Papageorgiou *et al.*, 1999), while ursolic and its isomer oleanolic acid have been strongly related to antibiotic, antiinflammatory, antitumoral, antidiabetic, hypolipidaemic, antiatherosclerotic, antiulcer and hepatoprotective properties (Es Saady *et al.*, 1994).

In the present study the biological activity of extracts of the aforementioned constituents in different solvents, in minimum and maximum optimum doses, against copper induced LDL oxidation is reported for the first time.

MATERIALS AND METHODS

Materials. Mastic oil, which is the essential oil of *P. lentiscus* resin, was obtained by steam distillation of CMG (1.5 atm steam pressure, 105–100 °C). The colloid-like residue is the insoluble material that occurs after dilution of CMG in diethylether and methanol. CMG from the 'normal' and 'liquid' type collections and *P. terebinthus* resin, were kindly donated by the Chios Mastic Growers' Association. The neutral fraction, acidic emulsion and acidic fractions (from both Na₂CO₃ and NaOH) of *P. lentiscus* resin were obtained according to the fractionation process proposed by Barton and Seoane (1956).

Acacia and tragacanth gums, as well as ursodeoxycholic and oleanolic acids, were purchased from Aldrich (Milwaukee, WI), while dammar resin was from Fluka (Buchs, G), storax gum, 18- α -glycyrrhetic and ursolic acids were from Sigma (St Louis, MO). Alkannin was



Scheme 1. Chemical formulae of naturally occurring triterpenes.



Scheme 2. Chemical formulae of naturally occurring naphthoquinones (pigments).

purchased from Carl Roth (Karlsruhe, G), shikonin from Ikeda Corp (Tokyo, J) and naphthazarin from Fluka. Lupeol and amyrin were from Extrasynthese (Genay, F). The chemical formulae of the pure triterpenes and naphthoquinones used are presented in Schemes 1 and 2, respectively.

Sterilized LDL containing 6.3 mg protein 400 mg LDL and thiobarbituric acid were purchased from Sigma and malonaldehyde from Aldrich (Steinheim, G). Other reagents and chemicals were of analytical grade from Merck (Darmstadt, G).

Extraction and dilution procedures. CMG (0.5 g) was extracted according to the solvent sequence methanol/water 60:40, v/v and hexane. The first extracting solvent (5 mL \times 2) after removal from the residual gum, was evaporated under a stream of nitrogen and the residue was diluted with the second solvent (250 μ L \times 2), which was transferred to a new screw capped glass tube and appropriate volumes were used for the biological tests, e.g. 2.5, 5, 10, 25 and 50 μ L, corresponding to the respective weight (mg) in the test cuvette (2 mL), shown in Table 1. The CMG fractions, as well as the

Table 1. Biological tests expressed as TBARS values (μM) and % protection (% Pr) for Chios mastic gum (CMG) extracts (M/W, H)

Test ($n = 4$)	CMG (mg)		Incubation period		
			1 h	3 h	6 h
oxLDL	–	TBARS ^a	1.412 \pm 0.292	2.294 \pm 0.352	2.694 \pm 0.345
		TBARS net ^{a,b}	1.412 \pm 0.292	2.194 \pm 0.437	2.471 \pm 0.387
1	2.5	TBARS ^a	0.724 \pm 0.260	0.755 \pm 0.151	0.833 \pm 0.225
		TBARS net ^{a,b}	0.724 \pm 0.260	0.655 \pm 0.300	0.610 \pm 0.285
		% Pr ^{a,c}	48.7 \pm 27.7	70.1 \pm 24.1	75.3 \pm 19.5
2	5.0	TBARS ^a	0.677 \pm 0.165	0.742 \pm 0.136	0.808 \pm 0.158
		TBARS net ^{a,b}	0.677 \pm 0.165	0.642 \pm 0.292	0.585 \pm 0.236
		% Pr ^{a,c}	52.0 \pm 23.8	70.7 \pm 23.9	76.3 \pm 18.3
3	10.0	TBARS ^a	0.641 \pm 0.145	0.664 \pm 0.184	0.675 \pm 0.161
		TBARS net ^{a,b}	0.641 \pm 0.145	0.564 \pm 0.318	0.452 \pm 0.238
		% Pr ^{a,c}	54.5 \pm 23.1	74.3 \pm 24.6	81.7 \pm 18.4
4	25.0	TBARS ^a	0.146 \pm 0.142	0.404 \pm 0.174	0.450 \pm 0.134
		TBARS net ^{a,b}	0.146 \pm 0.142	0.304 \pm 0.312	0.227 \pm 0.220
		% Pr ^{a,c}	89.6 \pm 23.0	86.1 \pm 24.4	90.8 \pm 18.0
5	50.0	TBARS ^a	0.139 \pm 0.120	0.160 \pm 0.162	0.223 \pm 0.157
		TBARS net ^{a,b}	0.139 \pm 0.120	0.060 \pm 0.305	0.000 \pm 0.235
		% Pr ^{a,c}	90.1 \pm 22.3	97.2 \pm 24.3	99.9 \pm 18.3
oxLDL-blank	–	TBARS ^a	0.000 \pm 0.001	0.100 \pm 0.259	0.223 \pm 0.175
Significance tests					
Test 1 versus oxLDL test			$p < 0.00003$	$p < 0.000008$	$p < 0.0000005$
Test 5 versus oxLDL test			$p < 0.00001$	$p < 0.000002$	$p < 0.0000009$

For oxLDL and oxLDL blank Tests see Materials and Methods.

LDL, low density lipoprotein; H, hexane; M/W, methanol/water (60:40, v/v).

CMG (mg) oxLDL test plus the extract from the indicated quantity (finaling in the cuvette).

^a Thiobarbituric acid reactant substances mean value $\pm t_{v,\alpha}$ (standard deviation of the mean, SD_i), = critical value (5.841) of t for $v = (n - 1) = 3$, % P (confidence) = 100 (1 - 2 α) = 99% (or significance level $p = 0.01$).

^b Net oxidation = [(TBARS value of Test i) - (TBARS value of oxLDL blank)] and SD_{net i} (standard deviation for Test i) = (SD_i² + SD_{blank}²)^{1/2}.

^c % Pr = [(TBARS net of oxLDL) - (TBARS net of Test i)] \times 100 / (TBARS net of oxLDL) and SD = [(SD_{net i})² + (SD_{net i})²]^{1/2} \times 100 / (TBARS net of oxLDL).

Table 2. Biological activity of Chios mastic gum (2.5 mg, final in the cuvette) extracts, with different solvent combinations, against the *in vitro* LDL oxidation, expressed as % Protection (% Pr)

<i>P. lentiscus</i> resin ($n = 4$)	% Pr ^a			
	M/W fol. H	M fol. M	EA fol. EA	H fol. H
Acidic emulsion	8.9 \pm 9.2	–	–	–
Neutral fraction	19.6 \pm 18.7	–	–	–
'Liquid collection'	39.8 \pm 21.0	57.2 \pm 18.4	27.1 \pm 18.4	54.9 \pm 18.4
Acidic fraction (NaOH)	65.0 \pm 18.7	–	–	–
Acidic fraction (Na ₂ CO ₃)	65.1 \pm 21.3	–	–	–
Collofonium like residue	73.0 \pm 19.3	–	–	–
'Normal collection'	75.3 \pm 19.5	74.7 \pm 19.5	73.2 \pm 18.9	69.0 \pm 22.5
Mastic oil (liquor)	77.8 \pm 19.3	–	–	–

^a % Pr (6 h TBARS value) H, M/W and SD as in Table 1. EA, ethylacetate.

other gums and resins, were extracted according to the above method and tested for their antioxidant effect at a final concentration of 2.5 mg, shown in Tables 2 and 3, while three new different extracting solvent combinations were also used for comparison, following the same extraction sequence, described above.

LDL oxidation test (oxLDL). The method of LDL oxidation by copper ions (oxLDL) proposed by Balla *et al.* (1991) was applied with slight modifications. In brief, 1.0 mL of LDL suspension in PBS (200 μg protein) was incubated with 1.0 mL CuSO₄ (5 μM final concentration) as the oxidizing agent at three time periods (1 h, 3 h and 6 h at 37 °C) and the resulting thiobarbituric

acid reactant substances (TBARS), mainly malonaldehyde, were measured spectrophotometrically at 532 nm, after reaction with thiobarbituric acid solution (2 mL TBA, 1% in NaOH). An oxLDL-blank test was used in the absence of copper ions or malonaldehyde.

Biological activity tests. The biological activity tests (Table 1) were performed by using different volumes of the extract of CMG. Each volume was evaporated under a stream of nitrogen in different glass tubes and each residue was resuspended in 10 μL of ethanol. This ethanol volume has previously been proved to have no effect on the biological test (Gordon and Weng, 1992) and also in these experiments (data not shown). In each

Table 3. Biological activity of gum and resin (2.5 mg) extracts, using different solvent combinations, against the *in vitro* LDL oxidation expressed as % protection (% Pr)

	% Pr ^a			
	M/W fol. H	M fol. M	EA fol. EA	H fol. H
Gums (<i>n</i> = 4)				
Storax	66.5 ± 15.8	78.8 ± 21.2	70.9 ± 19.1	63.0 ± 18.8
Acacia	72.8 ± 22.6	72.4 ± 18.9	62.2 ± 19.0	55.4 ± 19.7
Tragacanth	53.5 ± 18.6	58.5 ± 18.6	57.6 ± 18.4	62.2 ± 20.7
Resins (<i>n</i> = 4)				
Dammar	59.0 ± 18.9	51.6 ± 18.9	61.1 ± 20.2	64.1 ± 19.4
<i>P. terebinthus</i>	49.5 ± 18.5	45.5 ± 19.9	28.4 ± 20.2	27.0 ± 19.7

^a % Pr (6 h TBARS values) and SD as in Table 1. M/W, fol., H, M and EA as in Table 2.

Table 4. Biological activity of naturally occurring substances (in different concentrations, final in the cuvette) against the *in vitro* LDL oxidation, expressed as % protection (% Pr)

	% Pr ^a		
	2.5 mg	20 µM	10 µM
Triterpenes (<i>n</i> = 4)			
Lupeol	58.7 ± 18.3 (2.92 mM) ^b	34.4 ± 20.0	19.5 ± 25.6
Glyccyr-acid	61.0 ± 18.6 (2.65 mM) ^b	50.2 ± 28.8	46.5 ± 19.7
Amyrin	62.6 ± 18.2 (2.92 mM) ^b	28.3 ± 21.3	31.6 ± 21.7
Ux (synthetic)	63.2 ± 18.0 (3.18 mM) ^b	73.6 ± 20.2	53.6 ± 18.1
Ursolic acid	70.7 ± 18.6 (2.73 mM) ^b	69.4 ± 18.6	68.1 ± 19.0
Oleanolic acid	67.9 ± 19.0 (2.73 mM) ^b	67.6 ± 18.7	63.1 ± 18.5
Naphthoquinones (<i>n</i> = 4)			
Naphthazarin	56.2 ± 18.3 (6.57 mM) ^b	55.6 ± 18.4	54.4 ± 18.2
Shikonin	62.3 ± 21.6 (4.34 mM) ^b	62.1 ± 18.2	60.8 ± 18.0
Alkanin	76.5 ± 20.5 (4.34 mM) ^b	59.1 ± 22.3	58.7 ± 19.6

^a % Pr (6 h TBARS values) and SD as in Table 1.

Glyccyr-acid, 18-a-glyccyrhethinic acid; Ux, ursodeoxycholic acid.

^b Final concentration corresponding to 2.5 mg of the substance.

Table 5. Effect of Ur and OI (naturally occurring in Chios mastic gum) and of Ux (Synthetic) in 2.5 mg overall quantities in the cuvette, against the *in vitro* LDL oxidation, expressed as % Protection (% Pr)

Tests (<i>n</i> = 4)	mg			% Pr ^a
	Ux	Ur	OI	
1	0.6	1.9	–	68.7 ± 19.5
2	1.9	0.6	–	69.9 ± 22.2
3	1.25	0.62	0.62	70.7 ± 20.9
4	1.25	1.25	–	72.6 ± 21.0
5	0.62	0.62	1.25	70.7 ± 18.5
6	0.62	1.25	0.62	75.0 ± 20.9
7	0.83	0.83	0.83	76.2 ± 20.3

^a % Pr (6 h TBARS values) and SD as in Table 1. Ux, ursodeoxycholic acid; Ur, ursolic acid; OI, oleanolic acid.

tube the oxLDL test was performed adding the appropriate solution, as previously described in the oxLDL sub-section. The same working sequence as above was used for the biological tests in Tables 2–5.

Polyphenol content. The total polyphenol content of CMG and other gums, expressed as caffeic acid, as previously described (Gundfinger, 1981), was measured spectrophotometrically using Folin-Ciocalteu reagent.

Data and statistical analysis. Data are expressed as the mean ± standard deviation. Comparable variances were tested for significance by Student's *t*-test.

RESULTS AND DISCUSSION

As shown in Fig. 1, the TBARS values tended to stabilize after the 6 h incubation and thus no further incubation periods were carried out. Therefore the % Pr refers to the 6 h incubation period. All the materials and substances exhibited *in vitro* biological protection against LDL induced oxidation, except for the neutral fraction and acidic emulsion of CMG, the 'normal collection' being the most effective. The protective activity of the substances tested, ranged from 19.5% to 63.1%, when comparing equal weights. In Fig. 1 some

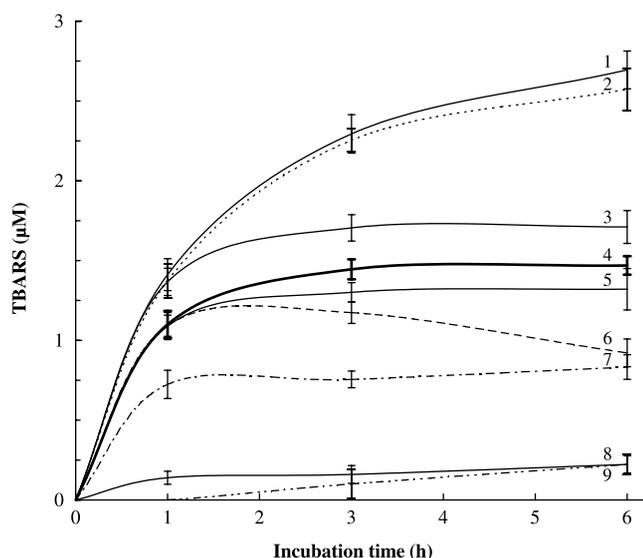


Figure 1. Representative biological tests against the copper ion induced LDL oxidation of different materials presented in Tables 1–5. M/W, fol. and H as in Table 2. Ux, Ur and Ol as in Table 5. For ox LDL and oxLDL blank tests see Materials and Methods. Curves (1) oxLDL (2) 2.5 mg *P. lentiscus* acidic emulsion (from M/W fol. H) (3) 2.5 mg *P. lentiscus* 'liquid collection' (from M/W fol. H) (4) 2.5 mg *P. terebinthus* (from M/W fol. H) (5) 2.5 mg Storax (from M/W fol. H) (6) Vitamin E (20 µM) (7) 2.5 mg *P. lentiscus* (from M/W fol. H) or 2.5 mg of Ux, Ur and Ol mixture in equal amounts (8) 50 mg *P. lentiscus* (from M/W fol. H) (9) oxLDL blank.

representative tests of the examined samples are presented, while the vitamin E effect is presented for comparison, because the examined concentration (20 µM) has been previously reported as the maximum, above which the protective effect does not increase (Gordon and Weng, 1992). As shown in Fig. 1 and Table 1, the effect of 2.5 mg of *P. lentiscus* resin extract, was almost equal to that of 5 mg, indicating the minimum quantity for the protective effect, while 50 mg was shown to be the quantity required for the complete inhibition of LDL oxidation. In all the samples examined, the TBARS values tended to stabilize between 3 and 6 h of incubation and thus the incubation period was not extended further. For comparative purposes, a protection factor, expressed as % protection (% Pr) factor, is used. An example of the calculation of the % Pr is given in Table 1.

Also investigated was which fraction of the resin *P. lentiscus* was responsible for the biological activity. Thus, the resin was fractionated into subfractions, according to the scheme proposed by Barton and Seoane (1956). The results show that mastic oil, colofonium-like residue and both the acidic fractions of NaOH and Na₂CO₃, were potent against LDL oxidation, while the neutral fraction and the acidic emulsion were both quite inactive. As shown in Table 2, all the extracts from CMG 'normal collection' obtained from different organic solvent combinations, exhibited remarkable protective activity ranging between 69.0% and 75.3%, the methanol/water (60:40, v/v), followed by hexane, being the most effective. The activity of mastic oil and colofonium-like residue was almost equal to that of the 'normal collection' CMG. The 'liquid collection' CMG appeared to have considerably reduced activity, indicating that the chemical constituents of *P. lentiscus*

from 'normal' and 'liquid' type collection probably differed.

Protective activity was recorded for the other gums and resins examined, using the different extracting solvents, as shown in Table 3. The most polar fraction of acacia and storax gums, which were obtained after methanol/methanol extraction, were the most effective, exhibiting an almost equal activity to the respective fraction of CMG. *P. terebinthus* showed almost half the activity compared with CMG and the other gums (except for tragacanth), with the polar fractions being twice as potent as the non-polar fractions. In dammar resin, however, the non-polar constituents (after hexane/hexane extraction) contained most of the protective activity.

The pure triterpenes amyryl and oleanolic acid, which are natural constituents of CMG (Papageorgiou *et al.*, 1997), with a relatively high protective activity (Table 4), did not exceed the overall activity of the crude product (75.3%), suggesting a synergistic effect between the active compounds. As also shown in Table 4, the isomers oleanolic and ursolic acids exhibited almost the same % Pr, indicating that the structural differences (Scheme 1) do not influence the biological activity. The above findings support the tests shown in Table 5, in which increasing amounts of oleanolic acid led to a maximum activity of the mixtures. Lupeol and 18- α -glycyrrhetic acid, which are triterpenes with structures similar to that of oleanolic acid (Scheme 1) and are constituents of *P. lentiscus* and *Glycyrrhiza glabra*, respectively, appeared also to have considerable activity.

Similar protective activity was observed for hydroxynaphthoquinones (Table 4) ranging from 54.4% (naphthazarin) to 60.8% (alkannin), indicative of the low influence of the molecular structure on the intensity of the biological activity. As indicated, the side chain of alkannin and shikonin (Scheme 2) increases the protective activity against LDL oxidation, while the stereochemistry of the phenolic hydroxyl group of the side chain of alkannin and shikonin, seems to play a secondary role. The grade and the percentage of polymerization of hydroxynaphthoquinones may also be a critical factor in their biological activity.

A final concentration of 20 µM in the cuvette appears to be the upper limit for maximum LDL protection, except for amyryl, lupeol, glycyrr-acid and alkannin, since no significant enhancement of biological activity was observed for 2.5 mg of each substance. A final concentration of 10 µM almost equalized the effect of 20 µM in most cases, and is the concentration of oleuropein that exhibited approximately 50% protection against LDL oxidation (Visioli *et al.*, 1995). Oleuropein is one of the main polyphenols of olive fruit and oil, and consists of linked hydroxytyrosol, elenolic acid and glucose.

Extraction with methanol/water is the usual procedure for the isolation of polar constituents from natural products and especially of polyphenols. The total polyphenol content in the methanol/water extract was found to be (in mg/kg): CMG, 271; storax gum, 323; acacia gum, 72; tragacanth gum, 130; dammar resin, 576; *P. terebinthus*, 1120. These values are within the range of polyphenols in olive oil (100–800 mg/kg) (Boskou, 1996), which is the major fat in the Mediterranean diet. Polyphenols and other simple phenols isolated from olive fruit and oil, i.e. oleuropein, hydroxytyrosol and

its derivatives, have been previously recognized as potent inhibitors of LDL oxidation, *in vitro*, induced either by metal ions (Visioli *et al.*, 1995; Visioli and Galli, 1994) or by horseradish peroxidase/hydrogen peroxide (Wieland *et al.*, 1993) or ferrylmyoglobin (Laranjinha *et al.*, 1999) or UV-light (Caruso *et al.*, 1999) or macrophage cells (Aviram and Fuhrman, 1998). Similar protective effects have been reported for the polyphenols of red wine, e.g. quercetin (Kerry and Abbey, 1997) and as cancer chemo preventors from green and black tea, e.g. catechins (Yang *et al.*, 2000). Other beneficial effects of natural polyphenols have also been reported, such as endothelial lung protection from herbs and species from Crete, Greece (Lionis *et al.*, 1998), immune system enhancement from olive oil (Stupans *et al.*, 2000), anticancer activity from olive oil (Owen *et al.*, 2000) and endothelial protection (Visioli *et al.*, 1998). The polyphenol species of the extracts in the present study will be further investigated, as the aforementioned health benefits of polyphenols might also apply to CMG and other resin polyphenols. As concluded from Table 2, other biologically potent non-polar substances or substances of medium polarity, should be present in CMG, as they eluted with hexane and ethyl acetate, respectively. The fact that *P. lentiscus* exhibits a higher protective effect than *P. terebinthus*, even though its phenolic content is much lower, enhances the hypothesis that substances other than polyphenols also contribute to the LDL protective activity. The nature of these substances is also under investigation.

It is noteworthy that part of the biological activity is retained in the acidic fraction and in the colophony-like residue (Table 2). Since the latter is easily removed from the total resin of *P. lentiscus*, a pharmaceutical study for its further development would be of interest. CMG by 'liquid collection' exhibited approximately half the biological effect of the 'normal collection', a

fact that should be taken into consideration when referring to its production process. It is likely that the extraction of resin with phytohormones rather than incisions, leads to a chemically modified liquid-like product, something that probably modifies its biological activity. It would be of interest to study the chemical composition of *P. lentiscus* by 'liquid collection'. Of the materials examined, mastic oil is the one that is directly consumed as a food additive, and also holds the highest LDL protective activity. Thus, its further development would be of interest and it could be considered to contribute to the beneficial effects of the Mediterranean diet, through its pharmaceutical and nutraceutical properties against CHD. In a recent study by our scientific team (Andrikopoulos *et al.*, 2002), the majority of the biologically active substances were found to be excreted in the saliva after chewing CMG in normal quantities (1–2 pieces of chewing gum) and chewing time (0.25–1.0 h), the 'normal collection' product exhibiting twice as much benefit.

The high biological activity of alkannin, almost equal to that of CMG, could be considered as an additional advantage. Thus, alkannin could be used as a nutraceutical, and also in oral pharmaceutical preparations with the additional activity of protecting LDL from oxidation. Shikonin could also be used for the same purpose. Natural resins and gums, such as acacia gum, tragacanth gum, storax gum and dammar resin, are used in pharmaceutical preparations and when taken orally, could protect LDL from oxidation.

In the present study, a novel beneficial property for human health, (CHD prevention through the protection of LDL from oxidation), of naturally occurring gums, resins, triterpenes and naphthoquinones, with previously well-known pharmaceutical usages, was demonstrated, which contributes to the knowledge of these materials.

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