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Alpinia zerumbet Potentially Elevates High-Density Lipoprotein Cholesterol Level in Hamsters

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In folkloric plant medicines, *Alpinia zerumbet* (AZ) has been popularly recognized as an exellent hepatoprotector. To search for a good high-density lipoprotein cholesterol (HDL-C) elevating herbal preparation, we examined AZ for its antioxidant and hypolipidaemic bioactivities, especially its HDL-C elevating activity. AZ seeds contain 0.51% essential oils (SO), which are comprised of monoterpenoids, oxygenated monoterpenoids, sesquiterpenoids, oxygenated sesquiterpenoids, aldehydes, acid, and esters. Gas chromatography/mass spectrometry analysis indicated that most of the monoterpenes and sesquiterpenes were recoverable in pentane eluent, whilst the oxygenated monoterpenoids and sesquiterpenoids remained in ether eluent. The high contents of rutin, quercetin, and polyphenolics in ethanolic extract of AZ seeds exhibit moderate antilipoperoxidative but potent DPPH free radical scavenging bioactivities. Conclusively, both seed powder (SP) and SO are effective hypolipidaemics with amazingly potent HDL-C elevating capabilities. On the basis of hepatoprotectivity, SP is a more feasible hypolipidemic agent as well as a promising HDL-C elevating plant medicine.

KEYWORDS: ***Alpinia zerumbet*** **seed essential oils; terpenes and sesquiterpenes; hamsters; antioxidant hypolipidemics; HDL**

INTRODUCTION

Alpinia zerumbet (Pers.) Burtt and Smith (AZ) belongs to one species of Zingiberaceae. Traditional folkloric experiences indicate that its roots and stems are beneficial to trauma and peptic ulcer. Its seeds are often used as aromatic stomachics. The essential oils obtained from *Alpinia speciosa* possess relaxation and anticonvulsive bioactivities on rat ileum muscles (*1*). The major chemical constituents present in its roots, leaves, and stems are sesquiterpenoids and diterpenoids (*2*). Specifically, it also contains 4-terpineol (*3, 4*), two active pharmacologically active compounds cardamonin and alpinetin (*5*), diarylheptanoids, blepharocalyxins C−E, blepharocalyxins D and E (in an AZ relative species *Alpinia blepharocalyx* seeds) (*6*), dihydro-5,6-dehydrokawain (DDK), and 5,6-dehydrokawain (DK) (*7–9*). DDK is a water-soluble constituent present in AZ roots. Besides, Morita et al. isolated four sesquiterpenopids, β-eudesmol, nerolidol, humulene epoxide II, and 4α-hydroxydihydroagarofuran, from AZ seeds (*10*). In addition, AZ is enriched with quercetin and rutin (*11*).

These compounds demonstrated a prominent suppressive effect on the growth of colon cancers and human HT-1080 fibrosarcoma cells (*6*), strong antiproliferative activity against both the acute and the chronic artificially induced peptic ulcer in rats, and excellent antiproliferative activity against both the acute and the chronic artificially induced peptic ulcer in rats (*7*). Moreover, DDK is a potent scavenger for the singlet oxygen species. Currently, AZ attracts many researchers for its good antilipoperoxidative bioactivity against in vivo reactive oxygen species (ROS).

Among all, the mostly cited compounds are flavonoids, a category of the secondary metabolites that exhibit vasodilative and vaso-strengthening bioactivities, which are effective in the prevention of strokes and hypertensiveness (*8–10*). Furthermore, quercetin is popularly recognized as a strong metallic ion chelator, a free radical scavenger, a xanthine oxidase inhibitor, and an antilipoperoxidative and antioxidative constituent (*11*), widely cited as a good healthcare agent, involving the prevention of cardiovascular diseases and peptic ulcers (*11*). Alternatively, the antiallergic action was reported to be due to its inhibitory effect on histamine release from macrophages, which is effective in the prevention of cataract and antiinflammation. Literature elsewhere has indicated that isoflavonoids exhibit antibacterial,

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antiallergic, antimutative, and anticoagulant acitivties (*12*). Similarly, rutin has been considered to exhibit antioxidative, antiinflammatory, antimutagenic, and antitumorigenic bioactivities. It is a rather good therapeutic for the improvement of corneal bleeding, capillary strokes, and coronary thrombosis (*13–15*).

The present study isolated the AZ essential oils (SO) and examined their active constituents using gas chromatography/mass spectrometry (GC/MS) analyses, especially rutin and quercetin. Pentane and ether were used to further fractionate the essential oils. In antioxidative activities including DPPH scavenging, ferrous ion chelating and antilipoperoxidative assays were performed. In addition, we compared the hypolipidemic activity of SO with that of seed powder (SP) in hamsters fed a 0.1% cholesterol diet. We found that both SO and SP indeed are good hypocholesterolemics and hypotriglyceridemics. Considering the safety, SP is more beneficial when the elevation of serum high-density lipoprotein cholesterol (HDL-C) levels is concerned.

MATERIALS AND METHODS

AZ Seeds. Fresh AZ seeds were collected from Nan-Tou County, an area located in Middle West Taiwan. Proximate analysis was performed as directed by the AOAC (*16*). Five major items including the contents of moisture, ash, crude fibers, crude proteins, and crude fat were analyzed.

Aqueous Extract (AE). Powder (10 g) was accurately weighed and transferred into a 200 mL Erlenmeyer flask. Distilled water (200 mL) was added and refluxed at 100 °C for 2 h. The reflux was filtered with the aid of aspiration, except for the first filtrate (A). The residue was added with distilled water (100 mL) and refluxed for 3 h. The mixture was filtered through a filter paper with aspiration to obtain filtrate B. The two filtrates were combined and evaporated at 50 °C under reduced pressure. The residue was dried in an oven to constant weight to estimate the yield. The final product was redissolved in distilled water to 25 mL and stored at −20 °C for further assays (the AE).

Ethanolic Extract (EE). Fresh AZ seeds were ground to a crude powder with mesh size #20. The powder (10 g) was accurately weighed and transferred into a 200 mL Erlenmeyer flask. Ethanol (200 mL) was added and refluxed at 40 °C for 2 h. The reflux was filtered with the aid of aspiration, except for the first filtrate (A). The residue was added with fresh ethanol (100 mL) and refluxed for 3 h. The mixture was filtered through a filter paper with aspiration to obtain filtrate B. The two filtrates were combined and evaporated under reduced pressure. The residue was dried in an oven to constant weight to estimate the yield. The final product was redissolved in ethanol to 25 mL and stored at −20 °C for further assays (the EE).

Determination of Total Polyphenolics. The method of Taga et al. was followed as previously described by Lin et al. (*17*). The authentic sample of gallic acid and the sample solutions as indicated were respectively dissolved in methanol/water (60:40, v/v), which was previously acidified with 0.3% HCl to the indicated concentrations. The remaining procedures were performed as previously mentioned. The absorbance of the final colored solution was measured at 750 nm against a reagent blank. The quantity of polyphenolics was calculated from the standard curve and expressed as mg gallic acid equivalent (GAE)/100 g of extract.

Determination of Rutin and Quercetin. The method of Fuleki was followed for quantitative determination of rutin and quercetin as previously described by Lin et al. (*17*). Aliquots of 20 μL of the filtrate were injected into the injection port and analyzed with high-performance liquid chromatography (HPLC; Hitachi L-2130 pump, Hitachi, Japan). Standard and sample rutin and quercetin were respectively dissolved in methanol to the desired concentrations. The remaining procedures were carried out as previously reported by Lin et al. (*17*) using an HPLC attached to a detector L-2400 UV and an Hitachi L-2130 pump. A column RP-18GP250 Mightysil (l = 250 mm; i.d. = 4.6 mm; thickness = 0.32μm; Kanto Chemical Co., Inc., Japan) was used for separation. The sample solutions to be analyzed were respectively filtered through a 0.45 μm filter (3 mm Millex Filter, Millipore). Aliquots of 20 μL of the filtrate were analyzed. The same mobile phase and elution conditions were adopted. The calibration curves were respectively established for rutin and quercetin by plotting the peak area vs each corresponding concentrations, from which quantitations of rutin and quercetin were achieved.

Preparation and Analysis of AZ Seed Essential Oils—Steam Distillation with Extraction. AZ SP (500 g) was added with distilled water to a total weight of 2000 g.The steam distillation proceeded for 3 h with a slight modification to collect the essential oils. The sample essential oils were dehydrated with anhydrous sodium sulfate and filtered through a 0.45 μm Micropore (3 mm Millex Filter, Millipore). The essential oils obtained were weighed, and the yields were calculated. The products were stored at 20 °C for further use.

Fractionation with an Adsorptive Gel Column. Samples of essential oils (0.2 g) were accurately measured and transferred into the adsorptive gel column (id = 2.0 cm and l = 15 cm). The fractionation was performed first with *n*-pentane (50 mL) and then with ether (50 mL). The eluents were respectively collected as fractions 1 and 2 (F-1 and F-2). The eluting solvents were driven of with nitrogen blowing. The residues were used for further GC/MS analyses for volatile components.

GC/MS Analysis. A gas chromatography GC HP 6890 attached to a HP5973MSD detector and a capillary column DB-1 (h = 60 m; i.d. = 0.25 mm; and membrane thickness, 0.25 μm) was used. Nitrogen was used as the carrier gas and operated at a flow rate of 1 mL/min. The temperature at the injection port was set to 250 °C. The ionization potential used was 70 eV, where the temperature of the ion source was held at 230 °C. Initially, the temperature was set at 40 °C for 10 min, then programmed at 2 °C/min up to 240 °C, and held at this temperature for 30 min. The flux ratio was set at 80:1.

Quantification of Volatile Constituents. Aliquots (1.0 μL) of the essential oils were respectively measured out with a GC microsyringe from the native essential oils, F-1 and F-2, and analyzed with the GC and GC/MS. Quantification of each constituent was calculated from the integrated diagrams.

The amount of each constituent in fractions F-1 and F-2 was calculated by eq 1:

$$Q = A \times Y \tag{1}$$

where Q = the quantity of each volatile constituent in fractions F-1 and F-2, A = the percent peak area in the gas chromatograms occupied by each constituent, and Y = the recovery yield of the essential oils.

Identification of Constituents in Essential Oils. A reference mixture of *n*-paraffins (C_5−C_{25}) was used to calculate the retention indices (RI) from the retention time (t_R) for each component according to the definition of Kavat's GC retention indices (shown in eq 2). By referring to the documented data, each exact constituent was deduced. Alternatively, by comparing the GC/MSD spectra, each component was qualitatively matched out and confirmed by GC/MS. The determination for volatile structures was based on the TNO (*18*), the Browse-Wiley Computerized Data Base, the NSB Computerized Data Base, and the cited standard spectra of standard chemicals.

The equation used for Kavat's GC RI calculations was

$$\mathrm{RI} = [\log(T_r')n + 1 - \log(T_r')/\log(T_r')_u - \log(T_r')n] \tag{2}$$

where $T_r' = (T_r - T_m)$, which means the calibrated and corrected values of RI for each constituent; n = the carbon number of *n*-alkanes; T_m = the retention time of methane in the separation column; T_r = the retention time of chemical constituent in the separation column; and u = unknown compound.

Determination of DPPH Free Radical Scavenging Capability (FRSC). The method of Shimada et al. was followed as previously described by Lin et al. (*17*). After the reaction was completed, the optical density was measured at 517 nm with a Hitachi U-2001 spectrophotometer. α-Tocopherol and BHA were used as positive controls.

Determination of Ferrous Ion Chelating Capability (FICC). The method of Dinis et al. was followed as previously reported by Lin et al. (*17*). When the reaction was completed, the optical density was immediately taken at 562 nm with a spectrophotometer (Hitachi U-2001). EDTA was used as the positive control. The percent FICC was calculated as described (*17*).

Table 1. Ingredients of Experimental Animal Diets[a]

	composition (%)[b]							
ingredients	C	SP-1	SP-3	SP-5	SO-1	SO-5	SO-10	H
casein	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
sucrose	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
corn starch	50.0	40.0	37.9	36.8	39.9	39.9	39.9	39.9
corn oil	4.0	11.1	11.6	11.3	12.0	12.0	11.9	12.0
lard	1.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
cholesterol		0.1	0.1	0.1	0.1	0.1	0.1	0.1
mineral[c]	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
vitamin[d]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
cellulose	5.0	4.8	4.4	3.8	5.0	5.0	5.0	5.0
methionine	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
choline bitartrate	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
AZ								
SP		1.0	3.0	5.0				
SO					0.01	0.05	0.1	
total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

[a] Based on AIN-76 formula (AIN, 1977). [b] C, normal diet; H, high fat plus high cholesterol diet; SP-1, high fat and diet containing 1% SP; SP-3, high fat plus high cholesterol diet containing 3% SP; SP-5, high fat plus high cholesterol diet containing 5% SP; SO-1, high fat plus high cholesterol diet containing 0.01% seed essential oil; SO-5, high fat plus cholesterol diet containing 0.05% seed essential oil; and SO-10, high fat plus cholesterol diet containing 0.1% seed essential oil. [c] Mineral premix: $CaHPO_4 \cdot 2H_2O$, NaCl, $K_3C_6H_5O_7$, K_2SO_4, MgO_3, MnO_3, Fe-citrate, $ZnCO_3$, $CuCO_3$, KI, $NaSeO_3$, and $K_2SO_4 \cdot Cr_2(SO_4)_3 \cdot 24H_2O$. [d] Vitamin premix: thiamine hydrochloride, pyridoxine hydrochloride, riboflavin, nicotinic acid, vitamin B_{12}, retinyl palmitate, and vitamins D_3, E, and K.

Antilipoperoxidative Capability on Lipid Micelles Determined by the Thiocyanate Method. According to the method of Mitsuda et al., the determination procedures proceeded as previously reported by Lin et al. (*17*). On completion of the reaction, the absorbance was measured at 500 nm with a Hitachi U-2800 spectrophotometer against the control reagent mixture. BHA and α-tocopherol were used as the positive controls.

Hamsters. Forty-eight male Syrian hamsters, aged 4−5 weeks, were purchased from the National Laboratory Animal Centre. All studies performed with hamster models were approved by the Hungkuang University Supervising Ethic Committee in accordance with the Helsinki Declaration of 1975.

The animal room was conditioned at 24 ± 1 °C with a relative humidity maintained at 40−60%. The light cycle was changed every 12 h (light, 6:00 a.m.−7:00 pm; dark, 7:00 pm−6:00 a.m.). Water and meal takings were ad libidum. For the first 2 weeks, hamsters were acclimated by supplying only regular diets for experimental hamsters (Fu-Sow brand; Long-Live brand). The hamsters were then randomly grouped into eight groups, six in each stainless cage. On the basis of the formulation of AIN-76 (31), the experimental meals were prepared according to the formula listed in **Table 1**. Each diet was thoroughly mixed to ensure a homogeneous compositional distribution and stored at 4 °C. These hamster groups were fed respectively with control diet (group C), high fat plus high cholesterol diet (group H), and high fat with high cholesterol diet plus SP 1, 3, and 5%, respectively, which were respectively designated as groups SP-1, SP-3, and SP-5. The other three groups were fed with high fat with a high cholesterol diet plus SO 0.01, 0.05, and 0.1% and denoted as groups SO-1, SO-5, and SO-10, respectively.

The experimental period extended to 8 weeks, during which the body weight and amount of diets consumed were recorded every 2 days until the end of experiment. After 8 weeks of feeding, the hamsters were fasted for 12 h and CO_2-anethesized to collect the blood from the abdominal aorta. Blood was immediately centrifuged at 3200 rpm for 15 min using a Kubota-3740 centrifuge to separate and collect the sera, which were used later for determination of the TG, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and HDL-C. After the animals were euthanized, the livers were dissected, picked up, and rinsed with saline; the adsorbed water was sucked off of the surface, and the samples were weighed.

Table 2. Proximal Composition of *AZ* Seeds[a]

composition	%
moisture	17.38 ± 0.22
dry matter	82.62 ± 0.22
NFE	48.58 ± 0.05
crude fat	12.07 ± 0.96
crude ash	5.98 ± 0.01
crude fiber	24.46 ± 0.84
crude protein	8.91 ± 0.20

[a] Data expressed as means ± SD ($n = 3$) on a dry basis. NFE (nitrogen-free extract) = 100% − (% crude fat + % crude protein + % crude fiber + % ash).

Analysis of Serum Lipoproteins. For blood collection, experimental hamsters were fasted for 12 h immediately before they were euthanized. Hamsters were CO_2-anesthesized. Blood was collected from the abdominal artery. The whole blood obtained was centrifuged at 3200 rpm (Kubota-3740) for 20 min. Sera were collected for further assay of TG, TC, LDL-C, and HDL-C. The enzyme GDP-PAP triglyceride kit (Teco Diagnostics) was used. The determination methods for both serum TC (STC) and serum TG (STG) directed by the manufactuter were followed as previously described by Lin et al. (*17*). After the reaction was completed, the absorbance was measured at 520 nm.

As previously described by Lin et al. (*17*), the entire procedure for the determination of serum LDL-C was processed according to the method of Pisani et al. The colored reaction product was measured spectrophotometrically at 600 nm.

The enzyme-linked spectrophotometric method for the determination of serum HDL-C developed by Matsuzaki et al. was followed, and the entire procedure was performed as previously described by Lin et al. (*17*). On completion of the reation, the absorbance was read at 600 nm.

Hepatic Lipid. The method of Folch et al. was followed as described by Lin et al. (*17*). The final combined extract was made to volume 10 mL with the same extraction solution and stored at −20 °C for further analysis. Similarly, the assay methods were performed as described in the section STC and STG. The optical densities were respectively measured at 520 nm. The amounts of TC and TG were calculated.

Hepatic Phospholipids Levels. The extraction of hepatic phospholipids contents was carried out according to the method of Folch et al. as previously described by Lin et al. (*17*). After the procedures were completed to yield a colored solution at ambient temperature, the absorbance was measured at 820 nm. The content of hepatic phospholipids was calculated from the calibration curve established using standard sample supplied by the manufacturer.

Statistics. Data obtained in the same group were analyzed by analysis of variance and Student's *t* test with computer statistical software SPSS 10.0 (SPSS, Chicago, IL). Statistical Analysis System (2000) software was used to analyze the variances, and Duncan's multiple range tests were used to test their significances of differences between paired means. The significance of difference was judged by a confidence level of $p < 0.05$.

RESULTS

Proximate Compositional Analysis. The crude fat, crude ash, crude fiber, and crude protein contents in AZ seeds were 12.07, 5.98, and, 24.46, and 8.91%, respectively. Besides, AZ seeds contained a rather high nitrogen-free extract (NFE) fraction (48.58%) (**Table 2**).

Major Chemicals Found in the AEs vs EEs. The EE showed a 3-fold total phenolic content when compared with that in the AE. Specifically, the AE contained lower contents of rutin and quercetin as compared with the EEs. These contents were 3.76 and 6.06 mg/100 g for rutin and 3.78 and 46.29 mg/100 g for quercetin, respectively, in the AEs and the EEs (**Table 3**).

Chemical Identification by GC/MS. The recovery of steam-distilled volatiles from AZ whole seeds was 0.51%. Fifty-three

Table 3. Yield and Composition of Polyphenolics, Rutin, and Quercetin, Respectively, in the Aqueous and the EEs[a]

item	AE	EE
yield (mg/100 g)	7.60 ± 0.52 a	7.16 ± 0.72 a
total polyphenolics	664 ± 2 a	2033 ± 1 b
rutin	3.76 ± 0.04 a	6.06 ± 0.03 b
quercetin	3.78 ± 0.49 a	46.29 ± 0.02 b

[a] Values expressed as means ± SD ($n = 3$). Data in the same row with different letters are significantly different ($p < 0.05$).

compounds were identified including 12 monoterpenoids, 10 oxygenated monoterpenoids, 22 sesquiterpenoids, five oxygenated sesquiterpenoids, three aldehydes, one acid, and eight esters. The most abundant were monoterpenoids accounting for a subtotal of 77.58%. The oxygenated monoterpenes were relatively very rare, amounting up to 2.16% only. The next higher set were the sesquiterpenoids, and their oxygenated equivalents, respectively, showed 9.14 and 6.74% in total (**Table 4**). The aldehyde, acid, and ester contents were all low, accounting respectively for 0.03, 0.38, and 0.23% only. Thus, the major flavor and fragrance of AZ seeds could be ascribed to categories of monoterpernoids and sesquiterpenes. In monoterpenoids, the major components were (in order of abundance) as follows: sabinene (33.93%), β-pinene (30.5%), α-pinene (6.65%), β-ocimene (2.79%), and 1-phellandrene (1.97%) (**Table 4**). While in sequiterpenes and their oxygenated alike, *t*-murolo, δ-cadinene, 1,6-germacradien-5-ol, *trans*-caryophyllene, and γ-cadiene were the most prominent components (**Table 4**). In addition, an unusual component torreyol was found in the oxygenated sesquiterpenoids. In the ester category, three extraordinary compounds, endobarnyl acetate, *trans*-pinocarvyl acetate, and neryl acetate, were also found. Furthermore, by fractionation with pentane and ether, the recoveries were 55 ± 0.03 and 35 ± 0.01%, respectively, by pentane and ether. As can be seen, most of the monoterpenes and sesquiterpenes were recovered in the pentane eluent, whereas the ether eluent was abundant in oxygenated monoterpenoids and oxygenated sesquiterpenoids. Worth mentioning, some oxygenated compounds originally not found or only presented in trace in the native essential oils were detected in relatively huge amounts in the ether eluent, for example, 1.8-cineole, *t*-cadinol, methyl geranate, bornyl acetate, myrtenyl acetate, α-terpeinenyl acetate, and neryl acetate etc. (**Table 4**).

DPPH Scavenging Effect. The EE of AZ seeds showed promising DPPH FRSC when compared with the positive controls BHA and α-tocopherol (**Figure 1**). At a concentration of 2 mg/mL, the FRSC reached 14 and 64%, and at a concentration of 6 mg/mL, it reached 48 and 86%, respectively, by the AEs and the EEs. Above a concentration of 8 mg/mL, leveling off around 90%, the EE was achieved (**Figure 1**).

FICC. Both the AEs and the EEs did not show significant FICC as compared with EDTA. Relatively huge dosages were required. The AE showed only moderate FICC, whereas the EE was far inferior in this regard, being almost ineffective (**Figure 2**).

Antilipoperoxidation. Both the AEs and the EEs suppressed lipid peroxidation for 36 h at dosages of 0.5−20 mg/mL. The ethanolic showed better antilipoperoxidative than the AEs; yet, they were slightly inferior to the positive controls α-tocopherol and BHA in this regard (**Figure 3**).

Hypolipidemic Effect. After treated with specified diets as indicated (**Table 1**) for 8 weeks, the feed consumptions did not show any significant variation among all groups. At the end of week 2, the body weights in all groups began to appear significantly different. After 4 weeks, a significant difference in body weight was seen for group H (high fat plus high cholesterol meal) as compared to all other groups ($p < 0.05$). The body weight increases revealed that group H had early obesity since week 8 ($p < 0.05$) (**Table 5**).

STC. After 8 weeks of feeding, the STC level was significantly raised to 332 mg/dL in group H as compared with the control (145.58 mg/day) ($p < 0.05$), which was moderately suppressed by feeding the SO groups and SP groups, being more prominently effective by SO-5 and SP-5, although having reached 291.0 and 279.7 mg/dL, respectively ($p < 0.05$), nonetheless far in excess than the control value (145.6 mg/dL) ($p < 0.05$) (**Table 6**).

STG Levels. A similar situation was found with STG. The STG level elevation in group H was shown to have been effectively reduced by feeding with SO and SP meals, prominently effective in groups SO-1, SO-5, SP-3 and SP-5, which gave very plausible final levels of 137.5, 133.3, 132.0, and 122.8 mg/dL, respectively ($p < 0.05$) (**Table 6**).

Serum LDL-C and HDL-C Levels. Levels of LDL-C were effectively reduced by SO-5, SP-3, and SP-5 meals, similar to STC and STG, mostly effective in groups SO-5, SP-3, and SP-5, by which values of LDL-C were suppressed to 66.3, 67.2, and 56.6 mg/dL, respectively. In contrast, levels of LDL-C in groups H, SO-1, and SP-1 were only reduced to 111.8, 92.0, and 79.1 mg/dL, respectively ($p < 0.05$) (**Table 6**). Levels of HDL-C were all maintained above 202.3 mg/dL in comparison with group H (98.3 mg/dL) and the control (186.1 mg/dL) ($p < 0.05$). More importantly, ratios of LDL-C/HDL-C were reduced to below 0.5 in all SO- and SP-fed groups, with better results observed in groups SO-5, SP-3, and SP-5, all of which had attained ratios to 0.3. The second beneficial was group SP-1 that revealed a ratio of 0.4. In contrast, groups SO-1 and SO-10 showed a slight default in this respect ($p < 0.05$) (**Table 6**).

Liver Weight and Ratio of Liver to Body Weight (L/B). As for the liver weight, all of the groups at the end of the 8 week treatment showed increased weights to over 7.7 g for group SO-10 to 8.4 g for group SO-1, although an insignificant difference was found among all SO- and SP-fed groups, but all were significantly different when compared with the control (liver weight = 3.1g; L/B = 3.5) (**Table 7**). The L/B ratios were all around 7.1−7.8 in all tested groups as compared to 3.5 of the control. Nonetheless, the high level of HTG found in the group H (20.42 mg/g) was effectively suppressed by feeding seed essential oil SO-1, SO-5, and SP meals SP-3 and SP-5. Astonishingly, group SO-10 was seen to have a high level of HTG (18.5 mg/g) and HTC (16.4 mg/g) (**Table 7**), as similarly observed in STG and STC (**Table 6**).

Hepatic Phospholipids. Interestingly, the hepatic phospholipids level was the highest with group SO-10, reaching a level of 39.3 mg/g, as compared with that of group H (20.4 mg/g) and the control value (43.8) ($p < 0.05$) (**Table 7**).

DISCUSSION

Proximate Compositional Analysis. The unusually high content of crude fiber in AZ seeds could be attributed to its seed outer cortex, which may play a rather important role in hypolipidemic bioactivity.

Major Chemicals Found in the AEs vs EEs. The EE contained huge amount of polyphenolics (2033 mg/100 g) and moderately abundant of rutin (6.06 mg/100 g) and quercetin (46.29 mg/100 g), whereas the AE consisted of far lower polyphenolics (664 mg/100 g), rutin (3.76 mg/100 g) and

Table 4. Major Composition of Volatiles in the Seed Essential Oils of AZ

compounds	RI[b]	MW	formula	CAS no.	composition (%)[a] SO[c]	SOP[c]	SOE[c]
			monoterpene hydrocarbons				
α-thujene	923	136	$C_{10}H_{16}$	353313	0.2 ± 0.02	–[d]	–
α-pinene	932	136	$C_{10}H_{16}$	80-56-8	6.65 ± 0.28	6.41	–
camphene	942	136	$C_{10}H_{16}$	79-92-5	0.11 ± 0.03	–	–
β-pinene	977	136	$C_{10}H_{16}$	127-91-3	30.55 ± 0.02	34.86	–
β-myrcene	984	136	$C_{10}H_{16}$	123-35-3	0.97 ± 0.06	1.11	–
1-phellandrene	995	136	$C_{10}H_{16}$	99-83-2	1.97 ± 0.58	1.89	–
α-terpinene	1007	136	$C_{10}H_{16}$	99-86-5	0.12 ± 0.11	–	–
sabinene	1029	136	$C_{10}H_{16}$	3387-41-5	33.93 ± 0.09	26.52	–
β-ocimene	1043	136	$C_{10}H_{16}$	3779-61-1	2.79 ± 0.01	4.47	–
γ-terpinene	1051	136	$C_{10}H_{16}$	36284	0.17 ± 0.04	0.23	–
α-terpinolene	1078	136	$C_{10}H_{16}$	586-62-9	0.12 ± 0.01	0.15	–
alloocimene	1080	136	$C_{10}H_{16}$	73-84-7	–	0.02	–
				subtotal	77.58	75.66	–
			oxygenated monoterpenes				
trans-sabinene hydrate	1019	154	$C_{10}H_{18}O$	–	+[e]	–	+
1.8-cineole	1025	154	$C_{10}H_{18}O$	470-82-6	–	–	20.5
α-fenchone	1067	152	$C_{10}H_{16}O$	–	0.3 ± 0.01	–	0.99
cis-sabinene hydrate	1082	154	$C_{10}H_{18}O$	–	0.04 ± 0.02	–	–
linalool	1112	154	$C_{10}H_{18}O$	78-70-6	0.17 ± 0.06	–	0.25
camphor	1115	152	$C_{10}H_{16}O$	76-22-2	0.09 ± 0.01	–	–
1-terpineol	1130	154	$C_{10}H_{18}O$	586-82-3	0.14 ± 0.02	–	0.25
4-terpineol	1162	154	$C_{10}H_{18}O$	562-74-3	0.61 ± 0.07	–	2.28
α-terpineol	1176	154	$C_{10}H_{18}O$	98-55-5	0.76 ± 0.03	+	2.71
cis-piperitol	1208	154	$C_{10}H_{18}O$	–	0.05 ± 0.01	–	0.12
				subtotal	2.16	–	27.1
			sequiterpene hydrocarbons				
bicycloelemene	1282	204	$C_{15}H_{24}$	–	+	–	–
α-chamigrene	1284	204	$C_{15}H_{24}$	19912-83-5	+	–	–
α-cubebene	1339	204	$C_{15}H_{24}$	17699-14-8	0.08 ± 0.01	+	–
elixene	1344	204	$C_{15}H_{24}$	3242-08-8	–	0.05	–
α-muurolene	1361	204	$C_{15}H_{24}$	–	0.04 ± 0.01	0.06	–
α-copaene	1370	204	$C_{15}H_{24}$	3856-25-5	0.21 ± 0.06	0.38	+
(−)-sinularene	1374	204	$C_{15}H_{24}$	–	0.03 ± 0.03	0.05	–
β-elemene	1382	204	$C_{15}H_{24}$	515-13-9	0.56 ± 0.01	0.98	–
α-gurjunene	1402	204	$C_{15}H_{24}$	489-40-7	0.07 ± 0.04	0.11	–
α-bergamotene	1405	204	$C_{15}H_{24}$	–	+	0.04	–
trans-caryophyllene	1411	204	$C_{15}H_{24}$	87-44-5	1.05 ± 0.02	1.9	+
calarene	1419	204	$C_{15}H_{24}$	17334-55-3	0.04 ± 0.01	0.05	–
alloaromadendrene	1439	204	$C_{15}H_{24}$	25246-27-9	0.06 ± 0.04	0.12	–
α-humulene	1445	204	$C_{15}H_{24}$	6753-98-6	0.79 ± 0.05	1.4	–
aromadendrene	1452	204	$C_{15}H_{24}$	109119-91-7	0.68 ± 0.02	1.39	–
germacrene-D	1470	204	$C_{15}H_{24}$	23986-74-5	0.65 ± 0.01	+	+
β-selinene	1475	204	$C_{15}H_{24}$	17066-67-0	0.05 ± 0.02	0.11	–
epi-bicyclosesquiphellandrene	1479	204	$C_{15}H_{24}$	54324-03-7	0.19 ± 0.01	0.34	–
bicyclogermacrene	1484	204	$C_{15}H_{24}$	100762-46-7	0.39 ± 0.07	0.81	–
α-muurolene	1487	204	$C_{15}H_{24}$	31983-22-9	0.54 ± 0.12	1.01	–
γ-cadiene	1501	204	$C_{15}H_{24}$	39029-41-9	0.98 ± 0.03	2.04	–
δ-cadinene	1512	204	$C_{15}H_{24}$	843-76-1	2.73 ± 0.05	4.71	–
				subtotal	9.14	15.5	–
			oxygenated sequiterpenes				
torreyol	1525	222	$C_{15}H_{26}O$	1943-97-3	0.17 ± 0.04	–	–
1,6-germacradien-5-ol	1565	222	$C_{15}H_{26}O$	-	1.78 ± 0.04	–	7.94
t-cadinol	1582	222	$C_{15}H_{26}O$	1474790	+	–	8.56
elemol	1595	222	$C_{15}H_{26}O$	639-99-6	0.16 ± 0.02	–	0.6
t-muurolol	1633	222	$C_{15}H_{26}O$	19912-62-0	4.63 ± 0.03	–	7.43
				subtotal	6.74	–	24.53
			aldehydes				
nonanal	1082	142	$C_9H_{18}O$	124-19-6	–	–	0.2
phellandral	1202	152	$C_{10}H_{16}O$	23963-70-4	+	–	–
cuminic aldehyde	1257	148	$C_{10}H_{12}O$	122-03-2	0.03 ± 0.05	–	+
				subtotal	0.03	–	0.2
			acid				
palmitic acid	2313	256	$C_{16}H_{32}O_2$	21096	0.38 ± 0.03	–	0.4
				subtotal	0.38	–	0.4
			esters				
endobarnyl acetate	1205	196	$C_{12}H_{20}O_2$	76-49-3	0.08 ± 0.01	–	–
methyl geranate	1257	182	$C_{11}H_{18}O_2$	2349-14-6	+	–	0.1
bornyl acetate	1266	196	$C_{12}H_{20}O_2$	5655-61-8	–	–	0.26
trans-pinocarvyl acetate	1276	194	$C_{12}H_{18}O$	–	0.15 ± 0.02	–	0.57
myrtenyl acetate	1301	194	$C_{12}H_{20}O_2$	1118-39-4	–	–	0.13
methyl cinnamiate	1310	162	$C_{10}H_{10}O_2$	103-26-4	–	–	+
α-terpinenyl acetate	1328	196	$C_{12}H_{20}O_2$	80-26-2	–	–	0.09
neryl acetate	1339	196	$C_{12}H_{20}O_2$	141-12-8	–	–	0.35
				subtotal	0.23	–	1.5

Table 4. Continued

compounds	RI[b]	MW	formula	CAS no.	composition (%)[a] SO[c]	SOP[c]	SOE[c]
			miscellaneous compounds				
cryptone	1152	138	$C_9H_{14}O$	500-02-7	0.29 ± 0.01	−	0.85
				subtotal	0.29	−	0.85
				total	96.55	91.16	30.05

[a] Each value is the mean of triplicate determinations. [b] Retention index, using paraffin (C_5−C_{25}) as a reference. [c] SO, steam-distilled AZ seed essential oil; SOP, pentane-fractionated, steam-distilled *A. zerumet* seed essential oil; and SOE, ether-fractionated, steam-distilled AZ seed essential oil. [d] −, not detected. [e] +, less than 0.01 (peak area).

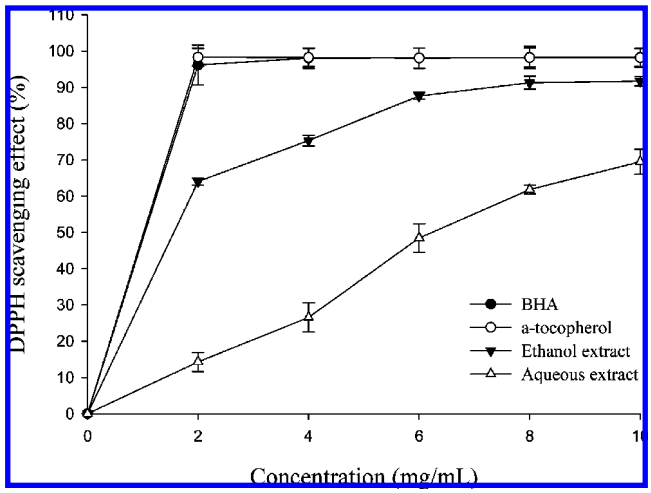

Figure 1. Scavenging effects of the aqueous and EEs of AZ seeds on 1,1-diphenyl-2-picrylhydrazyl free radicals (DPPH). Data are expressed as means ± SD ($n = 3$).

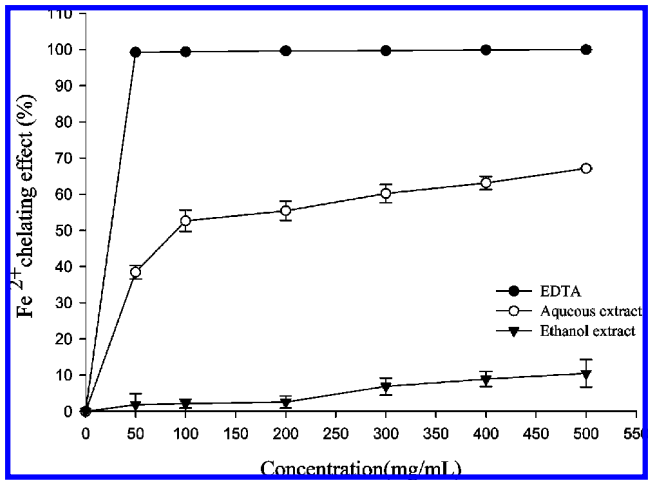

Figure 2. Chelating capabilities of the aqueous and EEs of AZ seeds on ferrous ions. Data are expressed as means ± SD ($n = 3$).

quercetin (3.78 mg/100 g), implicating that stronger bioactivity could be exhibited by the EE. The difference of polyphenol content between the AEs and the EEs could be due to two reasons: the higher processing temperature used for aqueous extraction and the polarity of these polyphenolics.

Chemical Identification by GC/MS. Most of the monoterpenes and sesquiterpenes were found to have been recovered in pentane eluent; however, they were totally absent in ether fraction. Conversely, the oxygenated counterparts only appeared in the ether fraction, indicating that the oxygenated components with higher polarity were more easily soluble in the more polar ether eluent. Worth mentioning, in addition to this, some components that were originally not found in the steam-distilled essential oils appeared in the individual factions, such as 1,8-cineol, methyl geranate, bornyl acetate, myrtenyl acetate, α-terpinenyl acetate, and neryl acetate, implicating that the concentration effect of such an adsorptive gel column fractionation could be available for further separation and concentration of these flavors concerned. Especially worth mentioning is the presence of camphor in AZ seed essential oils (0.09%). Although very rare (0.09%), it is very unusual because the occurrence and distribution of camphor in the natural environment is extremely rather limited. In addition, one of the oxygenated monoterpenoids, 1,8-cineole, was uniquely concentrated in ether eluent amounting up to 20.5%.

DPPH Scavenging Effect. Quercetin has been considered to be a potent free radical scavenger as well as an antilipoperoxidative. Obviously, more powerful capability of the EE of AZ seeds could be attributed to its higher phenolic content.

FICC. The conflicting effect of the AE and the EE on FRSC and FICC apparently implicates that totally different action mechanisms were operating respectively. Speculatively, the AE could contain some unknown compounds more responsive for ferrous chelaing; possibly, some water-soluble polycarboxylic and polyhydroxylic organic acids were playing important roles. As well-known, ferrous ions are very common active in vivo catalysts that initiate tremendous lipooxidative reactions, which in turn usually lead to irreversible tissue damage.

Antilipoperoxidation. As above-mentioned, quercetin has been considered to be a potent free radical scavenger as well as an antilipoperoxidative, while rutin is a very potent antioxidant. At dosages from 0.5 to 20 mg/mL tested, the two extracts showed comparable results; however, in prolonged incubation (longer than 36 h), the EE revealed slightly better results when antilipoperoxidation is concerned, which could be ascribed to its high polyphenolics as well as rutin and quercetin contents. Isoflavonoids are promising potent antioxidants. AZ seeds contain abundant amounts of rutin (6.06 mg/100 g) and quercetin (46.29 mg/100 g) in EE. In addition, 1,8-cineol was reported to be an effective local antiinflammatory and expectorant as well as a rather good free radical scavenger (*21*). Alternatively, 4-terpineol is beneficial to cardiovascular diseases and hypertension. α-Humulene and *trans*-caryophyllene possess potent antiinflammatory activities (*22*). In addition, 13 monoterpenoids, the monocyclic monoterpenoid compounds terpinolene, α-terpinene, and γ-terpinene, all have potent antioxidative capabilities (*23*).

Liver and L/B Ratio. Either SO or SP diets all increased the liver weights. The physiologically slight change in color in the beginning of feeding could be due to its temporary storage for increased contents of HTG and HTC (not shown). On continued feeding, decreasing levels of HTG and HTC were found depending on the amount of SP in diets, evidence that SP is a rather strong hepatoprotecting agent. Better results can be achieved by diets containing 6−10% of SP (i.e., SP-6 to SP-10).

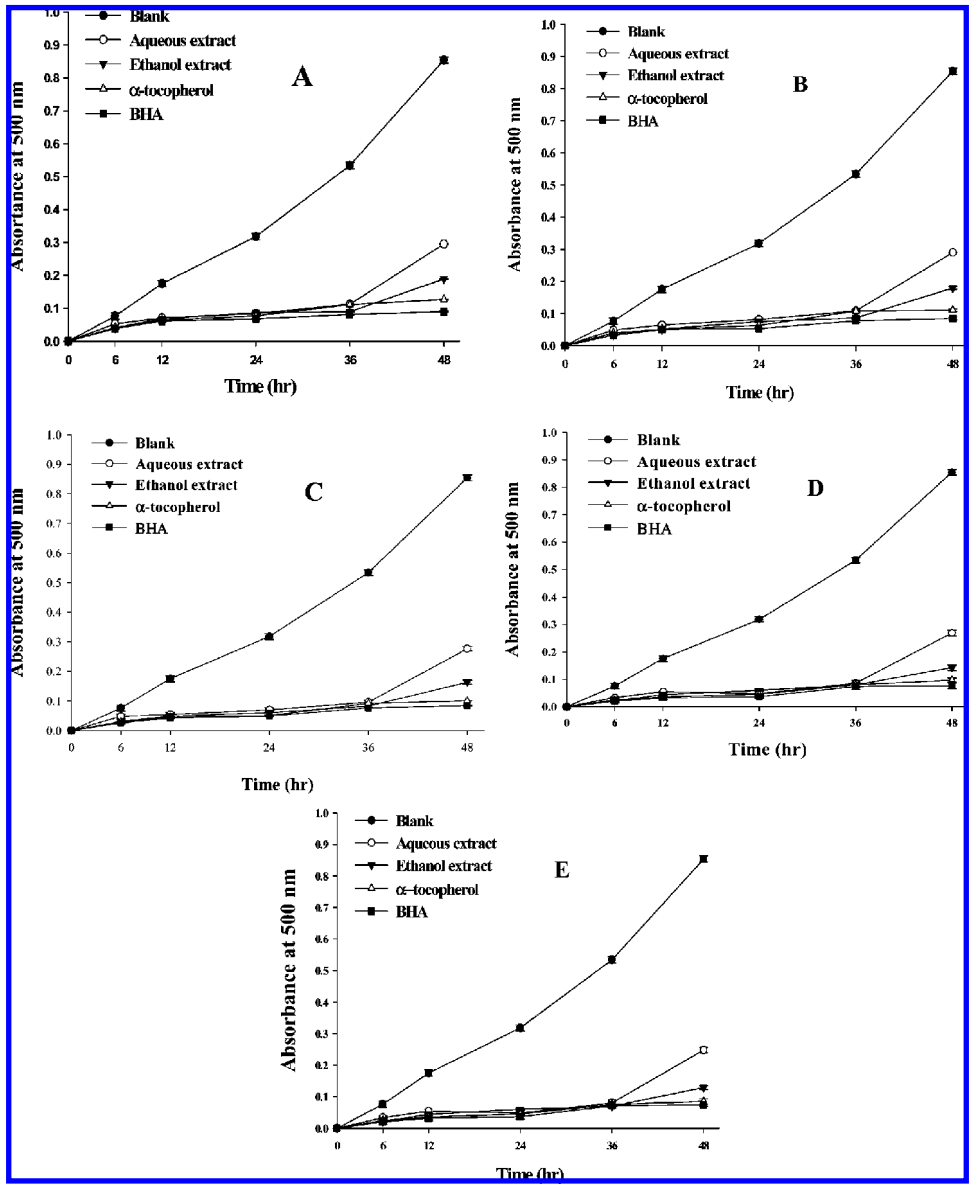

Figure 3. Antilipoperoxidative capabilities of the aqueous and ethanol extracts of AZ seeds on lipid peroxidation in micelles. Data are expressed as means ± SD from triplicate experiments. Concentrations of the aqueous and ethanol extracts used were 0.5 (**A**), 1.0 (**B**), 5.0 (**C**), 10.0 (**D**), and 20.0 mg/mL (**E**).

Table 5. Diet Comsumption vs Body Weight Gain during Feeding Period with AZ Seeds and Essential Oils[a]

	period						
	g				g		
	acclimation	fed			acclimation	fed	
group[b]	2 weeks	4 weeks	8 weeks	initial body weight (g)	2 weeks	4 weeks	8 weeks
H	5.5 ± 0.2 a	5.2 ± 0.3 a	6.2 ± 0.2 a	87.6 ± 2.5 a	98.8 ± 3.2 b	110.9 ± 4.2 c	116.2 ± 9.2 d
SO-1	5.7 ± 0.4 a	5.6 ± 0.4 a	6.1 ± 0.2 a	84.3 ± 3.5 a	96.5 ± 4.5 b	106.5 ± 9.3 b	107.7 ± 9.1 b
SO-5	6.2 ± 0.5 a	5.6 ± 0.5 a	6.3 ± 0.4 a	82.9 ± 4.7 a	95.7 ± 7.4 b	103.1 ± 5.8 b	103.9 ± 7.5 b
SO-10	5.6 ± 0.4 a	5.3 ± 0.4 a	6.2 ± 0.5 a	87.6 ± 8.9 a	98.1 ± 6.8 b	108.0 ± 6.7 b	108.1 ± 10.0 b
SP-1	5.4 ± 0.6 a	5.9 ± 0.5 a	6.3 ± 0.5 a	84.4 ± 9.7 a	96.0 ± 6.2 b	104.8 ± 7.3 b	106.2 ± 9.3 b
SP-3	5.2 ± 0.9 a	5.8 ± 0.2 a	6.5 ± 0.2 a	87.7 ± 6.4 a	94.4 ± 7.9 b	104.9 ± 7.7 b	105.4 ± 6.9 b
SP-5	5.8 ± 0.3 a	5.3 ± 0.4 a	6.4 ± 0.2 a	87.9 ± 3.8 a	94.3 ± 3.8 b	102.8 ± 5.8 b	104.8 ± 4.3 b
C	5.4 ± 0.5 a	5.2 ± 0.2 a	6.0 ± 0.2 a	82.6 ± 2.9 a	84.4 ± 2.3 a	90.3 ± 7.7 ab	94.6 ± 8.1 b

[a] Values are expressed as means ± SD ($n = 6$). Data in the same column with different superscripts are significantly different ($p < 0.05$). [b] H, high fat and cholesterol diet; C, normal formula; SO-1, high fat and cholesterol diet containing 0.01% AZ seed essential oil; SO-5, high fat and cholesterol diet containing 0.05% AZ seed essential oil; SO-10, high fat and cholesterol diet containing 0.1% AZ seed essential oil; SP-1, high fat and cholesterol diet containing 1% AZ SP; SP-3, high fat and cholesterol diet containing 3% AZ SP; and SP-5, high fat and cholesterol diet containing 5% AZ SP.

STC. Although the reductions in levels of STC by either SO or SP were not that promising enough when compared with the control, speculatively, a more prolonged feeding could have reached more satisfactory results, implicating the hypocholes-

Table 6. Effect of Different AZ Diets on Serum Lipid Levels of Syrain Hamsters[a]

	mg/dL				
groups	STC	STG	LDL-C	HDL-C	LDL-C/HDL-C
H	332.0 ± 2.1 A	237.8 ± 7.0 A	111.8 ± 6.8 A	98.3 ± 5.1 D	1.1 A
SO-1	300.0 ± 8.2 B	137.5 ± 5.7 BC	92.0 ± 5.1 B	205.4 ± 5.4 AB	0.5 ± 0.0 B
SO-5	291.0 ± 5.3 BC	133.3 ± 8.0 BC	66.3 ± 1.2 D	221.1 ± 2.7 B	0.3 ± 0.0 CD
SO-10	303.0 ± 4.3 B	156.3 ± 2.3 B	92.2 ± 3.3 B	202.3 ± 3.9 AB	0.5 ± 0.1 B
SP-1	297.2 ± 3.1 B	155.0 ± 3.7 B	79.1 ± 3.4 C	209.3 ± 4.1 AB	0.4 ± 0.0 C
SP-3	290.0 ± 5.2 BC	132.0 ± 2.9 BC	67.2 ± 3.1 CD	214.5 ± 7.6 AB	0.3 ± 0.0 CD
SP-5	279.7 ± 5.3 C	122.8 ± 5.6 C	56.6 ± 4.5 DE	220.9 ± 3.1 A	0.3 ± 0.1 D
C	145.6 ± 5.1 D	104.3 ± 7.6 D	44.0 ± 2.9 E	186.1 ± 6.1 C	0.2 ± 0.1 E

[a] Values are expressed as means ± SD ($n = 6$). Data in same column with different superscripts are significantly different ($p < 0.05$).

Table 7. AZ Seeds Showed Promising Hepatoprotective Lipidemic Characteristics[a]

			mg/g		
groups	liver wt (g)	L/B ratio[b](%)	HTG	HTC	HPL
H	8.0 ± 0.9 A	7.7 ± 1.3 A	20.4 ± 1.5 A	20.5 ± 1.4 A	20.4 ± 2.2 C
SO-1	8.4 ± 0.7 A	7.8 ± 0.9 A	17.5 ± 1.3 C	15.1 ± 0.6 B	37.2 ± 4.3 B
SO-5	8.0 ± 0.7 A	7.9 ± 0.4 A	17.0 ± 1.0 CD	14.0 ± 1.1 C	37.1 ± 5.9 B
SO-10	7.7 ± 0.5 A	7.1 ± 0.5 A	18.5 ± 1.3 B	16.4 ± 1.4 C	39.3 ± 4.5 B
SP-1	8.0 ± 0.7 A	7.2 ± 0.5 A	18.2 ± 1.9 B	13.0 ± 0.5 CD	35.9 ± 5.2 B
SP-3	8.2 ± 0.6 A	7.1 ± 0.3 A	17.4 ± 1.7 C	12.4 ± 0.8 CD	35.4 ± 3.3 B
SP-5	8.0 ± 0.6 A	7.3 ± 0.5 A	16.5 ± 1.5 D	12.1 ± 0.6 CD	34.5 ± 5.9 B
C	3.1 ± 0.4 B	3.5 ± 0.3 B	16.6 ± 1.8 D	10.9 ± 0.4 D	43.8 ± 2.5 A

[a] Values are expressed as means ± SD ($n = 6$). Data in each column with different letters are significantly different ($p < 0.05$). HTG, hepatic triglycerides; HTC, hepatic TC; and HPL, hepatic phospholipids. [b] L/B ratio, liver to body weight ratio.

terolemic effect of AZ seeds, although moderate at the initial stage, and could be very prominent in the long run. Quercetin is able to significantly reduce the activity and mRNA levels of various enzymes involved in hepatic fatty acid synthesis. Rutin reduces only a few of the parameters for lipogenesis, suggesting that a reduction in hepatic lipogenesis is the mechanism underlying the hypolipidemic quercetin (*24*). As quercetin appeared more abundantly than rutin in AZ seeds, more effective suppression of fatty acid synthesis could be expected. Conversely, Park et al. found in rat models that rutin was capable of reducing serum cholesterol, triglycerides, and oxidative product levels, although not very effective in suppressing the activity of liver HMG-CoA reductase, yet effectively inhibited the activity of acyl coenzyme A cholesterol acyltransferase (ACAT) (*25*). Moreover, AZ seeds contain profound amount of fibers, which accelerate bile acid secretion and metabolism by forming conjugates with bile acids, enhancing excretion in feces, and reducing the recycled bile acid metabolites. Shinnick et al. demonstrated a similar hypocholesterolemic effect with bile acid pool in rats using oatmeal (*26*). Thus, the hypolipidemic activity of AZ seeds probably can be attributed to the complicated synergistic mechanism exerted by its profound amounts of rutin, quercetin, and crude fibers. As mentioned, increased SP in diets could be more beneficial to retain a normal STC level.

STG Levels. AZ seeds are effective hypotriglyceridemics as well as hypocholesterolemics. As mentioned in the above, quercetin and rutin can significantly reduce the activity and mRNA levels of various enzymes involved in hepatic fatty acid synthesis. The potent and complicated hypolipidemic can be ascribed to the synergistic mechanism exerted by its profound amounts of rutin, quercetin, and crude fibers. Results also indicate that SP diets would be more beneficial in this regard.

Serum LDL-C and HDL-C Levels. Elevated levels of HDL-C were seen in all SO- and SP-fed groups. Concomitantly, apparent suppression of LDL-C and elevation of HDL-C levels was exhibited by all SO and SP diets. Accumulating evidence has revealed that 1,8-cineole, α-terpinene, and γ-terpinene together with the isoflavonoid rutin and quercetin, as well as polyphenolics, are excellent LDL-C suppressing agents. Amazingly, highly elevated levels of HDL-C were seen by feeding on SO and SP diets. More favorable concentration ranges were SO-5, SP-3, and SP-5. More importantly, the ratio of LDL-C/HDL-C was well-controlled by either SO or SP feedings. Worth noting, too, a high essential oil ingredient such as SO-10 could induce unfavorable physiological status. One discrepancy arised here that the sum of LDL-C plus HDL-C actually exceeded that of STC value; the deviation may have been caused by several reasons: (i) the interkit deviation as usually encountered in determination of the prostate specific antigen (PSA), which may range from 46 to 227%, and (ii) the operational defects have not completely hydrolyzed the bound form cholesterols, which remain for further investigation. Although the absolute liver weights were increased in all groups, the appearance of livers obtained from group SP-5 was still normal in color and texture, implicating that higher dosages of SP could be more beneficial and healthy when hepatoprotective bioactivity is concerned. As is well-known, the risk factors pertinently related with atherosclerosis are hypercholesterolemia, hypertriglyceridemia, and a suppressed level of in vivo antioxidative capability (*27*). Cholesterol feeds were reported to stimulate VLDL secretion, increase serum levels of VLDL-TG, cholesteryl esters (CE), and LDL, and conversely to suppress the HDL-C concentration. The level of HDL-C is always negatively correlated with atherosclerosis (*28, 29*). Surprisingly, AZ seeds and its essential oils are potent HDL-C elevating plant medicines, especially the SP that has been confirmed to be nontoxic (data not published), HDL-C enhancing as well as LDL-C/HDL-C suppressing in nature. Furthermore, rutin is capable of reducing serum cholesterol, triglycerides, and oxidative product levels (*25*); with the aid of profound fiber content in AZ seeds, the cholesterol levels were greatly reduced. Alternatively, HMG-CoA reductase, cholesterol transfer protein (CETP), and ACAT are positively correlated with de novo synthesis of cholesterol, and activation of CETP accelerated the increase of serum cholesterol levels (*30*); speculatively, the latter was inhibited by some constitutes yet unclear in AZ essential oils.

Hepatic Lipid Parameters. Rutin is capable of reducing serum cholesterol, triglycerides, and oxidative product levels (*25*). Alternatively, as mentioned in the above, high fiber content can be very beneficial to reduce TC, thus both levels of HTG and HTC were effectively suppressed.

In summary, AZ essential oil accounts for 0.51% in seeds, which comprises 53 kinds of constituents including 11 monoterpenoids, nine oxygenated monoterpenoids, 21 sesquiterpenoids, five oxygenated sesquiterpenoids, three aldehydes, one acid, and eight esters. Among which, the relatively more abundant are monoterpenoids, sesquiterpenoids, and their oxygenated derivatives. Most parts are recoverable by pentane elution, while the major parts of the oxygenated monoterpenoids and oxygenated sesquiterpenoids are extractable by ether. AZ seeds contain profound crude fibers and flavonoids (rutin and quercetin) in addition to extremely high contents of polyphenolics in the EE. The latter exhibits potent DPPH free radical scavenging activity, being more effective as an antilipoperoxidative than the AE. Finally and the most attractive, AZ seeds and its essential oils are potent HDL-C elevating plant medicines. Especially, AZ SP is nontoxic and extremely strong HDL-C enhancing as well as LDL-C/HDL-C suppressing in nature. Conclusively, AZ SP is a potent HDL-C elevating (equivalently LDL-C/HDL-C ratio-suppressing) plant medicine. It can be used as a good hepatoprotector and antiatherosclerotic.

LITERATURE CITED

(1) Maria, A. C.; José, H. L.; Andrelina, N. C.; David, N. C.; Manassés, C. F. Myorelaxant and antispasmodic effects of the essential oil of *Alpinia speciosa* on rat ileum. *Phytother. Res.* **2000**, *14* (7), 549–551.

(2) Hong, X.; Hui, X. D.; Keng, Y. S. Labdane diterpenes from *Alpinia zerumbet*. *Phytochemistry* **1996**, *42*, 149–151.

(3) Lahlou, S.; Galindo, C. A.; Leal-Cardoso, J. H.; Duarte, G. P. Cardiovascular effects of the essential oil of *Alipinia zerumbert* leaves and its main constituent, terpinen-4-ol, in rats: Role of the autonomic nervous system. *Planta Med.* **2002**, *68*, 1097–1102.

(4) Lahlou, S.; Interaminense, L. F.; Leal-Cardoso, J. H.; Duarte, G. P. Antihypertensive effects of the essential oil of *Alpinia zerumbet* and its main constituent, terpine-4-ol, in DOCA-salt hypertensive conscious rats. *Fundam. Clin. Pharmacol.* **2003**, *17*, 323–330.

(5) Krishna, B. M.; Chaganty, R. B. Cardamonin and alpinetin form the seed of *Alpinia speciosa*. *Phytochemistry* **1973**, *12*, 238.

(6) Yasuhiro, T.; Mohammad, S.; Arjun, A.; Banskota, H.; Kadota, S. Blepharocalyxins C-E: Three novel antiproliferative disarylheptanoids from the seeds of *Alpinia blepharocalyx*. *Tetrahedrom Lett.* **2000**, *41*, 5903–5907.

(7) Hsu, S. Y. Effective compositions of *Alpinia zerumbet* rhizoma and stems—The peptic ulcer protectant. *J. Chin. Pharm. (Taiwan)* **1988**, *40*, 41–48.

(8) Mpalantinos, M. A.; de Moura, R. S.; Parente, J. P.; Kuster, R. M. Biologically active flavonoids and kava pyrones form the aqueous extract of *Alpinia zerumbet*. *Phytother. Res.* **1998**, *12*, 442–444.

(9) Masuda, T.; Mizuguchi, S.; Tanaka, T.; Iritani, K.; Takeda, Y. Isolation and structure determination of new antioxidative ferulic acid glucoside esters form the rhizome of *Alpinia speciosa*, a Zingiberaceae plant used in Okinawan food culture. *J. Agric. Food Chem.* **2000**, *48*, 1479–1484.

(10) Morita, M.; Nakanishi, H.; Morita, H.; Mihashi, S.; Itokawa, H. Structure and spasmolytic activities of derivatives form sesquiterpenes of *Alpinia speciosa* and *Alpinia japonica*. *Chem. Pharm. Bull.* **1996**, *44*, 1479–1484.

(11) Knekt, P.; Kumpulainen, J.; Jarvinen, R.; Rissanen, H.; Heliovara, M.; Reunanen, A.; Hakulinen, T.; Aroma, A. Flavonoid intake and risk of chronic diseases. *Am. J. Clin. Nutr.* **2002**, *76*, 560–568.

(12) Mizui, T.; Sato, H.; Hirose, F.; Doteuchi, M. Effect of antiperoxidative drugs on gastric damage induced by ethanol in rats. *Life Sci.* **1987**, *41*, 755–763.

(13) Trnovsky, J.; Letoureneau, R.; Haggog, E.; Boucher, W.; Theoharides, T. C. Quercetin-induced expression of rat mast cell protease and accumulation of secretory granules in rat basophilic leukaemia cell. *Biochem. Pharmacol.* **1993**, *46*, 2315–2316.

(14) Pisha, E.; Pezzuto, J. M. Fruits and vegetables containing compounds that demonstrate pharmacological activity in humans. In *Economic and Medical Plant Research*; Wagner, H., Hikino, H., Fransworth, N. R., Eds.; Academic Press: London, United Kingdom, 1994; Vol. 6, pp 189−233.

(15) Yildizogle-Ari, N.; Altan, V. M.; Altinkurt, O.; Ozturk, Y. Pharmacological effects of rutin. *Phytother. Res.* **1991**, *5*, 19–23.

(16) AOAC (Association of official Analytic Chemists). In *Official Methods of Analysis*, 13th ed.; Horwitz, W., Ed.; AOAC: Washington, DC, 1984.

(17) Lin, L. Y.; Peng, C. C.; Yang, Y. L.; Peng, R. Y. Optimization of bioactive compounds in buckwheat sprouts and their effect on blood cholesterol in hamsters. *J. Agric. Food Chem.* **2008**, *56*, 1216–1223.

(18) TNO. *Compliation of Mass Spectrao Valite Compounds in Food*; Central Institute of Nutrition and Food Research-TNO: Zeist, The Netherlands, 1988.

(19) American Institute of Nutrition (AIN). Report of the American Institute of Nutrition ad hoc committee on standards of nutritional studies. *J. Nutr.* **1977**, *107*, 1340−1348.

(20) Yamauchi, R.; Tatssumi, Y.; Asano, M.; Kato, K.; Ueno, Y. Effect of mental salts and fructose on the autooxidation of methyl linoleate in emulsions. *Agric. Biol. Chem.* **1988**, *52* (3), 849–850.

(21) Neda, M. D.; Biljana, B.; Marina, S.; Biserka, M.; Milan, M. Antimicrobial and antioxidant activities of three *Mentha* species essential oils. *Planta Med.* **2003**, *69*, 413–419.

(22) Fernandes, E. S.; Passos, G. F.; Medeiros, R.; da Cunha, F. M.; Ferreira, J.; Campos, M. M.; Pianowski, L. F.; Calixto, J. B. Anti-inflammatory effects of compounds alpha-humulene and (−)-*trans*-caryophyllene isolated from the essential oil of *Cordia verbenacea*. *Eur. J. Pharmacol.* **2007**, *22*, 1–9.

(23) Ruberto, G.; Baratta, M. T. Antioxidant activity of selected essential oil components in two lipid model systems. *Food Chem.* **2000**, *69* (2), 167–174.

(24) Odbayar, T. O.; Badamhand, D.; Kimura, T.; Takahashi, Y.; Tsushida, T.; Ide, T. Comparative studies of some phenolic compopunds (quercetin, rutin, and ferulic acid) affecting hepatice fatty acid synthesis in mice. *J. Agric. Food Chem.* **2006**, *54*, 8261–8265.

(25) Park, S. Y.; Bok, S. H.; Jeon, S. M.; Park, Y. B.; Lee, S. J.; Jeong, T. S.; Choi, M. S. Effect of rutin and tannic acid supplements on cholesterol metabolism in rats. *Nutr. Res. (N. Y.)* **2002**, *22*, 283–295.

(26) Shinnick, F. L.; InK, S. L.; Marlett, J. A. Dose response to a dietary oat bran fraction in cholesterol-fed rat. *J. Nutr.* **1990**, *120*, 561–568.

(27) Halliwell, B. Antioxidants and human disease: A general introduction. *Nutr. Rev.* **1997**, *55*, S44−S52.

(28) Murakami, S.; Kondo, Y.; Toda, Y.; Kitajima, H.; Kameo, K. Effect of taurine on cholesterol metabolism in hamsters: Up-regulation of low density lipoprotein (LDL) receptor by taurine. *Life Sci.* **2002**, *70*, 2355–2366.

(29) Miyazaki, A.; Koga, T. Pravastin sodium, an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase, decreases serum total cholesterol in Japanese White rabbits by two different mechanisms. *Atherosclerosis* **2002**, *162*, 299–306.

(30) Foger, B.; Chase, M.; Amar, M. J.; Vaisman, B. L. Cholesteryl ester transfer protein corrects dysfunctional HDL and reduces aortic atherosclerosis in lecithin: Cholesterol (LCAT)-transgenicmice. *J. Biol. Chem.* **1999**, *274*, 36912–36920.

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