RESEARCH ARTICLE

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Received: 26.07.2023
Acceptance: 05.05.2024
DOI: 10.18521/ktd.1332992

This article was written from the thesis and a part of it was presented as an oral at the 2nd International Dr. Safiye Ali Congress on Multidisciplinary Studies in Health Sciences in 2022.

Konuralp Medical Journal
e-ISSN1309–3878
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Effects of Walnut Septum on The Enzyme Pathways Associated with Plasma Cholesterol Level

ABSTRACT

Objective: Cholesterol is crucial compound that plays pivotal role in cellular function in living organisms. Its excess or deficiency in plasma can lead to destruction and disintegration of cell membrane structure. Maintaining balanced intake of cholesterol in diet and seeking medical treatment, if necessary, can help prevent these negative effects. Furthermore, people often resort to natural and herbal remedies, such as walnut septum. Due to dearth of scientific data regarding effects of walnut septum on cholesterol metabolism, this research was undertaken to explore its potential effects.

Methods: Analysis was begun by extracting septum using various solvents. Resulting extracts were then analyzed using GC-MS, and compounds were identified by using an integrated library database. To detect effects of extracts on cholesterol esterase and HMG-CoA reductase, a colorimetric method was employed.

Results: Monophenol, 2,4-Di-tert-butylphenol, 2,6-Di-tert-butylphenol, ethyl linoleate, and butyl linoleate were some of compounds detected by GC-MS scanning. The highest inhibitions were observed in the enzymatic analysis, with a rate of 3.2% (acetone) in the HMG-CoA reductase analysis and 13.6% (water) in the cholesterol esterase analysis.

Conclusions: Although the walnut septum extract contains various chemical compounds, our in vitro analysis data suggest that there is no inhibitory effect at therapeutic level on enzyme pathways that regulate plasma cholesterol levels, namely HMG-CoA reductase and cholesterol esterase. We believe that further research is necessary to comprehensively evaluate its effects on other pathways.

Keywords: Walnut Septum, HMG-CoA Reductase, Cholesterol Esterase, GC-MS.

Ceviz Septumunun Plazma Kolesterol Düzeyi ile İlişkili Enzim Yolakları Üzerine Etkileri

ÖZET

Amaç: Kolesterol, canlı organizmaların hücresel fonksiyonunda rol oynayan önemli bir bileşiktir. Kan plazmasındaki fazlalığı veya eksikliği, hücre zar yapısının tahribine ve parçalanmasına neden olabilmektedir. Diyette, dengeli kolesterol alımını sürdürmek ve gerekirse tıbbi tedavi almak, bu olumsuz etkilerin önlenmesine yardımcı olabilir. Ayrıca, halk arasında genellikle ceviz septumu gibi doğal ve bitkisel tedavilere başvurulmaktadır. Ceviz septumunun kolesterol metabolizması üzerindeki etkilerine ilişkin bilimsel verilerin yetersizliği nedeniyle, bu araştırma potansiyel etkilerini araştırmak için yapılmıştır.

Yöntem: Analiz, çeşitli çözücüler kullanılarak septumun ekstrakte edilmesiyle başlatıldı. Elde edilen ekstraktlar daha sonra GC-MS'te analiz edildi ve bileşikler, entegre kütüphane veri tabanı kullanılarak tanımlandı. Ekstraktların kolesterol esteraz ve HMG-CoA redüktaz üzerindeki etkilerini saptamak için kolorimetrik yöntem kullanıldı.

Bulgular: GC-MS taraması ile tespit edilen belirgin bileşikler arasında monofenol, 2,4-Di-tert-bütilfenol, 2,6-Di-tert-bütilfenol, etil linoleat ve bütil linoleate bulunmaktadır. HMG-CoA redüktaz analizinde en yüksek inhibisyon %3,2 oranıyla aseton ekstraktında gözlenirken kolesterol esteraz analizinde %13,6 oranıyla sulu ekstrakta gözlendi.

Sonuç: Ceviz septum ekstraktı, çeşitli kimyasal bileşikler içermesine rağmen, plazma kolesterol seviyelerini düzenleyen enzim yolaklarından HMG-CoA redüktaz ve kolesterol esteraz üzerinde, in vitro olarak, terapötik seviyede inhibitör etkisinin olmadığını göstermektedir. Diğer yollar üzerindeki etkilerini kapsamlı bir şekilde değerlendirmek için daha fazla araştırmanın gerekli olduğuna inanmaktayız.

Anahtar Kelimeler: Ceviz Septum, HMG-CoA Redüktaz, Kolesterol Esteraz, GC-MS.

INTRODUCTION

Recently, new cholesterol drugs have been investigated by screening inhibitors of transmembrane protein Hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase, which facilitates transformation of HMG-CoA to mevalonate the critical point of control in cholesterol production (1). In addition, research on inhibitors of cholesterol esterase (CE), which plays a role in absorption of cholesterol and other lipids from the intestines, is also underway (2). One of the most critical factors in exploring these drugs; cholesterol is an essential molecule for stability of cell membrane structure. Thus, its amount must be kept within narrow limits (3). When there is excess or deficiency of cholesterol in plasma and other tissues, it can lead to destabilization of the cell membrane and disruption of apoptosis and necrosis mechanisms (4). Hypercholesterolemia leads to decrease in membrane viscosity, impairs selective permeability of the membrane by preventing pore closure, and causes formation of necrotic cell masses and tissue damage by affecting matrix metabolism (5). Amount of extrinsic cholesterol intake can be lessened by diminishing activity of pancreatic cholesterol esterase, which facilitates passage of cholesterol and lipids from diet from intestines into bloodstream (2). Internal balance of cholesterol levels is maintained by regulating the activity of HMG-CoA reductase, the enzyme involved in intracellular cholesterol synthesis. Despite their side effects, statins, which were initially derived from fungi but can now be synthesized, are the most effective class of drugs for inhibiting HMG-CoA reductase activity. To avoid negative effects associated with side effects, drugs with fewer side effects are preferred for regulating cholesterol levels (1). Based on this, there has been examination and analysis of new natural and herbal products that may offer safer response. Walnut, which has been recommended for medicinal use for a long time, is one of the plants that has been investigated. Various parts of the plant, such as fruit, seed, oil, leaf, and bark, have been subject of research for their diverse therapeutic effects (6). The therapeutic effects of walnut have been attributed to presence of unsaturated fatty acids, plant sterols, and polyphenols in its structure, according to reports (6-8). There are studies on the effects of walnut kernel (9), leaf (10) and green husk (11) on disorders related to cholesterol and lipid metabolism. However, although there is a belief among people that the walnut septum is effective, there is no literature data available on its potential effects on cholesterol metabolism, except for antibacterial (12), antitumoral (13), antidiabetic (14), and antioxidant (12) studies. In this study, various solvents (water, ethanol, methanol, acetone, hexane, and cyclohexane) were used to extract the walnut septum, followed by gas chromatography mass spectrometry scanning to identify its compounds. In the study was aimed to investigate whether the walnut septum had inhibitory effect on both CE and HMG-CoA reductase enzymes.

MATERIAL AND METHODS

Extract Preparation: The septum of walnuts acquired from local vendor was separated and solutions of 10% (w/v) were prepared with distilled water, ethanol, methanol, acetone, hexane and cyclohexane, and, then extracted at room temperature for 24 hours. One of aqueous solutions was separately boiled for one hour. All solutions were filtered and stock powder extracts were obtained.

GC-MS Analysis: Analysis was carried out on an Agilent 7890A GC System equipped with an Agilent 5975C inert Mass Selective Detector (MSD) with Triple Axis Detector (TAD). The mass spectrometry ion source was electron ionization (EI), and its source temperature was set to 230˚C. The carrier gas helium was used in the GC chromatography column (Agilent HP5-MS, 30m × 0.25mm × 0.25µm), and injector temperature was fixed at 200˚C. For splitless mode, 5mg/ml samples (distilled water powder extracts were dissolved in methanol and then injected) were injected into device with injection volume of 1.0 µL. Oven temperature was held at 40˚C for 5 minutes before being incremented to 100˚C at a rate of 5˚C per minute (5˚C/min). It was kept at 100˚C for 5 minutes and then increased to 225˚Cat a rate of 20˚C (20˚C/min) and maintained at 225˚C for 8 minutes in gradient mode. The analysis time was 33.25 minutes. The components' mass spectra in scanned samples were identified through comparison with integrated library database, such as the Wiley Registry of Mass Spectral Data, 7th Edition, and the NIST (National Institute of Standards and Technology 98 Library).

Analysis of the HMG-CoA Reductase Enzyme Activity: In the analysis performed according to instructions of HMG-CoA reductase commercial kit (Bio Vision K588-100), lyophilized HMG-CoA reductase, HMG-CoA, and NADPH were dissolved in 550 µL, 1300 µL and 440 µL distilled water, respectively. Extracts were dissolved in distilled water on a water bath, and solutions of 10 mg/ml were prepared, which were then diluted 1:5, 1:10 and 1:50. To run experiment in a 96-well plate, a reaction mixture (for one well; HMG-CoA 12 µL, NADPH 4 µL, and HMG CoA reductase assay buffer 174 µL) was prepared. Atorvastatin (10 mM) was transferred to inhibitor control well and 2 µL of extract to test wells. Then 3 µL of HMG-CoA reductase assay buffer, 5 µL of HMG-CoA reductase and 190 µL of the reaction mixture were transferred onto them. For enzyme well; 5 µL of HMG-CoA reductase, 5 µL of HMG-CoA reductase assay buffer, and 190 µL of the reaction mixture were transferred. After all transfers

were performed, they were kept at 37°C for 10 minutes and absorbances of 340 nm were read spectrophotometrically. Percent inhibition values were determined according to equation that "% inhibition= [(A (enzyme)- A (enzyme + extract))/ A(enzyme)] x 100".

Analysis of the Cholesterol Esterase Enzyme Activity: Volumes of 50 µL, 30 µL, 20 µL and 10 µL of the previously prepared 1:5 dilution were used for in vitro analysis performed according to reference method (15). Reaction, in which enzyme activity was measured without using the extracts, was taken as "control". Activator sodium taurocholate (12mM) (Sigma) was dissolved in 100 mM sodium phosphate and 100 mM NaCl (pH=7) assay buffer and kept at 0°C. Pancreatic cholesterol esterase (Sigma 26745) stock solution (0.018 U/mL) was prepared with 100 mM sodium phosphate buffer (pH=7), and diluted with same buffer and kept at 0 °C. Substrate para-nitrophenyl butyrate (Sigma), which was prepared as 4 mM with acetonitrile, was diluted 1:4 with assay buffer. Amounts were transferred to 96-well plate as shown in Table 1. The well-plate without substrate was kept at 30°C for 10 minutes, and at end of time, the substrate was added, and reaction was started. Absorbance was read at 408 nm, and percent inhibition values were determined using the equation "% inhibition= [(A (enzyme)- A (enzyme + extract))/ A(enzyme)] x 100".

Table 1. Content of cholesterol esterase enzyme analysis

Group	Test buffer	Distilled water	Sodium taurocholate	Para-Nitrophenyl butyrate	Cholesterol esterase	Extract
Enzyme (control)	80 µL	100 µL	100 µL	20 µL	10 µL	0
100 µg sample	80 µL	50 µL	100 µL	20 µL	10 µL	50 µL
60 µg sample	80 µL	70 µL	100 µL	20 µL	10 µL	30 µL
40 µg sample	80 µL	80 µL	100 µL	20 µL	10 µL	20 µL
20 µg sample	80 µL	90 µL	100 µL	20 µL	10 µL	10 µL

RESULTS

The compounds and the percentages data obtained by GC-MS scanning of walnut septum extracts prepared in different solvents are given in Table 2. Percentages of monophenol, 2,4-Di-tert-butylphenol, 2,6-Di-tert-butylphenol, ethyl linoleate and butyl linoleate were higher than other compounds. In colorimetrically measured enzyme data, inhibition rate of septum extracts on the HMG-CoA reductase enzyme was 3.2%, while rate of atorvastatin was 88.7% (Table 3). Inhibitory effect on the cholesterol esterase enzyme was seen in the distilled water extract with 13.6, but no inhibiting effect was observed in the other samples except for acetone (Table 4).

DISCUSSION

A parallel relationship is observed between deterioration of cholesterol balance in a body and increase in lipid metabolism-dependent disorders (16). Statins are the most common drugs used to restore balance (1). Additionally, it is outlined that walnut species are recommended because of their unsaturated fatty acid which has a therapeutic effect on disorders related to lipid metabolism (17). Presence of secondary metabolites such as phenolic compounds, which play a role as antioxidants in oxidative stress, makes the walnut species even more valuable (18).

Unsaturated fatty acids, one of chemical components in structure of walnut plant, have been seen in seed part (kernel) (19). According to a study by Pei et al, oleic acid, linoleic acid, and linolenic acid were common fatty acid components in the kernels (20). In a 1998 study that analyzed septum compounds, triacyl-glycerides predominated among the lipid components. It was reported that the fatty acids included in their formation were palmitic, stearic, oleic, linoleic, and linolenic (21). When the fatty acid content of the septum was examined in a thesis study, it was found that linoleic acid was the most abundant, followed by lesser amounts of oleic acid, gamma-linolenic acid and alpha-linolenic acid (22). Through our GC-MS scanning (Table 2), we identified that ethyl and butyl esters of linoleic acid (ethyl linoleate and butyl linoleate) stood out from fatty acid profiles of septum extracts.

The phenolic fractions of septum analyzed by Bezhuashvifi and Kurashvili were rich in low molecular mass aromatic substances, involving gallic, syringic, 4-hydroxybenzoic, protocatechuic, p-coumaric, ferulic, and vanillic acids (21). In the work of Rusu et al., gallic acid, protocatechuic acid, gentisic acid, catechin, syringic acid, epicatechin, vanillic acid, ferulic acid, p-coumaric, hyperoside, isoquercitrin, chlorogenic acid, and quercitrin were identified (23). Liu et al. found 75 phenolic compounds, including phenolic acids, tannins, and flavonoids, among which were quercetin-rhamnose-hexoside, kaempferol-rhamnoside quercetin-3-O-glucoside first identified (24). Fourteen phenolic compounds (gallic acid, catechin, phthalic acid, vanillin, ethyl gallate, kaempferol, dihydroquercetin, taxifolin-3-o-α-L-arabinofuranoside, quercetin-3-rhamnoside, quercetin-3-o(4-o-acetyl)-α-L-rhamnopyranoside, propyl gallate, blumenol B and vanillic acid) were reported by Hu et al. in the septum content analysis (25).

Table 2. Retention time and percentage values of the compounds seen as a result of scanning the extracts in GC-MS.

	A		B		C		D		E		F		G	
Compound name	R.T. min	% of total	R.T. min	% of total	R.T. min	% of total	R.T. min	% of total	R.T. min	% of total	R.T. min	% of total	R.T. min	% of total
1-Hexadecene	-	-	-	-	22.641	1.745	18.107	0.005	24.297	4.542	-	-	-	-
1-P-Menthene	-	-	-	-	-	-	21.220	0.060	-	-	-	-	-	-
1-Tetradecene	-	-	-	-	-	-	-	-	22.647	4.144	-	-	-	-
2,4-Di-tert-butylphenol	23.757	1.012	-	-	23.757	5.503	-	-	23.757	5.460	23.752	68.028	-	-
2,6-Di-tert-butylphenol	-	-	-	-	-	-	-	-	-	-	-	-	23.767	42.945
3-Carene	-	-	-	-	-	-	13.245	0.109	-	-	-	-	-	-
4-Vinylphenol	20.192	6.631	20.307	0.901	-	-	-	-	-	-	-	-	-	-
9-Hexadecenoic acid	-	-	16.213	2.111	-	-	-	-	-	-	-	-	-	-
Acrylic acid	-	-	-	-	-	-	-	-	25.236	23.194	-	-	-	-
Alpha terpinolene	-	-	-	-	-	-	15.772	0.118	-	-	-	-	-	-
Arachidonic acid trimethylsilyl ester	8.082	0.342	-	-	-	-	-	-	-	-	-	-	-	-
Butyl linoleate	-	-	-	-	25.744	84.769	25.583	55.128	-	-	-	-	-	-
Butylated hydroxytoluene	-	-	-	-	-	-	-	-	-	-	-	-	26.543	5.996
Carane	-	-	-	-	-	-	21.962	0.041	-	-	-	-	-	-
Caryophyllene	18.314	0.361	14.869	0.688	-	-	16.675	0.005	-	-	-	-	-	-
Citronellol	-	-	15.009	0.707	-	-	-	-	23.513	2.523	-	-	-	-
Cyclopentolate	23.363	1.086	-	-	-	-	-	-	-	-	-	-	-	-
Dinoseb acetate	22.833	0.366	-	-	-	-	-	-	-	-	-	-	-	-
Docosahexaenoic acid	-	-	17.712	0.481	-	-	17.603	0.008	-	-	-	-	-	-
Docosane	20.976	1.281	-	-	21.458	0.143	-	-	22.413	0.330	23.592	3.566	29.143	1.943
Dodecane	19.590	6.101	19.585	6.570	19.580	0.336	-	-	21.547	0.840	19.590	0.687	11.865	1.656
Eicosane	-	-	-	-	23.591	1.543	-	-	26.668	21.137	29.215	3.306	4.149	3.017
Ethinamate	22.242	0.633	17.884	0.464	-	-	17.297	0.004	-	-	-	-	-	-
Ethyl linoleate	-	-	-	-	-	-	25.755	22.345	-	-	-	-	-	-
Ethyl linolenate	13.966	0.324	-	-	-	-	-	-	-	-	-	-	-	-
Eugenol	22.309	0.964	-	-	-	-	-	-	-	-	-	-	-	-
Farnesane	-	-	-	-	22.102	0.092	-	-	21.251	0.683	14.973	0.197	-	-
Gamma terpinene	23.638	9.094	23.633	15.483	-	-	14.838	0.064	-	-	-	-	-	-
Gamma terpineol	-	-	-	-	-	-	-	-	7.278	0.884	-	-	-	-
Geranial	-	-	-	-	-	-	21.106	0.106	-	-	-	-	-	-
Geranyl acetate	-	-	-	-	-	-	22.569	0.082	-	-	-	-	-	-
Geranyl linalool isomer B	6.572	0.367	-	-	-	-	-	-	-	-	-	-	-	-
Guaiacol	15.808	10.734	-	-	-	-	-	-	-	-	-	-	-	-
Hemimellitene	-	-	-	-	-	-	-	-	12.607	3.666	-	-	-	-
Heneicosane	-	-	-	-	23.264	0.264	-	-	21.899	0.652	27.975	1.873	27.991	2.975
Heptadecane	-	-	-	-	23.352	0.274	-	-	-	-	23.357	0.455	26.398	2.146
Hexadecane	-	-	-	-	21.541	0.122	-	-	-	-	24.343	2.306	22.730	2.323

(Notes; R.T. : Retention time, "-": No detection, Extracts= A: Macerated in distilled water, B: Boiled in distilled water, C: Macerated in acetone, D: Macerated in ethanol, E: Macerated in methanol, F: Macerated in hexane, G: Macerated in cyclohexane)

Table 3. Percentage of inhibition of walnut septum extracts on the HMG-CoA reductase enzyme

Extracts	% Inhibition	In-well concentration
A	0.8	20 µg/mL
	1	5 µg/mL
	1.4	2 µg/mL
B	0.7	20 µg/mL
	1.6	5 µg/mL
	2.3	2 µg/mL
C	2.4	20 µg/mL
	1.8	5 µg/mL
	3.2	2 µg/mL
D	1.9	20 µg/mL
	2	5 µg/mL
	2.4	2 µg/mL
E	0.2	20 µg/mL
	2.7	5 µg/mL
	1.6	2 µg/mL
F	1.8	20 µg/mL
	2.3	5 µg/mL
	2.2	2 µg/mL
G	2.6	20 µg/mL
	3.1	5 µg/mL
	2.7	2 µg/mL
H	88.7	56 µg/mL

Notes; Extracts= A: Macerated in distilled water, B: Boiled in distilled water, C: Macerated in acetone, D: Macerated in ethanol, E: Macerated in methanol, F: Macerated in hexane, G: Macerated in cyclohexane, H: Atorvastatin

Table 4. Percentage of inhibition of walnut septum extracts on the cholesterol esterase enzyme

Extracts	% Inhibition	In-well concentration
A	No inhibition	333 µg/mL
	No inhibition	200 µg/mL
	8.1	133 µg/mL
	13.6	67 µg/mL
B	No inhibition	333 µg/mL
	2.7	200 µg/mL
	6.3	133 µg/mL
	7.2	67 µg/mL
C	1.8	333 µg/mL
	No inhibition	200 µg/mL
	No inhibition	133 µg/mL
	No inhibition	67 µg/mL
D	No inhibition	333 µg/mL
	No inhibition	200 µg/mL
	No inhibition	133 µg/mL
	No inhibition	67 µg/mL
E	No inhibition	333 µg/mL
	No inhibition	200 µg/mL
	No inhibition	133 µg/mL
	No inhibition	67 µg/mL
F	No inhibition	333 µg/mL
	No inhibition	200 µg/mL
	No inhibition	133 µg/mL
	No inhibition	67 µg/mL
G	No inhibition	333 µg/mL
	No inhibition	200 µg/mL
	No inhibition	133 µg/mL
	No inhibition	67 µg/mL

Notes; Extracts= A: Macerated in distilled water, B: Boiled in distilled water, C: Macerated in acetone, D: Macerated in ethanol, E: Macerated in methanol, F: Macerated in hexane, G: Macerated in cyclohexane

The phenolic compounds in the septum extracts we prepared using solutions of different polarities and identified by GC-MS scanning (Table 2) have a higher percentage distribution of substances such as 2,4-Di-tert-butylphenol (in hexane extract), 2,6-Di-tert-butylphenol (in cyclohexane extract) and monophenol (in distilled water extract).

These findings suggest that the reason for differences in results obtained from the aforementioned studies was due to differences in procedures used and habitats of septum.

Synthesis rate of cholesterol is determined by its level in cell. Cellular synthesis of cholesterol can be prevented by inhibiting HMG-CoA reductase, which is rate-limiting enzyme in cholesterol synthesis (26). As a result of the inhibition of HMG-CoA reductase, expression of LDL receptors, which lowers plasma concentration of cholesterol, is induced. Thus the amount of cholesterol can be regulated (27).

In our study, we investigated ability of walnut septum to obstruct HMG-CoA reductase using in vitro method. We observed that septum extracts prepared using solvents of different polarity were less effective than atorvastatin which inhibited HMG-CoA reductase by 88.7%. The highest inhibition in extracts at concentrations of 20 µg/mL and 5 µg/mL was determined in cyclohexane, at 2.6% and 3.1%, respectively, while the highest inhibition in 2 µg/mL extract was determined in acetone extract at 3.2% (Table 3). To our knowledge, there is no literature investigating inhibitory effect of walnut septum on HMG-CoA reductase activity, except for our study.

A study conducted by Oriakhi and Uadia (2020) investigated the effects of African walnut oil on HMG-CoA reductase enzyme activity in liver cells. It was reported that extract may inhibit HMG-CoA reductase activity, based on comparison of HMG-CoA reductase activity in liver cell lysates of animals fed normal diet and animals fed with African walnut oil. However, when the inhibition rate of walnut oil and atorvastatin on HMG-CoA reductase was compared after feeding with high cholesterol diet, the oil was not as effective as atorvastatin (28). Several studies investigating effects of different species on HMG-CoA reductase activity have reported that *Basella alba* (29), *Amaranthus viridis* (29) and *Piper sarmentosum* (29) species had anti-HMG-CoA reductase activity over 50% and that *Quercus infectoria* (30), *Rosa damascene* (30), *Myrtus communis* (30) and *Citrus aurantium* (31) flowers were strong inhibitors of HMG-CoA reductase. In another research on effectiveness of *Salvia* species on HMG-CoA reductase enzyme, researchers found that eight different *Salvia* species had inhibitory effect, although not as much as atorvastatin (32).

Pancreatic cholesterol esterase (CE) activity is one of the enzyme pathways associated with

plasma cholesterol level. It catalyzes cholesterol esters in intestinal lumen and mediates their entry into mucosal cells. These products are then transported into circulation via lymph, causing an increase in plasma cholesterol levels and contributing to develop and progress of atherosclerosis, a cardiovascular disease (3). To further this contribution, researchers are studying different plant species, their active ingredients, and chemical agents to investigate their potential to inhibit cholesterol esterase. In our study, we investigated effects of extracts prepared with solvents of different polarities on cholesterol esterase activity. We compared "normal enzyme activity of 100% " with "decreased enzyme activity" after treatment with the extracts. The highest inhibition rate (13,6 %) was observed in the extract that was kept in distilled water overnight, while we did not see any inhibition in the extracts prepared with ethanol, methanol, hexane and cyclohexane (Table 4). To the best of our knowledge, there are no previous studies on effects of walnut septum on cholesterol esterase activity, although there are studies on effects of other plant species on the enzyme. Thus, we have provided first record of its effectiveness of walnut septum extracts in inhibiting cholesterol esterase activity.

In one of these, it has been reported that effects on cholesterol esterase of clove (*Syzygium aromaticum*), which has an inhibitory effect as much as simvastatin, may be due to its phenolic content. Thus, it may contribute to prevention of hyperlipidemia by inhibiting cholesterol esterase activity and avoiding digestion and absorption of fats and cholesterol (33). According to another study, safflower (*Carthamus tinctorius*) and cassia (*Cassia angustifolia*) plants have a good inhibitory effect on CE, although not as much as simvastatin (34). Meanwhile there was no inhibitory effect of main bioactive component obtained from leaves of *Mangifera indica* (35) against CE, it was reported in literature that flower extracts of *Camellia nitidissima* (36), leaves of *Ecballium elaterium* (37), the bark of *Pinus brutia* (37), leaves of *Hedera helix* (37), *Dendrobium nobile* (38), *Citrus grandis* (39), grape seeds (40), hawthorn (38) and wolfberry (38) extracts had an inhibitory effect.

CONCLUSIONS

To clarify claims of cholesterol regulation by consuming walnut septum, we investigated effects of septum extracts on enzymes that are involved in enzyme pathways associated with plasma cholesterol levels. We determined that the septum did not have a noticeable inhibitory effect on either cholesterol esterase, which is responsible for absorption of cholesterol from intestines, or HMG-CoA reductase, which is responsible for intracellular cholesterol production, in vitro. Low rate of inhibitions led us to consider effects of various substances that emerged in content of walnut septum based on extraction method used.

Consequently, this research yielded worthy of note data indicating that the walnut septum extracts prepared in solvents of different polarities, despite having various chemical components, did not have a therapeutic level inhibitory effect on two different enzyme pathways that regulate the plasma cholesterol level. We consider that further research will be needed to evaluate its effects on other pathways deeply.

Acknowledgements: This article was created from the results of PhD thesis and the thesis project was supported by Düzce University Research Fund Project Number: 2019.04.03.1032. We thank Düzce University.

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