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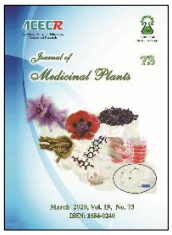

Research Article

Study of steroidal compounds from peel and seed of some pomegranate cultivars (*Punica granatum* L.) and investigating the effect of pomegranate seed oil on blood lipid levels in hypercholesterolemic rabbits

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ABSTRACT

Background: High level of blood cholesterol can cause diseases such as atherosclerosis, high blood pressure, cardiovascular diseases, and increase the chance of apoplexy and fatty liver. Unsaturated fatty acids play important roles in preventing cardiovascular diseases because they decrease total cholesterols and LDL-C in the blood. **Objective:** The study investigates some sterol compounds of Iranian pomegranate seed oil and their effects on decreasing the level of cholesterol in hypercholesterolaemia rabbits. **Methods:** Three different varieties of pomegranate were selected and oil extraction was done from powdered tissue by Soxhlet extractor. Then, the extract was transfused into GC-MS for Identification Sterol Compounds. Oil extracted from pomegranate was taken into the diets of hypercholesterolemia rabbits. **Results:** The results showed that the major phytosterols in pomegranate seed and skin tissues were stigmasterols, Δ5-avenasterols, campsterols and beta-sitosterols, among which the β-sitosterols most dominant phytosterols. Studying the influence of oil extracted on decreasing cholesterol in hypercholesterolemic rabbits show that using 5 and 10 g/kg extracted oil from pomegranate seeds in the diet of hypercholesterolemic rabbits (tested with cholesterol 1%) can decrease significantly total cholesterol, triglyceride and LDL-C of hyperlipidemia against neutral sample and it can increase significantly HDL-C. **Conclusion:** The role of the pomegranate seed oil in improving the lipid profile of the plasma and reducing undesirable fats may be can have a considerable effect on human health and lowering blood lipids and lowering the risk of cardiovascular disease. Increasing awareness of the potential capacity of this valuable fruit can help to play a better role in the various industries.

Abbreviations: LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; RSQ, Red Skin of Qom; WSR, White Skin of Rijab; BSY, Black Skin of Yazd; GC/MS, Gas Chromatography/Mass Spectrometry; TG, total triglyceride; TC, total cholesterol.

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1. Introduction

Today's high-polluted industrialized world has led to emerging diseases. One of them is blood cholesterol, which involved not only older people, but also most of the young people as well as the teens. Sometimes, high level of cholesterol doesn't show any signs, although it can lead to such diseases as atherosclerosis, high blood pressure, cardiovascular diseases, higher chance of apoplexy, fatty liver and so on [1]. Cholesterol level management is a process that should be observed continuously. The first approach to control cholesterol is to modify the diet. Decreasing meat and rice and increasing vegetables in daily diet are the most important points to be satisfied [2]. Phytosterols, including plant sterols and stanols, are steroidal compounds with chemical structures and biological functions like cholesterol. Using one or more mechanisms, phytosterols compete with the absorption of cholesterol in the intestines and decrease blood cholesterol level [3] and also they decrease the influence of triglyceride by decreasing the density of lipoprotein particles full of triglycerides that are produced by the liver [4]. The richest natural sources of phytosterols are herbal oils and the products made of them [5]. European Food and Drug Administration (FDA) confirms the roles of foods consisting of phytosterols and the diets including low levels of saturated fats in decreasing the risk of affliction to cardiovascular diseases [6]. Steroidal compounds are synthesized in the plants, whereas animals get the compounds by their diets [7]. Pomegranate (*Punica granatum* L.) is a beautiful shrub with fallen succulent leaves. Planting pomegranate and utilization of it has had a great history [8, 9]. Pharmaceutical use of the fruit is an ancient activity so that pomegranate had been a symbol of life, longevity, health, and immortality [10]. Several compounds of different tissues of pomegranate had been purified that have variant treatment, dietary, cosmetic, sanitary and industrial utilizations [8]. It was proofed that there are phytosterols specially β-Sitosterol in such tissues of the fruit as leaf, stem, seed and flower [11, 12]. In ancient medical texts, there were some believes about the influences of the fruit's compounds on decreasing the amount of cholesterol [13]. The studies show that daily drinking the pomegranate juice causes to decrease the level of bad cholesterol such as low density of lipoprotein (LDL-C) and triglyceride and to increase of good cholesterol such as high density of lipoprotein (HDL-C) [14]. Pomegranate seed oil includes considerable amounts of steroidal compounds which have great roles in decreasing the level of cholesterol [15]. The study investigates some sterol compounds of Iranian pomegranate seed oil using the approach of GC-Mass and their effects on decreasing the level of cholesterol in hypercholesterolemia rabbits. Since studies have been done to reduce the effect of pomegranates on reducing blood cholesterol on Iranian pomegranates fruit juice, the present study aimed to investigate the effect of pomegranate seed oil on cholesterol level, and could be offered a new strategy for the use of pomegranate seed waste in Iranian cultivars in reducing cholesterol blood.

2. Materials and Methods

2.1. Providing herbal samples

In order to extract the oil from seeds and skin tissues of pomegranate, samples from pomegranate collections were prepared by the Agricultural and Natural Resources Research Centre of Isfahan Province. Based on the skin colour of the fruits (white, red and black) and the coincidence of growth periods,

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threevarieties, Red Skin of Qom (RSQ), Sour White Skin of Rijab (WSR), and Sweet Black Skin of Yazd (BSY) were selected and then skin and seeds of fruits were sampled.

2.2. Extracting oil

To dry the seed and skin tissues of pomegranates, 100 g of each were placed in glass containers for 8 hours in an electrical oven at 65℃. They were pulverized, using an electrical mill. Then the powder was protected in aluminium foils of -20℃ for utilization in the next stages. Extracting oil from powdered tissues was done by using Soxhlet extractor by standard methods for 8 hours with N-hexane [16].

2.3. Identification sterol compounds

Herbal sterols are groups of steroid alcohols that form the main part of the unsaponifiable phase of the herbal oils. Measuring phytosterols of the herbal oils are a main indicator to identify the oils in order to qualify [17]. Therefore, five fruits of each cultivars of pomegranates were selected with a sufficient factor of cassation and three oil extractions were done from each fruit using Soxhlet method. The extracted oils related to each tissue were combined together and then were solved in methanol. Then, the mixture was transfused into GC-MS 6100 with column of DB-5 (length 30 m, internal diameter 0.25 mm). The process of calibration was done by using perflouro-3-butylamine. In this test, the GC method, ESS was selected and in considered method for Mass, the mass range was in 40-300, because there is not peak in range lower than 40. Method of EI was used in order to gain the mass spectrum. Helium was used as a bearer in the column of the device. Transfusion of oil and methanol mixture took 70 minutes. After transfusing the samples into the column, sufficient temperature table was programmed in the device (begin the program with 150℃ for 2 minutes, then scale up the temperature with the ratio of 5℃/min until reaching to 320℃ and finally fix in 320℃ for 5 minutes). Analyses of mass spectrums written by the device were compared with mass spectrums of the device's library. In order to decrease the volume of information and to facilitate analyses of the results, device data only were compared to sterols library. Purity is the main factor to accept the candidates that its maximum is 1000 and it shows a 100% matching. Purity equalled 800 is an accepting factor that shows a 80% matching and three first candidates are more important [18].

2.4. Investigating the influences of oil extracted from tissues on decreasing cholesterol

In order to investigate phytosterols' performances in decreasing cholesterols, oil extracted from pomegranate was taken into the diets of hypercholesterolemia rabbits. So, 20 New-Zealand matured rabbits weighted 1700-2000 g were prepared from the Institute of Razi. In order to adjust to the environment, the rabbits were kept for two weeks under a basic diet and standard circumstances of light (12 hours lightening), temperature (23-25 ºC) and humidity (40-70%). The rabbits were fed with a standard diet provided from the Institute of Pasteur. Before beginning the diet, 5 ml blood was cupped from a pestle vein before breakfast in order to determine the plasma level of cholesterol, triglyceride, LDL-C and HDL-C for confirming low level of lipid. One percent EDTA was used in order to prevent clotting. To provide full-cholesterol diet, cholesterol powder of MERCK (Germany) in 100 g boxes and olive oil were used so that 1 g of cholesterol powder was solved in 4 g olive oil and then after heating and homogenizing, was used to lubricate 100 g

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food (1 g cholesterol, 4 ml olive oil and 95 g food). Every day, the plate was full of food and the rabbits accessed to water. The rabbits were divided to 4 groups with 5 members. For 20 days, the first group was fed with standard diet and other groups were fed with full cholesterol diet (1%). At the end of the period, the process of cupping was done again from pestle vein and then plasma level of total cholesterol, triglyceride, LDL-C and HDL-C were identified. Feeding the groups continued for 25 days for the next stage, but 5 g/kg and 10 g/kg extracted oil of pomegranate seed (Soxhlet method) were added respectively to the diets of groups 3 and 4. After that, the process of cupping was done from pestle vein and plasma levels of total cholesterol, triglyceride, LDL-C and HDL-C were determined.

2.5. Lipid profile

The prepared blood samples placed at room temperature for 45 minutes to clot, then the blood tubes were centrifuged at 2000 g for 25 minutes at 4˚C temperature and the separated serum was transferred to new sterilized tubes and then stored at -20˚C until analysis was performed. The amount of triglyceride (TG), total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) of serum were determined by using biochemistry enzyme kits (Pars Azmon, Tehran, Iran) according to the manufacturer's instructions and measured by automated chemistry analyser (Hitachi Automatic Analyser 902, Roch, Basel, Switzerland). In order to determine low-density lipoprotein cholesterol (LDL-C), the following equation was used [19]:

$$LDL\text{-}C = TC-(TG/5)-(HDL\text{-}C)$$

2.6. Statistical analysis

Experiments were conducted in a completely randomized design. Analysis of variance of the data was performed using one-way ANOVA method and comparison of mean of data was done by the LSD method using SPSS version 16 software.

3. Results

3.1. Studying sterol compounds

The results of this study showed that the main phytosterols included stigmasterols, Δ5-avenasterols, campsterols and beta-sitosterols. The total amount of sterol varied from 639.88 mg to 427.39 mg in 100 g oil. The most abundant observed from two tissues of three cultivars of pomegranate is β-sitosterol and formed 65%-74% of all sterols (Table 1). Between tissues of seed and skin of the fruits, seed tissue has the maximum amount of phytosterols. The difference between phytosterols tissues of seed and skin was significant ($P \leq 0.05$). Between cultivars of pomegranate, BSY had the maximum amount of phytosterols. But, the difference between phytosterols of different cultivars wasn't significant ($P > 0.05$) neither for extracted samples of seed nor for ones extracted from skin. Fig. 1 shows phytosterols of skin and seed tissues related to three cultivars of pomegranate.

3.2. Studying Influence of Oil Extracted from Tissues of Pomegranate on Decreasing Cholesterol

Competing to abstract cholesterol from the intestine, phytosterols cause to decrease of cholesterol level of the blood [3]. Table 2 shows the results of phytosterols' performances to decrease cholesterols. After the test, for all groups other than group 1, total cholesterol increased significantly ($P \leq 0.05$) compared with before the test. Results of the study show a significant ($P \leq 0.05$) difference between total cholesterol of group 1 and other groups and also between group 2 and other groups. In groups 3

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and 4 total cholesterol decreases after the test compared with group 2. In contrast, there wasn't significant ($P > 0.05$) difference between groups 3 and 4 despite the decrease in cholesterol in group 4 compared to the group 3. The results show that there is a significant ($P \leq 0.05$) difference of triglyceride between group 1 and other groups. In addition, difference of triglyceride between group 2 and groups 3 and 4 was significant ($P \leq 0.05$).

Table 1. Results of the studying sterol compounds in three cultivars of pomegranate

Sterol Compound	Skin RSQ	Skin WSR	Skin BSY	Seed RSQ	Seed WSR	Seed BSY
Cholesterol	0.96	0.89	1.02	1.31	1.56	1.07
Brassicasterol	0.71	0.68	0.65	1.11	1.24	0.83
Cholоresterol	3.84	3.98	4.12	5.23	4.96	4.24
Campesterol	41.21	43.33	38.45	49.87	56.28	51.14
Stigmasterol	23.57	21.66	25.31	30.33	31.45	29.31
Beta-Sitosterol	327.39	308.53	316.76	465.88	489.29	448.67
Δ-5-Avenasterol	32.38	35.86	31.48	42.27	38.16	40.32
Δ-2,23-Stigmastadiano	5.89	6.23	6.12	6.77	8.84	7.61
Δ-7-Stigmasterol	2.98	2.34	2.88	3.96	4.23	3.53
Δ-7-Avenasterol	3.48	3.89	4.11	5.35	3.87	4.65
Total	**442.41**	**427.39**	**430.90**	**612.08**	**639.88**	**591.37**

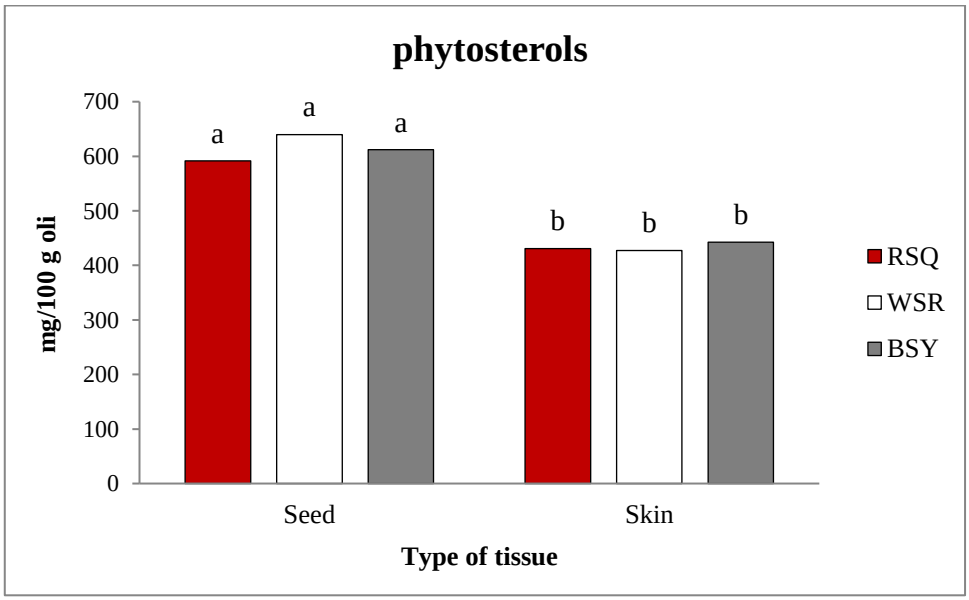

Fig. 1. Phytosterols of skin and seed tissues related to three cultivars of pomegranate.
RSQ: Red Skin of Qom, WSR: White Skin of Rijab, BSY: Black Skin of Yazd. Different letters indicate statistically significant differences ($P \leq 0.05$).

Despite of more decrease of triglyceride in group 4 against group 3, but the difference wasn't significant ($P > 0.05$). In all groups other than group 1, LDL-C increased significantly ($P \leq 0.05$) after the test against before that. The results of the study show that LDL-C of group 2 is significantly ($P \leq 0.05$) more than that for other groups. In groups 3 and 4, LDL-C decreases significantly ($P \leq 0.05$) against group 2, but there isn't significant ($P > 0.05$) difference between groups 3 and 4. The results did not show a significant ($P > 0.05$) difference of HDL-C between groups 1 and 2. HDL-C of groups 3 and 4 increased significantly ($P \leq 0.05$) against group 2. Despite of increasing HDL-C in group 4 against group 3, there wasn't significant ($P > 0.05$) difference.

Table 2. The results of phytosterols' performances to decrease cholesterols

Diet	Treatment	HDL-C	LDL-C	Total cholesterol	Triglyceride
Standard (Group 1)	-	26.3 ± 6.2^{b}	20.3 ± 5.34^{c}	48.3 ± 6.2^{c}	78.3 ± 4.78^{c}
Fatty (Group 2)	-	35.2 ± 6.63^{b}	541.7 ± 21.58^{a}	1703.2 ± 82.4^{a}	330.8 ± 18.36^{a}
Fatty (Group 3)	5 g/kg oil	69.4 ± 5.43^{a}	378.5 ± 41.2^{b}	1295.6 ± 96.4^{b}	211.2 ± 47.04^{b}
Fatty (Group 4)	10 g/kg oil	75.8 ± 9.21^{a}	335.3 ± 85.76^{b}	1182.7 ± 88.3^{b}	195.8 ± 16.9^{b}

The concentrations of all variables were calculated as mg/dl. Group 1: feeding with standard diet, Group 2: feeding with full cholesterol diet, Group 3: feeding with full cholesterol diet and 5 g/kg pomegranate's oil, Group 4: feeding with full cholesterol diet and 10 g/kg pomegranate's oil. Different study indicate statistically significant differences ($P \leq 0.05$).

4. Discussion

The results of the study showed that main phytosterols, on the base of increase abundance, included stigmasterols, Δ5-avenasterols, campsterols and beta-sitosterols. The compounds are the most current natural phytosterols and their qualitative characteristics were same. It seems that such characteristics follow a usual pattern for sterols in herbal oils. Pande & Akoh [20] in a study on six Georgian pomegranate cultivars, indicated that the major phytosterols of these six cultivars were brassicasterol, campesterol, stigmasterol and β-sitosterol, and that the levels of these phytosterols in the seed tissue were much higher than the pomegranate fruit, while the most abundant compound was β-sitosterol [20]. Caligiani *et al* [21] also in a study of pomegranate oil compounds, showed that this tissue had high content of phytosterol compounds, and the β-sitosterol (up to 8069 mg/kg) had the highest rates [21]. In this study, the total amount of sterol varied from 639.88 mg to 427.39 mg in 100 g oil. These data are consistent with the results reported by Caligiani *et al* [21]. Among the above mentioned sterols, sitosterols form 65-74% of all sterols and according to Caligiani et al [21] and Pande & Akoh [20], sitosterols are the most common phytosterols of the samples. Sitosterol contents reported in the study, were almost as same as reported amounts of them for wheat germ [21] and they were 2.5 to 12.3 times more for such oils as walnut, hazelnut, almond and peanut [22] and 2-32 times more for such herbal oils as corn, coffee, rice and specially olive oil, that are famous as the richest comestible oils [23]. The

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total amount of sterol, as well as Indicative sterols such as compressor, beta-sitosterol and stigmasterol, were considerable in the seeds of the examined specimens and so they can be used as complement sources of sterols in food and pharmaceutical industries. Sterols are fat-like parts of a membrane and they play basic roles in fluidity; so, they are necessary for different cell performances [24]. The compounds have such extensive environmental activities as anti-inflammatory, anti-cancer, antioxidation and antibacterial activities. On the other hand, the metabolites cause decreasing the cholesterols of blood serum [25-27]; therefore, as rich sources of pharmaceutical sterols, using pomegranate's seeds can be considered as a sufficient replacement for cholesterols of diets. Recently, effectiveness of herbal sterols to improve characteristics of cholesterols has been confirmed by European Administration of Food Safety [6].

Metabolic disorders and decrease of antioxidant power of blood in patients with high cholesterol cause increasing the production of free radicals and to decrease their ejection. Oxygen radicals and their combinational tendency to organic molecules and such different compounds as LDL-C cause to oxidation of them. Reaction of oxide form of LDL-C with oil layers of the cells' membrane cause to produce lipid oxide in them; These disorders cause to intensify arthrosclerosis and cardiovascular disease of the aorta in patients with high blood lipids [28]. Nowadays, it cleared that unsaturated fatty acids play important roles in preventing cardiovascular diseases because they decrease total cholesterols and LDL-C in the blood [29]. Antioxidant influences of pomegranate's seed oil and juice against lipid oxidation, particularly LDL-C, sweeping capacity of oxygen radicals and their influences on aggregation and activity of plackets were investigated in several researches [10, 30, 31]. Esmaillzadeh *et al* [13] investigates the influences of concentrated juice of pomegranate on cholesterol profiles of hyperlipidemic patients with diabetes II. They show that daily consuming 40 g pomegranate for 8 weeks can significantly effect on decreasing LDL-C, total cholesterol, LDL-C/HDL-C and total cholesterol/HDL-C. Investigating influence of pomegranate juice on patients with diabetes [13]. Rosenblat *et al* [35] show that daily consuming 50 ml for 3 month decreases significantly lipid level of serum peroxides, cell peroxides and abstraction oxidized cell of LDL-C and it significantly increases peroxonaze activity in the blood; the above-mentioned factors prevent Arthrosclerosis [35]. Results of the study show that using 5 and 10 g/kg extracted oil from pomegranate fruit in the diet of hyperlipidemic rabbits (tested with cholesterol 1%) can decrease significantly total cholesterol, triglyceride and LDL-C of hyperlipidemic against neutral sample and also it can increase significantly HDL-C. Aviram *et al* [31] examined the effects of pomegranate juice consumption on the lipid peroxidation in whole the plasma and changes in LDL and HDL lipoproteins in humans and mice. The results of their studies showed that pomegranate juice consumption reduced LDL accumulation and increased serum HDL levels by up to 20% in humans, and LDL levels decreased by 90% in mice. In treated mice, the size of atherosclerotic lesions decreased by 44% [31]. Sharifiyan *et al* [32] investigated the effect of extract of pomegranate fruit peel on diet of hypercholesterolemic rabbits. They showed that

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the use of pomegranate peel extract, despite a significant increase in serum antioxidant capacity of hypercholesterolemic rabbits, cannot improve the plasma lipid profile of the rabbits, and have no significant effect on levels of cholesterol, triglyceride and LDL-C compared to control group [32]. Al-Moraie *et al* [33] evaluated the effect of pomegranate juice consumption on three different dosage (1, 3 and 5 ml/kg b. wt.) in the diet of hypercholesterolemic rats over a period of 28 days. The results showed that oral administration of pomegranate juice significantly reduced total cholesterol, triglyceride, LDL-C, VLDL-C, and liver enzymes compared to control group, and the level of HDL-C and antioxidant enzymes increased significantly [33]. Bagri et al [11] examined the effect of pomegranate extract on diabetic rats. Their studies showed that the consumption of pomegranate extract at different dosage (250 and 500 mg/kg) for 21 days, significantly reduced the levels of fasting blood sugar, total cholesterol, triglyceride, LDL-C, VLDL-C and tissue fatty acid peroxidation levels, and significantly increased HDL-C levels, glutathione content and antioxidant enzymes compared to the diabetic control group [11]. Elbandy & Ashoush [34] in studying the effect of pomegranate seed oil, residues of seeds and their mixture on hypercholesterolemic rats, showed that rats fed with all three groups of pomegranate compounds improved the lipid profile of the plasma compared to the control group, and a significant decrease in total cholesterol, triglyceride, LDL-C [34]. The results of this study are in accordance with studies by Aviram *et al* [31], Esmaillzadeh *et al* [13], Rosenblat *et al* [35], Bagri *et al* [11], Al-Moraie *et al* [33] and Elbandy & Ashoush [34], but did not conform to study of Sharifiyan *et al* [32].

5. Conclusion

Pomegranate seed oil containing the considerable content of different phytosterols, has a high potential for cosmetic, nutritional, and health applications. Considering that the pomegranate seeds are part of the waste of the preparation of pomegranate juice processing, recycling these remaining seeds can be beneficial in many industries. Potential capacity of pomegranate seeds can have an impressive presence on nutrition and especially on the health processes. The role of the pomegranate seed oil in improving the lipid profile of the plasma and reducing undesirable fats may be can have a significant effect on human health and lowering blood lipids and lowering the risk of cardiovascular disease. Increasing awareness of the potential capacity of this valuable fruit can help to play a better role in the various industries. The study of pomegranate seed compounds in different cultivars and in different growth stages can help to clarify the hidden mystery of this valuable fruit and help to improve its nutritional and health habits.

Author contributions

Roksana Bayati performed the experiments and collected data; Hossein Ali Asadi-Gharneh guided aspects of the research and participated in writing of manuscript.

Conflict of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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References

1. Baharvand Ahmadi B, Eftekhari Z, Bahmani M, Jelodari M and Mirhoseini M. Overview of medicinal plants used for cardiovascular system disorders and diseases in ethnobotany of different areas in Iran. *J. Herb Med Pharmacol*. 2016; 5 (1): 39-44.

2. Rodriguez CJ, Cai J, Swett K, González HM, Talavera GA, Wruck LM, Wassertheil-Smoller S, Lloyd-Jones D, Kaplan R and Daviglus ML. High cholesterol awareness, treatment, and control among Hispanic/Latinos: results from the Hispanic Community Health Study/Study of Latinos. *J. the American Heart Association* 2015; 24; 4 (7): e001867.

3. Trautwein EA, Duchateau GS, Lin Y, Mel'nikov SM, Molhuizen HO and Ntanios FY. Proposed mechanisms of cholesterol-lowering action of plant sterols. *European J. Lipid Science and Technol*. 2003; 105 (3-4): 171-185.

4. Plat J and Mensink RP. Plant stanol esters lower serum triacylglycerol concentrations via a reduced hepatic VLDL-1 production. *Lipids* 2009; 44 (12): 1149.

5. Smet ED, Mensink RP and Plat J. Effects of plant sterols and stanols on intestinal cholesterol metabolism: suggested mechanisms from past to present. *Molecular Nutrition & Food Res*. 2012; 56 (7): 1058-1072.

6. Kongduang D, Wungsintaweekul J and De-Eknamkul W. Biosynthesis of β-sitosterol and stigmasterol proceeds exclusively via the mevalonate pathway in cell suspension cultures of Croton stellatopilosus. *Tetrahedron Letters* 2008; 16: 49 (25): 4067-4072.

7. Nguyen TT. The cholesterol-lowering action of plant stanol esters. *The Journal of Nutrition* 1999; 1: 129 (12): 2109-2112.

8. Varasteh F, Arzani K, Zamani Z, Tabatabaei SZ. Physico-chemical seasonal changes of pomegranate (*Punica granatum* L.) fruit' Malas-e-Torsh-e-Saveh'in Iran. In XXVII International Horticultural Congress-IHC2006: International Symposium on Asian Plants with Unique Horticultural 769. 2006; 13: 255-258.

9. Stover ED, Mercure EW. The pomegranate: a new look at the fruit of paradise. *HortScience*. 2007; 1: 42 (5): 1088-1092.

10. Lansky E, Shubert S and Neeman I. Pharmacological and therapeutic properties of pomegranate. In Symposium on production, processing and marketing of pomegranate in the Mediterranean region: advances in research and technology. Séminaires Méditerranéens (CIHEAM). 2000. (pp. 231-235).

11. Bagri P, Ali M, Aeri V, Bhowmik M and Sultana S. Antidiabetic effect of *Punica granatum* flowers: effect on hyperlipidemia, pancreatic cells lipid peroxidation and antioxidant enzymes in experimental diabetes. *Food and Chemical Toxicol*. 2009; 1: 47 (1): 50-54.

12. Rahimi HR, Arastoo M and Ostad SN. A comprehensive review of Punica granatum (pomegranate) properties in toxicological, pharmacological, cellular and molecular biology researches. *Iranian J. Pharmaceutical Res.* 2012; 11 (2): 385.

13. Esmaillzadeh A, Tahbaz F, Gaieni I, Alavi-Majd H and Azadbakht L. Cholesterol-lowering effect of concentrated pomegranate juice consumption in type II diabetic patients with hyperlipidemia. *International J. Vitamin and Nutrition Res*. 2006; 1: 76 (3): 147-151.

14. Fuhrman B, Volkova N, Aviram M.

Pomegranate juice inhibits oxidized LDL uptake and cholesterol biosynthesis in macrophages. *The J. Nutritional Biochem.* 2005; 1: 16 (9): 570-576.

15. Zak A, Zeman M, Vitkova D, Hrabak P and Tvrzicka E. Beta-sitosterol in the treatment of hypercholesterolemia. *Casopis Lekaru Ceskych*. 1990; 129 (42): 1320-1323.

16. Eikani MH, Golmohammad F and Homami SS. Extraction of pomegranate (Punica granatum L.) seed oil using superheated hexane. *Food and Bioproducts Processing*. 2012; 1: 90 (1): 32-36.

17. Matysik S, Klünemann HH and Schmitz G. Gas chromatography–tandem mass spectrometry method for the simultaneous determination of oxysterols, plant sterols, and cholesterol precursors. *Clinical Chemistry* 2012; 1: 58 (11): 1557-64.

18. Amirav A, Gordin A, Poliak M and Fialkov AB. Gas chromatography-mass spectrometry with supersonic molecular beams. *Journal of Mass Spectrometry* 2008; 43 (2): 141-63.

19. Lateef T and Qureshi SA. Centratherum anthelminticum and Withania coagulans improves lipid profile and oxidative stress in triton X-100 induced hyperlipidemic rabbits. *Group*. 2020; 1 (11.36): 1-22.

20. Pande G and Akoh CC. Antioxidant capacity and lipid characterization of six Georgia-grown pomegranate cultivars. *Journal of Agricultural and Food Chemistry* 2009; 10: 57 (20): 9427-9436.

21. Caligiani A, Bonzanini F, Palla G, Cirlini M and Bruni R. Characterization of a potential nutraceutical ingredient: pomegranate (*Punica granatum* L.) seed oil unsaponifiable fraction. *Plant Foods for Human Nutrition* 2010; 1: 65 (3): 277-283.

22. Maguire LS, O'sullivan SM, Galvin K, O'connor TP and O'brien NM. Fatty acid profile, tocopherol, squalene and phytosterol content of walnuts, almonds, peanuts, hazelnuts and the macadamia nut. *International J. Food Sciences and Nutrition* 2004; 1: 55 (3): 171-178.

23. Carretero AS, Carrasco-Pancorbo A, Cortacero S, Gori A, Cerretani L and Fernández-Gutiérrez A. A simplified method for HPLC-MS analysis of sterols in vegetable oil. *European J. Lipid Science and Technol*. 2008; 110 (12): 1142-1149.

24. Laloi M, Perret AM, Chatre L, Melser S, Cantrel C, Vaultier MN, Zachowski A, Bathany K, Schmitter JM, Vallet M and Lessire R. Insights into the role of specific lipids in the formation and delivery of lipid microdomains to the plasma membrane of plant cells. *Plant Physiol*. 2007; 1: 143 (1): 461-472.

25. Vivancos M and Moreno JJ. β-Sitosterol modulates antioxidant enzyme response in RAW 264.7 macrophages. *Free Radical Biology and Medicine* 2005; 1: 39 (1): 91-97.

26. Prieto JM, Recio MC and Giner RM. Anti-inflammatory activity of β-sitosterol in a model of oxazoloneinduced contact-delayed-type hypersensitivity. *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas*. 2006; 5 (3): 57-62.

27. Baskar AA, Al Numair KS, Gabriel Paulraj M, Alsaif MA, Muamar MA and Ignacimuthu S. β-sitosterol prevents lipid peroxidation and improves antioxidant status and histoarchitecture in rats with 1, 2-dimethylhydrazine-induced colon cancer. *J. Medicinal Food* 2012; 1: 15 (4): 335-343.

28. Aviram M. Modified forms of low density

lipoprotein and atherosclerosis. *Atherosclerosis* 1993; 4: 98 (1): 1-9.

29. Melgarejo P and Artes F. Total lipid content and fatty acid composition of oilseed from lesser-known sweet pomegranate clones. *J. the Science of Food and Agriculture* 2000; 80 (10): 1452-1454.

30. Schubert SY, Lansky EP and Neeman I. Antioxidant and eicosanoid enzyme inhibition properties of pomegranate seed oil and fermented juice flavonoids. *J. Ethnopharmacol*. 1999; 1: 66 (1): 11-7.

31. Aviram M, Dornfeld L, Rosenblat M, Volkova N, Kaplan M, Coleman R, Hayek T, Presser D and Fuhrman B. Pomegranate juice consumption reduces oxidative stress, atherogenic modifications to LDL, and platelet aggregation: studies in humans and in atherosclerotic apolipoprotein E–deficient mice. *The American J. Clinical Nutrition*. 2000; 1: 71 (5): 1062-1076.

32. Sharifiyan F, Movahedian-Attar A, Nili N and Asgary S. Study of pomegranate (*Punica granatum* L.) peel extract containing anthocyanins on fatty streak formation in the renal arteries in hypercholesterolemic rabbits. *Advanced Biomedical Res*. 2016; 5 (8): 1-6.

33. Al-Moraie MM, Arafat RA and Al-Rasheedi AA. Effect of pomegranate juice on lipid profile and antioxidant enzymes in hypercholesterolemic rats. *Life Science J.* 2013; 10 (3): 2717-28.

34. Elbandy MA and Ashoush IS. Phytochemicals in pomegranate seeds and their effect as hypolipidemic agent in hypercholesterolemic rats. *World J. Dairy & Food Sciences* 2012; 7 (1): 85-92.

35. Rosenblat M, Hayek T and Aviram M. Anti-oxidative effects of pomegranate juice consumption by diabetic patients on serum and on macrophages. *Atherosclerosis* 2006; 1: 187 (2): 363-371.

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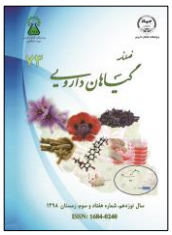

مقاله تحقیقاتی

مطالعه ترکیبات استروئیدی پوست و بذر برخی ارقام انار و بررسی اثر روغن بذر انار بر سطوح چربی خون در خرگوش‌های هیپرکلسترولمی

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اطلاعات مقاله

گل‌واژگان:
بتا-سیتوسترول
کلسترول
فیتوسترول
روغن هسته انار

چکیده

مقدمه: سطح بالای کلسترول می‌تواند باعث عوارضی مانند آترواسکلروز، فشار خون بالا، بیماری‌های قلبی-عروقی، افزایش احتمال سکته مغزی و کبد چرب شود. اسیدهای چرب غیراشباع در پیشگیری از بیماری‌های قلبی-عروقی نقش مهمی ایفا می‌کنند، زیرا باعث کاهش کلسترول و لیپوپروتئین کم‌چگالی در خون می‌شوند. **هدف:** هدف این مطالعه بررسی ترکیبات استرولی روغن بذر برخی ارقام انار و اثرات آن بر کاهش سطوح کلسترول خون در خرگوش‌های هیپرکلسترولمی می‌باشد. **روش بررسی:** سه رقم متفاوت انار انتخاب شدند و روغن بذر آنها بوسیله سوکسله استخراج و ترکیبات استرولی آنها توسط دستگاه گازکروماتوگراف متصل به طیف‌سنج جرمی شناسایی شدند. روغن استخراج شده در جیره غذایی خرگوش‌های هیپرکلسترولمی به‌کار برده شد. **نتایج:** نتایج نشان داد که مهم‌ترین فیتواسترول‌های موجود در بذر و پوست انار استیگمااسترول‌ها، آونا استرول‌ها، کمپسترول‌ها و بتا-سیتوسترول‌ها بودند و در میان آن‌ها بتا-سیتوسترول‌ها ترکیب غالب بود. بررسی تأثیر روغن استخراج شده بر کاهش کلسترول در خرگوش‌های هیپرکلسترولمی نشان داد کاربرد ۵ و ۱۰ گرم بر کیلوگرم روغن استخراج شده از بذرهای انار در رژیم غذایی خرگوش‌های هیپرکلسترولمی (تست شده با کلسترول ۱٪) میزان کلسترول کل، تری‌گلیسرید و لیپوپروتئین کم‌چگالی نمونه‌های هیپرلیپیدی را در مقایسه با نمونه‌های شاهد به طور معنی‌داری کاهش داد. همچنین توانست مقدار لیپوپروتئین پرچگالی را به طور معنی‌داری افزایش دهد. **نتیجه‌گیری:** نقش روغن بذر انار در بهبود پروفایل چربی‌های پلاسما و کاهش چربی‌های نامطلوب می‌تواند تأثیر قابل توجهی بر سلامت انسان و کاهش چربی‌های خون و کاهش خطر بیماری‌های قلبی-عروقی داشته باشد. افزایش آگاهی در مورد ویژگی‌های این میوه ارزشمند می‌تواند به کاربرد بهتر آن در صنایع مختلف کمک کند.

مخففها: (LDL-C) لیپوپروتئین کم‌چگالی؛ (HDL-C) لیپوپروتئین پرچگالی؛ (RSQ) انار پوست قرمز قم؛ (WSR) انار پوست سفید ریجاب؛ (BSY) انار پوست سیاه یزد؛ (GC/MS) گاز کروماتوگراف متصل به طیف‌سنج جرمی؛ (TG) گلیسیرید کل؛ (TC) کلسترول کل

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