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In-vitro Evaluation of Complex Forming Affinity of Total Saponins Extracted from *Ziziphus spina-christi* and *Quillaja saponaria* with cholesterol

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ABSTRACT

Saponins are high molecular weight glycosides, consisting of a sugar moiety linked to a triterpene or steroid aglycone. In recent years, they have received considerable attention because of their various biological activities including hepatoprotective, anti-tumor, antimicrobial, and anti-inflammatory activities. It has been shown that oral administration of some saponins may lead to prevention of hypercholesterolemia, the phenomenon which is the result of complex formation with cholesterol. Because of the presence of considerable amounts of saponins, the leaves of *Ziziphus spina-christi* have been traditionally used for washing heir and body. The objective of the present study was to extract and characterize total saponin from *Z. spina-christi* leaves, and also evaluate possible interaction between the saponin and cholesterol. The collected leaves of the plant were identified, dried, powdered and defatted with petroleum ether in a soxhlet apparatus. The air-dried powder was successively extracted with methanol, n-butanol and diethyl ether. Then foaming power of the extracted *Z. spina-christi* total saponin (ZTS) was measured using the Ross-Miles foam column method and the index of emulsification (E_{24}) of the extracted saponin was also determined. The results were compared to data from *Quillaja saponaria* total saponin (QTS), and sodium lauryl sulfate (SLS) as a potent synthetic surfactant. Using a Du-Nouy tensiometer, critical micelle concentrations (CMCs) of the saponins were determined by measuring surface tension as a function of surfactant concentration. The effect of complex formation with cholesterol was determined by measuring the changes in surface tension and critical micelle concentrations after addition of cholesterol in saponin solutions. The results indicated that ZTS and QTS due to relatively high surface activity were capable of reducing surface tension, and therefore forming complexes with cholesterol. It can be concluded that oral administration of total saponins may cause a reduction in cholesterol absorption through gastrointestinal system.

Keywords: Saponin, *Ziziphus spina-christi,* surface tension, cholesterol

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INTRODUCTION

Saponins are secondary metabolites synthesized by many different plant species [1]. They have many medicinal uses including anti-microbial, anti-tumor, anti-insect [2] hepatoprotective, haemolytic and anti-inflammatory activities [3-5]. They also lower serum cholesterol levels, which may casuse reduceing the risk of cardiovascular disease [6]. In addition, saponins are used in preparation of soaps, detergents, shampoos, beer and cosmetic [7]. These compounds are usually extracted from higher plants, while marine organism such as starfish, sponges and sea cucumbers are now considered as a rich source of saponins [8-11]. Hypercholesterolemia is a major cause of cardiovascular diseases including atherosclerosis and coronary heart disease. Many patients prefer herbal therapies due to adverse effects of synthetic anti-hyperlipidemic agents, and also their contraindications and allergic reactions. Therefore, it is necessary to develop new safe and effective cholesterol-lowering agents from natural sources [12]. It has been reported that saponins can form insoluble complexes with cholesterol and reduce blood cholesterol levels in humans [13]. Several studies have shown that administration of ginseng saponins may decrease serum cholesterol levels, especially LDL cholesterol, and increase HDL cholesterol levels [14]. Jung *et al*. in 2007 reported that saponin extracted from *Pleurospermum kamtschaticum* was effective in reducing hypercholesterolemia and hyperlipidemia [15]. It has been shown that micellar solutions of saponins extracted from the *Q. saponaria* are efficiently able to solubilize very large hydrophobic molecules such as cholesterol, phytosterols and phenanthrene [16].

Zizyphus spina-christi, is a multipurpose tree species belonging to the botanical family Rhamnaceae and native to the warm temperature and subtropical regions, among them North Africa, South Europe, Mediterranean conteries, Australia, Tropical America, South and East of Asia and Middle East [17]. *Z. spina-christi* has been used in folk medicine for the treatment of fever, pain, dandruff, wounds and ulcers, inflammatory conditions, asthma and to cure eye diseases [18]. *Z. Spina-christi* leaves contain four saponin glycosides: christanin A (jujubagenin), christanin B, C and D [19, 20]. The aim of the study was to extract total saponin from the plant, investigate its characteristics and determine the ability of the saponins to form complex with cholesterol.

MATERIAL AND METHODS

QTS was purchased from Biochemica, Swiss. Cholesterol and SLS were obtained from Merck, Germany. All of the solvents were of analytical grade.

Plant Materials

The leaves of plant were collected from Ahvaz (Iran), and indentified in Faculty of Agriculture, Shahid Chamran University, Ahvaz. The leaves of the plant were ground into powder and stored at room temperature (25ºC).

Extraction of total saponin

The powdered leaves of *Z. spina-christi* was defatted in a soxhelet apparatus with petroleum ether (boiling range of 40-60 ºC) for removing lipids and phenolic compounds. The air-dried powder was extracted with methanol for 48 h. The solvent was removed under vacuum by rotary evaporator (Heidolph, Germany) and the resulting brown residue was suspended in water, then centrifuged at 2500 rpm for 45 min, and the supernatant was separated and extracted with water saturated n-butanol. The butanol phase was concentrated in rotary evaporator at 80ºC and the dry residue was dissolved in the least methanol quantity (30 ml), and then precipitated by addition of diethyl ether. Finally, total saponin of the plant (ZTS) was freeze-dried (Operon, Korea) and stored at room temperature [21, 22, 23].

Foaming Ability

Different concentrations of ZTS, QTS and SLS (0.01-5 mg/ml) in double-distilled water were prepared. 5 ml of each concentration was added to three tubes and tubes were vortexed for 5 seconds. After one minute, the foam height was measured. The results of average foam height were plotted as a function of saponin concentration.

Determination of Emulsification Index (E_{24})

2 ml of aqueous solution of different concentrations (0-5 mg/ml) of ZTS, QTS and SLS were added to the three tubes containing 3 ml of liquid paraffin was added to each tube. Then they were vortexed at 2500 rpm for 2 min to form emulsion. The samples were stored at 25°C for 24 h and then thickness of emulsified layer was measured. The emulsification indexs (E_{24}) were plotted as a function of the concentration.

Surface Tension and Micelle Formation Studies

Using a stock solution, different dilutions of ZTS and QTS in volumetric flask were prepared and vortexed for 5 seconds and then were kept at room temperature for 12 h. Then surface tensions of the solutions were measured using Du-Nouy Ring Tensiometer at 25˚C. Mean values of surface tensions were plotted as a function of saponin concentration. A concentration, in which no more significant change in surface tension observed, was considered as critical micelle concentration (CMC) [24].

Impact of Cholesterol

The same method was utilized to evaluate the presence cholesterol on surface tension of different concentrations of saponin solutions.

Statistical Methods

To compare the results of foam height, emulsification indices and surface tension in different samples, univariate ANOVA and general linear model were utilized. In the presence of any differences, Tukey test was utilized to analyze the difference.

RESULTS AND DISCUSSION

The extracted total saponin of Seder was 2.3% of the primary total weights of the plant material. Regarding to the results, by enhancement in the concentration of ZTS, QTS and SLS, the foam height was increased to the maximum value. SLS as synthetic commercial surfactants showed a maximum foam height of 71.7±7.6 cm. Also, foam ability saponin of Seder was more than QTS (P<0.001) and there was no significant different between the foam ability saponin of ZTS and SLS. From the results of the present study, it is clear that foam production depended on the type and concentration of surfactants. Foaming ability of surfactants is a propriety which may help improve the existence of surfactants in a solution [25]. It was suggested that saponins are able to produce foam because of a combination of water-soluble suagar chain and non-polar aglycon [26]. Due to ability of them in producing of foam, it is believed that these compounds are good candidates for employing in shampoos instead of alkanolamides. Alkanolamides are often used to prepare stable foam in shampoos, but as results of producing nitrosamines, they are potentially carcinogenic compounds. So, ZTS can be substituted the alkanolamides in formulation of a shampoo [27]. It is suggested that adding of SLS may be improve ZTS and QTS he foaming properties.

The results of emulsion stability showed that there was a significant correlation between the saponins concentration and formation of emulsions (Fig 1). A comparative analysis between the total saponins and SLS which was utilized as positive control showed that emulsification ability of saponins was significantly lower than the synthetic surfactant (P<0.05). Also, E_{24} of QTS was more than ZTS (P<0.05). It has been reported that an emulsion is considered stable if its E_{24} corresponds to 50% or more [28].

According to the results, the value of E_{24} in concentration of 5 mg/ml for ZTS, QTS and SLS was 52, 57 and 72%, respectively. Surfactants are widely used in the food industry to form and stable emulsion-based food and beverages. They facilitate the formation of oil-in-water emulsions by positioning oil-water interfaces, reducing the interfacial tension and enhancing further droplet disruption. Furthermore, adsorbed surfactants provide a protective coating around oil droplets, which inhibits their aggregation and improves the long term stability of emulsions [29]. These results suggest that the saponins can be successfully used to stabilize oil-in-water emulsions. However, further investigation is necessary to study the influence of environmental stresses such as pH, ionic strength, temperature and long-term storage on the stability of the emulsions.

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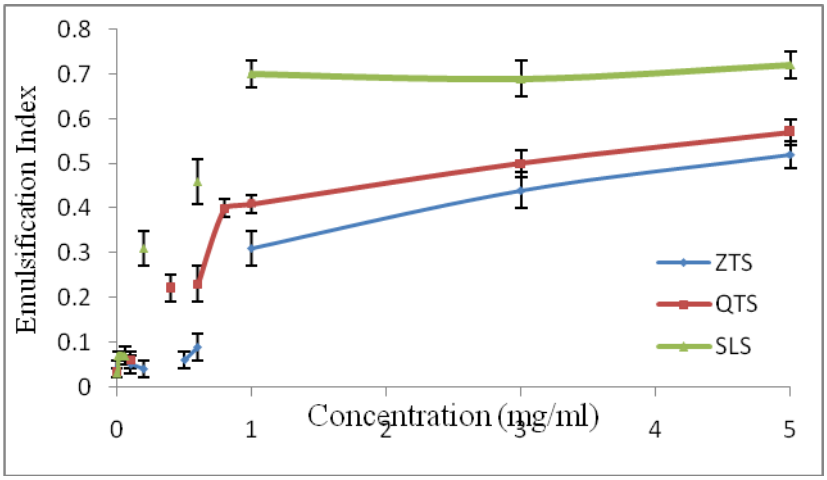

Figure 1: Emulsification indexes of different concentrations of ZTS, QTS and SLS (n=3)

The results of the measurement of surface tension of ZTS and QTS aqueous solutions saturated with cholesterol are shown in Fig (2). The CMC value of ZTS, QTS, ZTS solutions saturated with cholesterol and QTS solutions saturated with cholesterol was 0.572, 0.19, 0.49 and 0.055 mg/ml, respectively. According to the Fig (2) the presence of cholesterol in the saponin solutions decreased their surface tension. CMC is the important of property of saponins for application as surfactants. It is influenced by some factors such as temperature, solvent concentration, salt concentration and pH of aqueous phase. In addition, micelles formed in aqueous solutions can vary in size and shape depending on the saponin type [30]. Ribeiro *et al.* in 2013 investigated the functional properties of saponins from *Agave sisalana* and *Ziziphus joazeiro*. According to their results, the highest reduction in CMC values from *Ziziphus joazeiro* saponins was observed in neutral pH, near room temperature (25–30°C) and saline concentrations between 2 and 4%, while for *Agave sisalana* saponins, pH value between 3 and 4 with near room temperature; or pH value between 10–11 and temperature about 55–60 °C; and saline concentration of 2 to 6% were the optimal conditions [30].

Mitra *et al.* in 1997 reported the CMC values, between 0.51 to 0.77 g/L at 25°C, for *Quillaja* saponins from different commercial sources, while it can be decreased 0.35 g/L at 34°C [31]. Also, Stanimirova *et al*. in 2011 reported 0.25 g/L as CMC value for commercial quillaja saponins [16]. The differences between CMC values may be related to different methodologies that they used; diclorofluorescein method and surface tension method, respectively [16, 31]. In contrary to the previous, the mean value of CMC for *Quillaja* saponins in our study was 0.19 mg/ml. Also, in the other study, the value of CMC for QTS was 0.42 mg/ml [32]. A possible explanation for this difference may be due to difference in concentration and may be depended on the experimental conditions of during measurement.

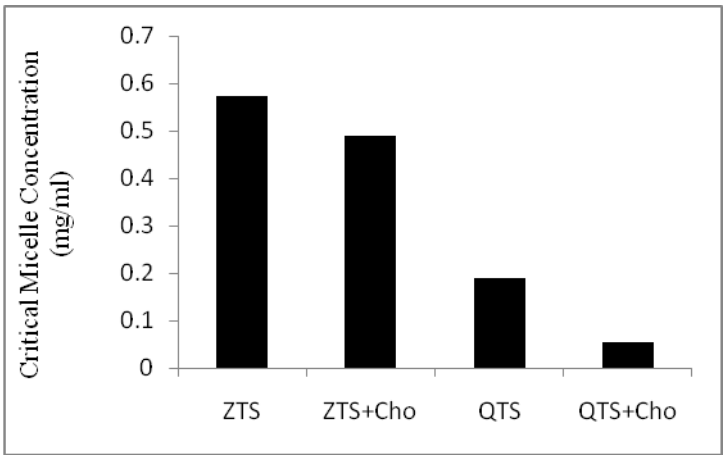

Figure 2: CMC values for ZTS and QTS

It is suggested that this effect may be associated with interaction of saponin aglycone with cholesterol [33]. It is known that the CMC values of a nonionic surfactant, such as the saponin, generally decrease with an increase in the temperature [34]. So, in case of, further experimental is necessary to investigate the influence on CMC value.

Due to surface activity of the saponins, oral administration of ZTS and QTS may reduce blood cholesterol. However, further investigation is necessary to determine their chemical reaction and also animal studies in order to evaluate their *in vivo* efficacy and toxicity.

CONCLUSION

The properties of saponin micelles change considerably with the addition of cholesterol. Based on the results, they may be suitable candidates for lowering cholesterol. The total saponin from ZTS and QTS can be suitable substitute for synthetic surfactants in drug. Also, since the saponins could able to make stable foam, they can be exploited for many applications in food, cosmetics and pharmaceuticals industries.

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REFERENCES

[1] Arif T, Bhosale JD, Kumar N, Mandal TK, Bendre RS, Lavekar GS, Dabur R. J Asian Nat Prod Res 2009; 11: 621-638.
[2] Dixon RA, Sumner LW. Plant Physiol 2003; 131: 878-885.
[3] Bink A, Pellens K, Cammue BPA, Thevissen K. The Open Mycology J 2011; 5: 29-38.
[4] Hu X, Neil SJ, Cai W, Tang Z. Functional Plant Biol 2003; 30: 901-907.
[5] Jyothi TC, Sindhu Kanya TC, Appu Rao AG. J Food Biochem 2007; 31: 1-13.
[6] Francis G, Makkar HPS, Becker K. Animal Feed Sci Technol 2005; 121: 147–157.
[7] Bhargava A, Shukla S, Ohri D. Industrial Crops and Products 2006; 23:73–87.
[8] Faizal A, Geelen D. Phytochem Rev 2013; 12: 877-893.
[9] Sharma V, Paliwal R. International J of Pharmacy and Pharmaceutical Sciences 2013; 5: 179-183.
[10] Oleszek W, Bialy Z. J Chromatogr A 2006; 1112: 78-91.
[11] Su X, Xue C, Li X, Gao X. Lou Y, Ding J. Clin Cellular Imunol 2011; 2: 1-5.
[12] EL-Farok DHM, EL-Denshry ES, Mahmoud M, Asaaf N, Emam M. Global J Pharmacol 2013; 7 (3): 298-306.
[13] Cheeke PR. J Anim Sci 2000; 77:1-10.
[14] Inoue M, Wu CZ, Dou DQ, Chen YJ, Ogihara Y. Phytomed 1999; 6: 257-265.
[15] Jung HJ, Nam JH, Park HJ, Lee KT, Park KK, Kim WB, Choi J. J Ethnopharmacol 2007; 112: 255-261.
[16] Stanimirova R, Marinova K, Tcholakova S, Denkov ND, Stoyanov S, Pelan E. Langmuir 2011; 27: 12486-12498.
[17] Basuny AM, Arafat SM. Farag HA. Int J Chem Natural Sci 2013; 1:29-36.
[18] Asgarpanah J, Haghighat E. African J Pharm Pharmacol 2012; 6: 2332-2339.
[19] Saad AH, Kadhim RB. IJRPA 2011; 2: 1802-1806.
[20] Waggas AM, Al-Hasani RH. American-Eurasian Journal of Scientific Research 2009:263-267.
[21] Soetan KO, Oyekunle MA, Aiyelaagbe OO, Fafunso MA. African J Biotechnol 2006; 5: 2405-2407.
[22] Moghimipour E, Sajadi Tabassi SA, Ramezani M, Lobenberg R. IJBMS 2003; 6: 9-19.
[23] Aqel H, Al-Charchafchi F, Ghazzawi D. Der Pharmacia Sinica 2012; 3: 349-356.
[24] Sinko PJ. Martin's Physical Pharmacy and Pharmaceutical Sciences, 6th ed. Lippincott William& Wilkins, Philadelphia. 2011, p: 389-390-621.
[25] Dehghan Noudeh G, Sharififar F, Khatib M, Behravan E, Ahmadi Afzadi M. African J Biotechnol 2010; 9: 110-116.
[26] Decroos K, Vincken JP, Koningsveld GA, Gruppen H, Verstraete W. Food Chem 2007; 101: 324–333.
[27] Aghel N, Moghimipour E, Raies Dana A. Iranian J Pharm Res 2007; 6: 167-172.
[28] Diab A, Gamal El Din S. African J Environ Sci Technol 2013; 7: 671-679.
[29] Yang Y, Leser ME, Sher AA, Julian McClements D. Food Hydrocolloids 2013; 30: 589e596.

[30] Ribeiro BD, Sales Alviano D, Weingart Barreto D, Zarur Coelho MA. Colloids and Surfaces A: Physicochem Eng Aspects 2013; 436: 736-743.
[31] Mitra S, Dungan SR: Micellar properties of quillaja saponin. J Agric Food Chem 1997; 45:1587-1595.
[32] Moghimipour E, Kooshapour H, Rezaee S, Khalili S, Handali S. Asian J Pharm Clin Res 2014: 17: 170-173.
[33] Weng A, Thakur M, Mallinckrodt B, Beceren-Braun F, Gilabert-Oriol R, Wiesner B, Eichhorst J, Böttger S, Melzigb MF, Fuchs H. J Controll Rel 2012; 164:74-86.
[34] Skurtys O, Aguilera JM. Food Hydrocolloids 2009; 23: 1810-1817.