African Journal of Biotechnology Vol.9 (1), pp. 110-116, 4 January
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Study of aqueous extract of three medicinal plants on cell membrane–permeabilizing and their surface properties

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Accepted 2 November, 2009

The aim of this study was to evaluate the effect of aqueous extract of three medicinal plants, *Artemisia dracunculus* L, *Cuminum cyminum* L and *Heracleum persicum* Desf, which contain saponins on biological membrane. Also in this study, some of their physicochemical properties were studied. At the first step, the aqueous extract of the plants were prepared, using maceration and then the extracts were lyophilized. 0.2 ml of RBC was added to 0.2 ml of different concentrations of each extract in McIvan's buffer, and then incubated in two different times and temperatures. The absorbance of the samples was determined by UV spectrophotometer. Among the three studied extracts, *A. dracunculus* L showed the highest hemolytic effect and the *Heracleum persicum* Desf showed the lowest one. The values of emulsification Index (E_{24}) and foam formation activity (F_h) showed for each extract the properties of surface activity. Regarding the results of this study, when considering the health of consumer, the use of aqueous extract of *H. persicum* Desf, with low hemolytic effect is preferred in pharmaceutical preparation. But if the hemolytic effect were considered, the use of aqueous extract of *A. dracunculus* L, with great hemolythc effect in comparison to the two other extract, is preferred.

Key words: *Artemisia dracunculus* L, *Cuminum cyminum* L, *Heracleum persicum* Desf, biological membrane, hemolysis.

INTRODUCTION

Saponins are from secondary metabolites of the plants which contain a steroid or triterpenoid aglycon attached to one or more sugar chains. They exhibit cell membrane - permeabilizing properties. Because of their foaming properties, saponins are used in the manufacturing of foods, beverages, toilet preparations and pharmaceuticals. Saponins are able to foam because of a combination of water-soluble suagar chain and non-polar aglycon. Their soapy character is due to their surfactant properties. Although saponins have been examined in many applications, especially in medicine, their natural role in plants is still a matter of discussion. Although saponins have many uses, especially in medicine, the membrane permeabilizing effect of saponins have been of great interest. In comparison to synthetic surfactants, the natural ones have attracted more attention because of their several advantages such as their diverse usage as emulsifier, foaming agent, functional foods, detergents and especially because of their safety and ease of preparations. Saponins are found in a number of medicinal plants (Price et al., 1987; Lacaile, 2005).

Absorption enhancing ability of surfactants in formulations with low absorption like peptides or proteins is used for drug delivery in non-injectable formulations. A board spectrum of surfactants used as enhancers includes

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bile salts, anionic detergents, glycerides and lysophospholipids. Morphological and biochemical studies on membrane of absorption sites showed that surfactants enhance membrane transport, followed by acute toxicity, but these effects were reversed after a long time. As a result, there is a pivotal relationship between permeability enhancement activity and acute toxicity; moreover, permeability enhancing effect of surfactants is not only related to their nature, but also depends on other characteristics like electrical charge, polarity and the membrane (Galembeck et al, 1998; Gould, 1996).

Permeability enhancers are agents that decrease or remove extra cellular layer resistance reversibly and allow the drug to pass through and between epithelial cells toward blood and lymph. Recently, enhancing drugs permeability through cellular membrane becomes one of the main topics in pharmaceutical researches (Muranishi, 1990).

Various models exist for evaluation of membrane toxicity of surfactants including single cell models using erythrocytes, erythrocyte ghosts, or liposomes. The erythrocyte model has been widely used as it presents a direct indication of toxicity of injectable formulations as well as general indication of membrane toxicity. Another advantage of erythrocytes model is that blood is readily available and that cells are easy to isolate from the blood; moreover, its membrane has similarities with other cell membrane (Robertis and Robertis, 1995).

Evaluating the permeability of enhancers using biological membranes plays an important role. Consequently, at the present study, the effects of aqueous extract of three medicinal plants of tarragon (*Artemisia dracunculus* L. Compositae), Persian cat parsnip (*Heracleum persicum* Desf ex Fisher, Apiaceae) and cumin (*Cuminum cyminum* L., Apiaceae) on biological membranes have been evaluated. The primary phytochemical screening has detected the presence of saponins in these plants (Price et al, 1987; Lacaile, 2005).

MATERIALS AND METHODS

Materials

All materials were of reagent grade unless otherwise mentioned. Aqueous extracts of *A. dracunculus* L., *C. cyminum* L. and *Heracleum persicum* Desf. were collected from Kerman province, (Iran). Sodium chloride, di-sodium hydrogen phosphate, citric acid (monohydrate), di-sodium phosphate and liquid paraffin were purchased from Merck (Germany). Drabkin's agent was supplied from Chimi-Daru (Iran).

Buffer and reagents preparation

McIlvaine's buffer was prepared as follows: solution 1, containing 21 g of citric acid (100 mM) and 8.775 g of sodium chloride (150 mM) made up to 1000 ml with deionized water, was mixed with solution 2, containing 28.4 g of di-sodium hydrogen phosphate (200 mM) and 8.775 g of sodium chloride (150 mM) made up to 1000 ml with deionized water, to produce the required pH of 7.0. Solution pH was measured by electrical pH-meter (TWT Metrohm, Germany).

Preparation of red blood cells suspension

Human blood was collected from a healthy individual with 46.7% hematocrit and added to four heparinized tubes. After centrifuging at 3000 rpm for 10 min (Hermle 230 ZA, Germany), plasma and buffy coat were removed and the erythrocytes were washed three times in at least five times of their volume with McIlvaine's buffer, pH = 7.0. Afterward, by adding McIlvaine's buffer, an erythrocyte suspension with 12% hematocrit was prepared and kept in 4°C for experiments (Gould et al., 2000).

Hemolytic method

A suspension of erythrocyte (200 µl) within a micro-tube was incubated for the required times with an equal volume of the test sample of extracts mixture, including aqueous extract of tested plants, prepared in McIlvaine's buffer, at 25 and 37°C. After incubation, the mixture was spun in a microcentrifuge at 3000 rpm for 35 s (Spectrafuge 161M, England) and 200 µl of the resulting supernatants was added to 3 ml of Drabkin's reagent. To assay for the amount of hemoglobin released, the absorbance of samples was assessed in 540 nm wavelength using spectrophotometer (Shimadzu, 3100, Japan). Positive controls consisted of 200 µl of uncentrifuged mixtures of erythrocyte suspensions and 200 µl of buffer, which was added to 3 ml Drabkin's reagent to obtain a value for 100% haemolysis. A negative control, included to measure the level of spontaneous haemolysis, comprised 200 µl buffer mixed with 200 µl erythrocytes, and after centrifugation for 35 s, a 200 µl sample of supernatant was added to 3 ml of Drabkin's reagent. Haemolysis percentage for each sample was calculated by dividing sample's absorbance on positive control absorbance (complete haemolysis) multiplied by 100 (Gould et al., 2000).

Determination of emulsification index

E_{24}, 5 ml of liquid paraffin was added to 5 ml of different concentrations of aqueous extract of tested extracts in a graduated tube and vortexed at high speed for 2 min. The emulsion stability was determined after 24 h. The E_{24} was calculated by measuring the emulsion layer formed (Carrillo et al., 1996).

Foam formation activity

Different concentrations of tested extracts were dissolved to 5 ml disodium phosphate buffer and shaken with vibrator for 5 s. The samples were put aside at 25°C for one min. F_h was measured as foam height in graduated cylinder (Dehghan Noudeh et al., 2008).

RESULTS AND DISCUSSION

The results of haemolysis induced by aqueous extracts were shown in Figures 1 - 4. Each concentration shows the mean of haemolysis percentage repeated in nine experiments. In order to compare the hemolytic effects of all extract, the concentration of each extract needed to induce 50% haemolysis was determined (data not shown). Results of E_{24} and F_h are presented in Figures 5 and 6, respectively.

Despite the fact that all of surfactants hemolytic activity

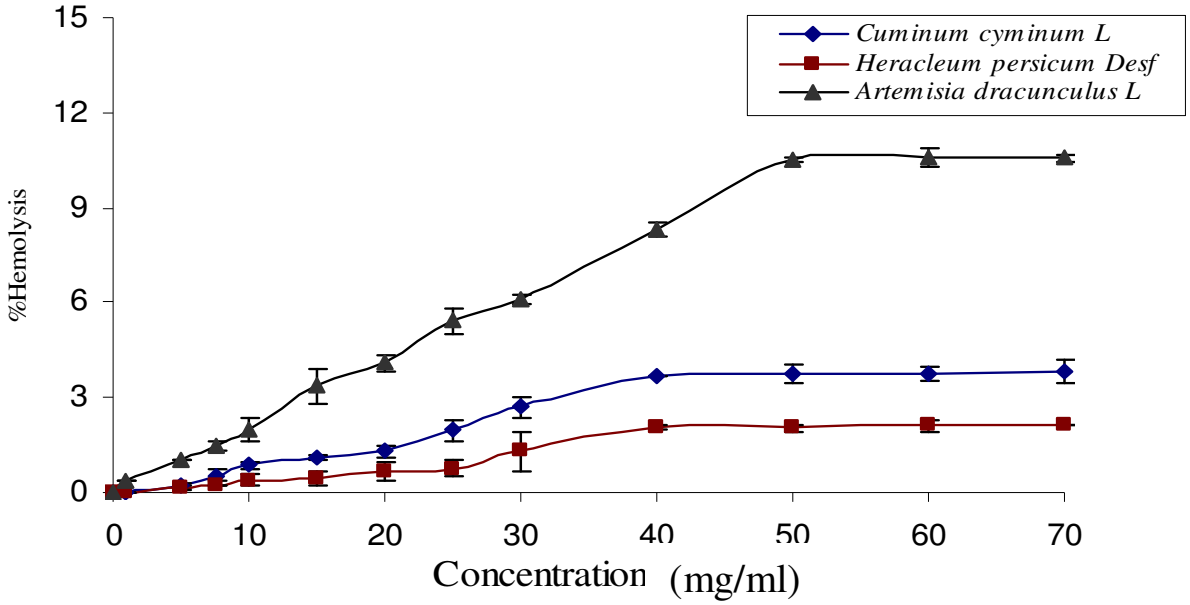

Figure 1. Hemolysis induced by *Cuminum cyminum* L, *Artemisia dracunculus* L. and *Heracleum persicum* Desf. aqueous extracts after 15 min at 25°C (n = 9).

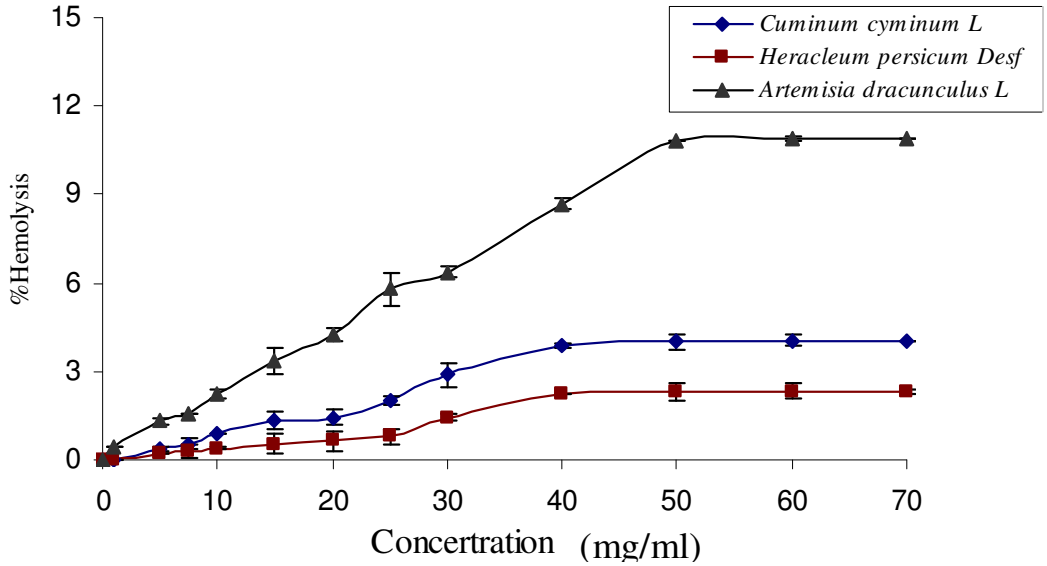

Figure 2. Hemolysis induced by *Cuminum cyminum* L, *Artemisia dracunculus* L. and *Heracleum persicum* Desf. aqueous extracts 30 min at 25°C (n = 9).

is not fully known, it is proposed that it may consist of processes in the following order: the surfactant may be absorbed and penetrate to the cell membrane, where it makes osmotic phenomenon by altering the permeability of membrane, which in turn causes the cellular lysis (Dehghan Noudeh et al., 2008). Biological membrane consists of a lipid bilayer which surrounds whole cell surface and proteins. Lipid bilayer structure is stabilized by non-covalent bonds among acyl groups and ionic bonds between polar heads and aqua. Haemolysis is due to red blood cells destruction which resulted from lysis of membrane lipid bilayer emulsion and cellular membrane destruction. As this haemolysis relates to concentration and potency of surfactant, this model can be used for evaluation of surfactants potency (Swenson and Curatolo, 1992). Regarding the high toxicity of synthetic surfactants, investigation for finding natural ones is of great interest. Among various sources for natural surfactants, saponins with special characteristics have been more considered. Saponins are used in industries for pre-

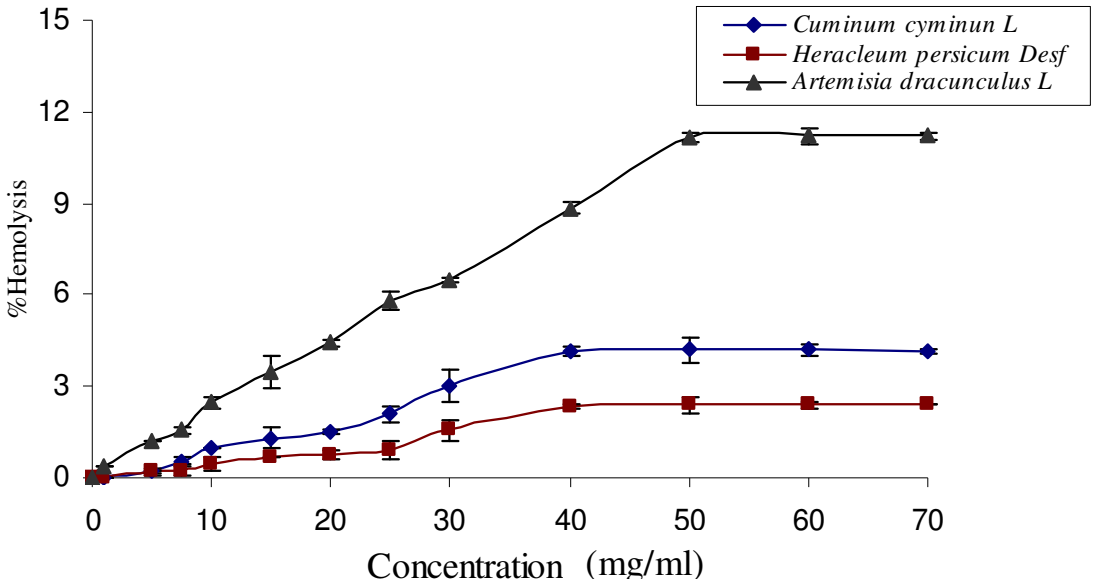

Figure 3. Hemolysis induced by *Cuminum cyminum* L, *Artemisia dracunculus* L. and *Heracleum persicum* Desf. aqueous extracts 15 min at 37°C (n = 9).

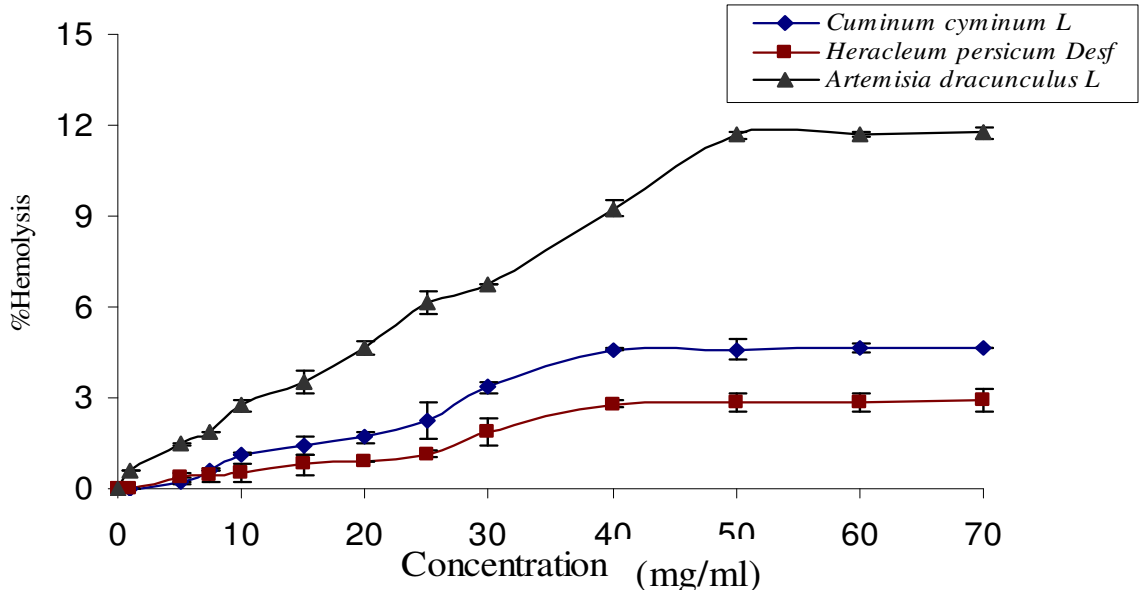

Figure 4. Hemolysis induced by *Cuminum cyminum* L, *Artemisia dracunculus* L. and *Heracleum persicum* Desf. Aqueous extracts 30 min at 37°C (n = 9).

paration of emulsions for photographic films and extensively in cosmetics, such as lipstick and shampoo. At the present study, three medicinal plant containing saponins have been evaluated for their activity on biological membranes. The results here show that hemolytic activity of aqueous extracts of tested plants increased as temperature arose. This can be attributed to liquid characteristic and fluidity of bilayer lipid of cell membrane. Therefore, some parts of the membrane can easily move throughout the surface and this characteristic is due to membrane phospholipids which convert to jelly in temperatures lower than physiologic temperature. This conversion of phospholipids helps in more stabilized and regular membrane and increases its resistance (Kleszczynska et al., 2005). The hemolytic activity of the aqueous extracts is increased in a dose-dependent manner (Figures 1 - 4). On the basis of Fick's law, diffusion flux from a membrane is proportional to concentration difference of both sides. So by increasing the concentration of saponin in extra membrane, it diffuses to intra membrane until it gets to a specific

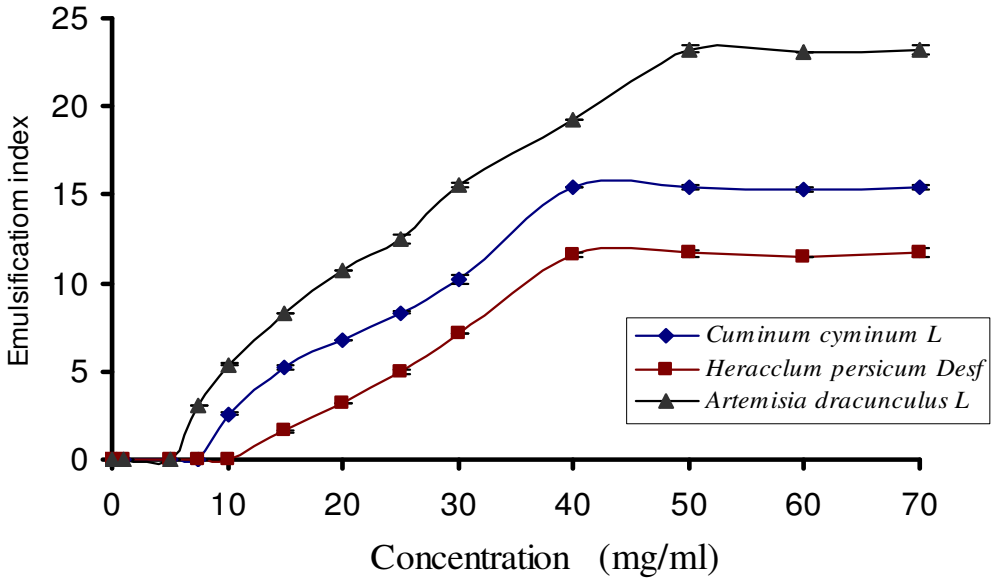

Figure 5. Emulsification index at different concentrations of *Cuminum cyminum* L, *Artemisia dracunculus* L. and *Heracleum persicum* Desf. aqueous extracts (n = 9).

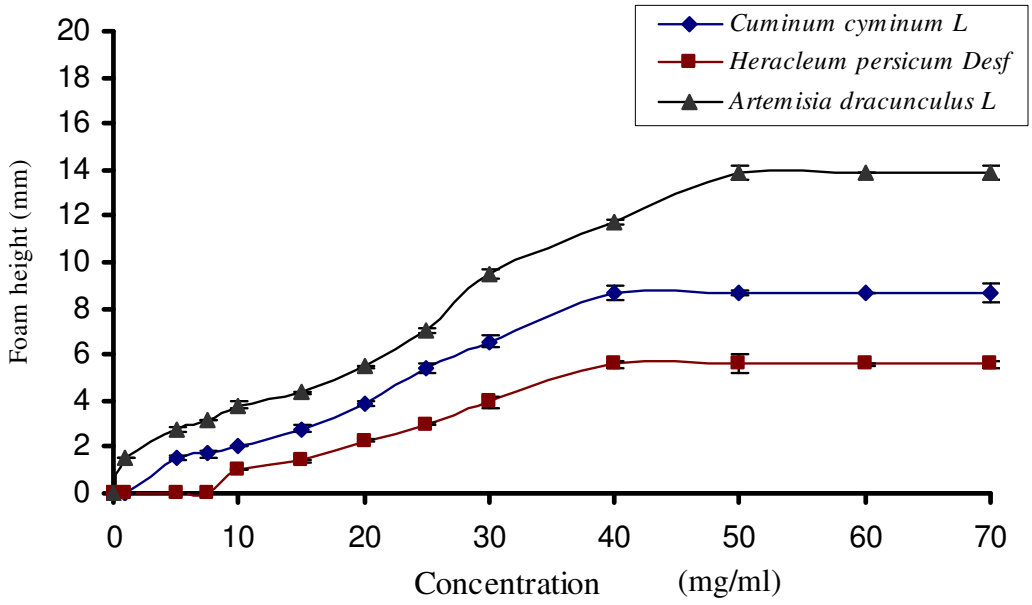

Figure 6. Foam formation activity at different concentrations of by *Cuminum cyminum* L, *Artemisia dracunculus* L. and *Heracleum persicum* Desf. aqueous extracts (n = 9).

concentration, which leads to membrane destruction and hemolytic effects (Kleszczynska et al., 2005). Furthermore the hemolytic activities of saponins are related to their chemical composition. Saponins with steroid aglycon have shown more haemolytic activity than those with triterpenoid agycons (Takechi and Tanaka, 1995). This activity is also related to their increased number of monosaccharide and the complexity of their glycidic moieties (Santos et al, 1996), acyl residues or the epoxy framework system (Oda et al., 2000).

The presence of fatty acids could also favor interactions between the saponin and membrane cholesterol promoting the haemolysis. The sugar side chains of saponins also affect heamolytic activity. The number of side chains influences both hemolytic activity and membrane permeability. Woldemichael and Wink, (2001) reported that saponins possessing two side chains induce lesshemolytic activity than saponins containing one

sugar (Tragner and Csordas, 1987). Contrary to this observation, Yamasaki et al. (1987) showed that increasing the amount of sugar side chains increased the membrane permeability for calcium ions (Araki and Rifkind, 1981). Considering all reports, the permeabilizing effect may be caused by the combination of target membrane composition, the type of the saponin side chain(s) and the nature of the aglycone (Woldemichael and Wink, 2001). This hemolytic activity increases in higher temperature because of more fluidity and permeability of the membrane (Kleszczynska et al., 2005). Our results showed that hemolytic effects of all tested extracts increase proportionally to the latency of incubation and the amount of contact duration with erythrocytes increasing (Figures 1 - 4). It is reported that the more contact duration of erythrocytes with a solution, including a surface active agent, the more is the amount of cellular lysis (Yamasaki et al., 1987). Adherence of a surface active agent such as saponins to erythrocyte's membrane is followed by their entrance that leads to alteration of the molecular structure of cell membrane, osmotic-colloid swelling and erythrocyte membrane rupture. Above mechanism depends on surfactant concentration, temperature and duration of contact with erythrocyte; and by increasing these factors, membrane permeability and haemolysis increase, which is caused by micelle production from surfactant and membrane phospholipids bilayer (Francis et al., 2002).

Another aspect of this study was to evaluate the membrane toxicity of samples. Any agents which have the ability to destroy the erythrocytes membrane can have similar effects on other cells membranes. Evaluating erythrocytes membrane stability is a proper criterion for determination of surfactant toxicity. According to our result, haemolysis was observed by increasing the incubation period and temperature. In 70 mg/ml and temperature of 37°C, *A. dracunculus* L. extract caused 11.47% of erythrocytes destruction, while *C. cyminum* L. and *H. persicum* Desf extracts caused 4.65 and 2.9% of destruction, respectively. Another potential property of surface active agents is their ability in inducing and stabilizing emulsions. Emulsifying index direct is related to surface tension and ability in micelle production. In this study the emulsifying index of the tested extracts has shown significant difference from each other (Figure 5) ($p < 0.05$). Regarding the surfactant activity of saponins, this activity of the extracts could be attributed to their saponins. Saponins have detergent or surfactant properties because they contain both water-soluble (one or more side chains of water-soluble sugars) and fat-soluble (a steroid or triterpenoid structure) components. Among the tested extracts, the extract of *A. dracunculus* L, has shown the most emulsifying index. These differences between the plants may be due to quantity or quality of their saponin contents. In this study, increasing the concentration of plant extracts leads to increase in emulsions stability; however, this trend was not the same in all surfactants (Figure 5). In general, the results of the present study show all the tested extracts, especially tarragon which, in less concentration (> 20 mg/ml), shows less heamolytic activity. In higher concentrations, similar to synthetic surfactants, this activity reaches to plateau. These findings indicate that the concentration of a plant extract for pharmaceutical aids is a critical point. The hemolytic activity of tarragon in concentrations about cmc increases significantly in comparison to synthetic surfactants such as tweens (Dehghan Noudeh et al., 2008). According to the hemolytic data and emulsifying index, extract of *A. dracunculus* L. had the least toxicity and the best properties for emulsification to be used in formulations. Foaming ability of surfactants is a propriety which may help improve the existence of surfactants in a solution; furthermore, this ability can be used in order to compare the detergency properties of detergents with high ability of foaming production. Foam production and stability depends on type and concentration of surfactants.

Our results also showed that extract of *C. cyminum* L had more ability to produce foam (Figure 6). Nowadays, there are a great deal of research is being carried out concerning the effect of surfactants on absorption. According to the results of the present study, extracts with different hemolytic effect, when considering the health of consumer, the use of aqueous extract of *H. persicum* Desf, with low hemolytic effect is preferred in pharmaceutical preparation, but if the hemolytic effect were considered, the use of aqueous extract of *A. dracunculus* L, with great hemolythc effect in comparison to the two other extract, is preferred.

ACKNOWLEDGMENTS

This work was taken from a pharmacy student thesis in Kerman University of Medical Sciences (Thesis No.531). This research was supported by a grant (No. 87/101) from Vice President for Research of Kerman University of Medical Sciences.

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