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Evaluation of the saponin green extraction from *Ziziphus spina-christi* leaves using hydrothermal, microwave and Bain-Marie water bath heating methods

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Abstract: Saponin as a biosurfactant was extracted from Iranian *Ziziphus spina-christi* leaves using three green extraction methods namely, autoclave, microwave and Bain-Marie heating methods. In this study, three solvents namely, methanol, ethanol and water were used to extract saponin. The results revealed that water, as compared to the methanol and ethanol, is a more suitable solvent to extract saponin from the *Z. spina-christi* leaves. The obtained results indicated that saponin extraction using autoclave provided more suitable physico-chemical properties along with a better yield. In fact, maximum foam volume (12.56 cm^3), color intensity (3.24% absorbance unit [a.u.]) and turbidity (1.39% a.u.) of the extracted solutions was obtained by the autoclave heating method. The high performance liquid chromatography (HPLC) results also illustrated that the amounts of extracted saponin using autoclave, Bain-Marie and microwave heating extraction methods were 14, 8.8 and 1.3 (intensity mV), respectively. The results obtained by HPLC were reconfirmed by Fourier transform infrared (FT-IR) analysis.

Keywords: autoclave; extraction; microwave; saponin; *Ziziphus spina-christi*.

1 Introduction

Surfactants are amphiphilic compounds with high potential activity to reduce surface tension between two immiscible fluids [1, 2]. Biosurfactants are biologically produced using numerous microorganisms or isolated from different plants and their derivatives. Biosurfactants have two main functional groups in their structure namely, hydrophobic and hydrophilic parts [3–7]. As compared to synthesized and commercial surfactants, biosurfactants have several advantages including being environmental friendly, higher surface tension reduction, biodegradability and selectivity in room temperature and normal pH, as well as lower toxicity [3, 5–7]. Due to their excellent emulsification, foamability, water binding capacity and wetting properties, biosurfactants have been widely used in different industries and products such as cosmetics, food, medicine, pharmaceuticals, and oil and gas production processes [8].

Saponin is one of the most important and applicable biosurfactants which is widely found in different plants, sea cucumber and starfish [9–11]. In fact, this secondary metabolite is part of the plant defense system against pathogens and herbivores [12]. Saponins are divided into two main categories which are triterpenoids and steroid glycosides attached to one or more sugar chains [13, 14]. This natural biosurfactant which is derived from soapwort has been widely used for centuries as household detergent due to its excellent foamability [10]. Saponins have been extracted from several plants and their products including soy milk, sugar beet, asparagus, strawberry and plum fruits [15–17]. *Ziziphus spina-christi* is one of the main sources of saponin which is known as “Sedr” and “Nebeq” in Iran and Arabic countries, respectively [18, 19]. There are around 50 species of *Ziziphus* distributed in tropical Asia, Africa and America [18, 19]. Several studies have indicated that *Z. spina-christi* leaves contain flavonoids, tannins, sterols, saponins and triterpenoids. Furthermore, 11 different cyclopeptide alkaloids exist in *Z. spina-christi* stem and root [20–23]. In Khuzestan, a southern province of Iran, *Z. spina-christi* leaves have been widely used as natural detergent and shampoo due to their saponin content [24].

For the extraction of saponins, two different extraction methods namely conventional and green have been developed [25–27]. Conventional methods including maceration, Soxhlet and reflux extractions are based on using a large amount of chemical solvents. However, in green processes such as ultrasound, microwave and accelerated solvent extractions which are based on water as the solvent, several advantages can be nominated such as lower extraction time with higher efficiency, minimum utilized

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chemical solvents and energy consumption, as well as prevention of the pollutions [25]. Therefore, the main objectives of the present study were to i) extract saponin from Iranian *Z. spina-christi* using three different green methods namely, microwave-assisted, autoclave-accelerated and accelerated water extraction and ii) evaluate the physico-chemical properties of the extracted saponin.

2 Materials and methods

2.1 Materials

Z. spina-christi dried leaves were purchased from a local market (Tabriz, Iran). Methanol, ethanol and distilled water as three different solvents were provided from Dr. Mojallali Chemical Complex Co. (Tehran, Iran).

2.2 Extraction of saponin

Z. spina-christi dried leaves were ground using a domestic miller (MX-GX1521, Panasonic, Tokyo, Japan). The provided dried powder was added into the distilled water, methanol and ethanol, as three different solvents, with a ratio of 1:20 g/ml and mixture solutions were exposed to three different extraction methods namely, microwave, autoclave and Bain-Marie. In the microwave-assisted extraction method, the mixture solutions were exposed and heated using a microwave oven (MG-2312W, LG Co., Seoul, South Korea) at a constant power of 800 W for 90 s with a 30 s interval of heating. In the autoclave-accelerated extraction method, the mixture solutions were placed into a laboratory autoclave which was set at a pressure of 15 psi and temperature of 121°C for 15 min. In the water extraction method, the prepared solutions were put into a laboratory Bain-Marie water bath in which temperature was adjusted in 50°C for 24 h. After that, the heated mixture solutions were filtered using No. 1 Whatman filter paper and the filtrates were then put in the laboratory oven at 60°C for 48 h to remove the solvents. Finally, 20 ml of distilled water was added into the solvent-free samples and shaken for 30 s. The samples were then used for the physico-chemical analysis.

2.3 Physico-chemical analysis

In order to identify the main functional groups in the filtrate mixture solutions containing saponin, Fourier transform infrared (FT-IR) measurements was performed. The FT-IR spectra of the provided *Z. spina-christi* extract were recorded on a Bruker Tensor 27 spectrometer (Ettlingen, Germany) using KBr pellets in the 4000–400 cm^{-1} region. In order to qualitatively evaluate the extraction yield, the prepared samples were shaken vigorously by hand for 30 s and the volume of the generated foam was measured. The extraction yield of the obtained saponin was then correlated to the foam volume. The amount of the saponin extracted was also monitored by the color intensity and turbidity tests. In fact, a lower concentration of bioactive compounds in aqueous solution leads to lower color intensity and turbidity [28]. In order to measure color intensity and turbidity of the extracts, a Jenway UV-Vis spectrophotometer 6705 (Stone, UK) in a 1 cm optical path quartz cuvette adjusted to wavelengths of 420 nm and 625 nm, respectively, was used. The absorbance unit (% a.u.) was used as unit of both color intensity and turbidity of the extracts. High performance liquid chromatography (HPLC) (Merck Hitachi, L-7100, Darmstadt, Germany) with a C 18 column and IR detector was used to identify and measure saponin in the extracts. The wavelength range was set from 250 nm to 500 nm. The samples were dissolved in HPLC grade methanol and injected into the system. Acetonitrile-water was used as the mobile phase and the sampling rate was 2 (points/second) such that total flow rate was kept at 0.70 ml/min. The amount of saponin in the samples was expressed by intensity mV unit.

2.4 Statistical analysis

Physico-chemical analysis of the prepared extracts was carried out in three replications. Data were interpreted by analysis of variance using Minitab v.16 statistical package (Minitab Inc., PA, USA). Tukey's comparison test was used to compare the mean values. All comparisons were made at 5% level of significance.

3 Results and discussion

3.1 Solvent selection

According to available data in the literature, water, methanol and ethanol have been extensively utilized in the extraction of saponin from the plant sources [29]. Therefore, these solvents were used in the present study to extract saponin from *Z. spina-christi*. The results of our study indicated that, as compared to the ethanol and methanol, the extraction of saponin using water provided higher extraction yield. This result was obtained when these three solvents were utilized to extract saponin from its source using a Bain-Marie water bath and then measuring the generated foam volume by shaking the extracts. Figure 1 shows the volume of the generated foam obtained

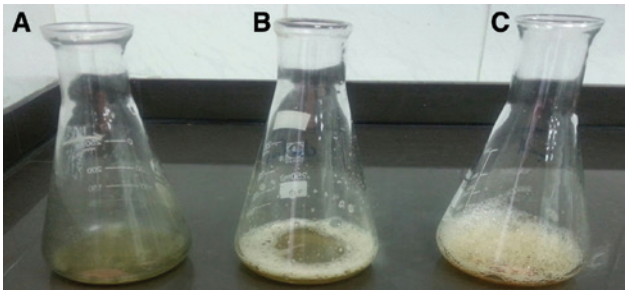

Figure 1: Generated foam by shaking the saponin extract from the *Ziziphus spina-christi* leaves using (A) ethanol, (B) methanol (C) and water.

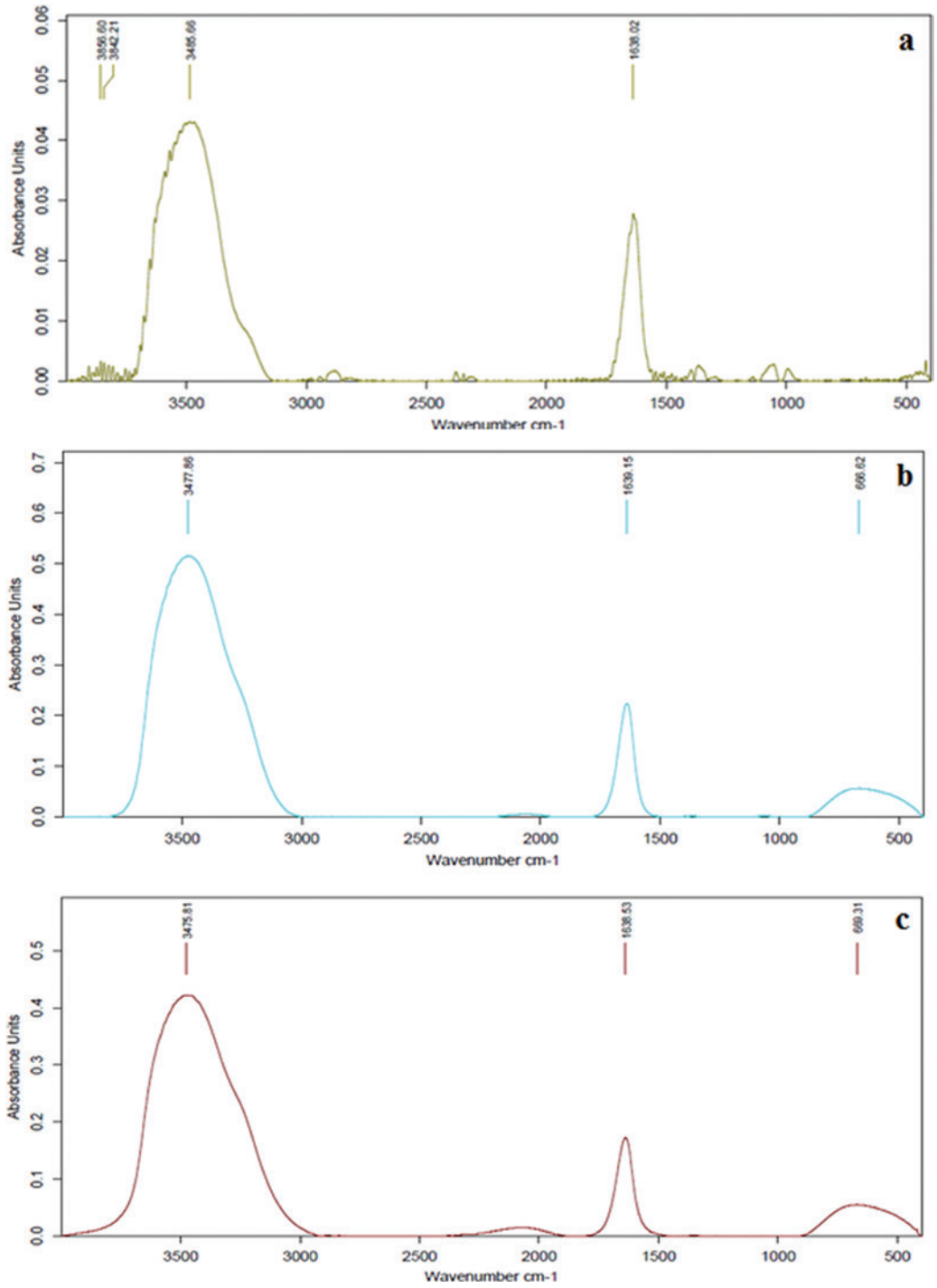

Figure 2: Fourier transform infrared (FT-IR) spectrums of the extracted saponin using (A) microwave, (B) autoclave and (C) Bain-Marie heating methods.

by shaking the saponin extracts. As can be seen, the foam volume is much higher in the case of water as compared to methanol and ethanol. Therefore, water was chosen as the green solvent to extract saponin from *Z. spina-christi* leaves via microwave, autoclave and Bain-Marie heating methods.

3.2 FT-IR analysis of the extracted saponin

In order to identify the main functional groups of the extracted saponin solutions, FT-IR spectra of the saponin extracts obtained using microwave, autoclave and Bain-Marie methods were determined (Figure 2). As clearly

observed in Figure 2A, there are two highlighted and sharp peaks centered at 3485.66 cm^{-1} and 1638.02 cm^{-1} for the extracted saponin solution by microwave heating. However, the saponin extract obtained using autoclave and Bain-Marie heating methods shows three highlighted peaks centered around 3475 cm^{-1}, 1640 cm^{-1} and 670 cm^{-1}. The absorption peaks centered around 3475 cm^{-1} and 1640 cm^{-1} are related to the hydroxyl group (OH) and amide I (NH) of the existing compounds of the extract, respectively. These results are consistent with the chemical structure of saponin shown in Figure 3. As clearly observed in this figure, saponin has a polyhydroxyl structure containing of the amide I group. The week absorption peak centered around 670 cm^{-1} in Figure 2B and C could be referred to the ring and skeletal modes of the main components.

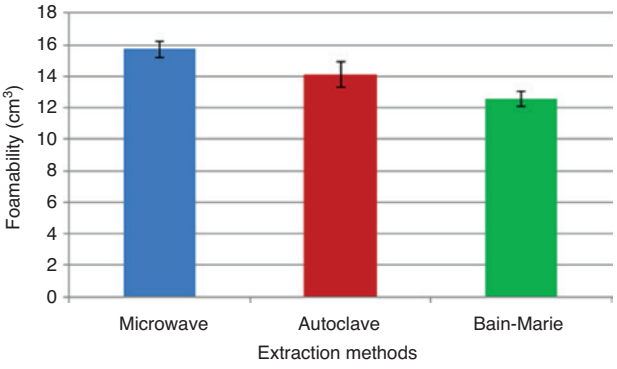

Figure 4: Foam volume generated by shaking the saponin extracts obtained using three different extraction methods. Data are mean values of three replications.

3.3 Foamability of the extracted saponin

Saponin as a biosurfactant has both hydrophilic and hydrophobic functional groups in its structure which can produce foam in water through simple shaking. In fact, the volume of the generated foam could be well correlated to the saponin concentration: the higher the foam volume, the higher the saponin concentration [3–7]. As observed in Figure 4, the generated foams of the saponin extracts obtained by the microwave and autoclave heating methods were higher than that obtained by the Bain-Marie method. The statistical analysis did not show significant ($p < 0.05$) difference between foam volumes produced by the saponin extracts using microwave and autoclave extraction methods.

3.4 Color intensity of the extracted saponin

The color intensity of the extracted saponin solutions via three different extraction methods is shown in Figure 5. As clearly observed in this figure, the color intensity of the saponin solutions by the microwave heating method had a minimum value of 2.11 (% a.u.) while the values of the color intensity using the other methods were high. Note that maximum color intensity was obtained for the saponin solution extracted by the autoclave method (3.24% a.u.). This can be explained by the fact that by increasing the extraction time, the yield of saponin extraction from the *Z. spina-christi* leaves increased, which in turn increased the color intensity of the solution. The obtained results indicated that there were significant ($p < 0.05$) differences between the color intensity value of the saponin solution extracted by the three different methods.

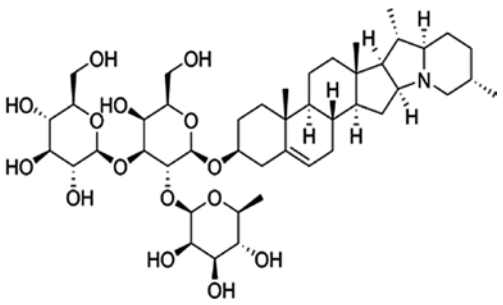

Figure 3: Saponin chemical structure.

3.5 Turbidity of the extracted saponin

The turbidity values of the extracted saponin solutions by using microwave, autoclave and Bain-Marie heating

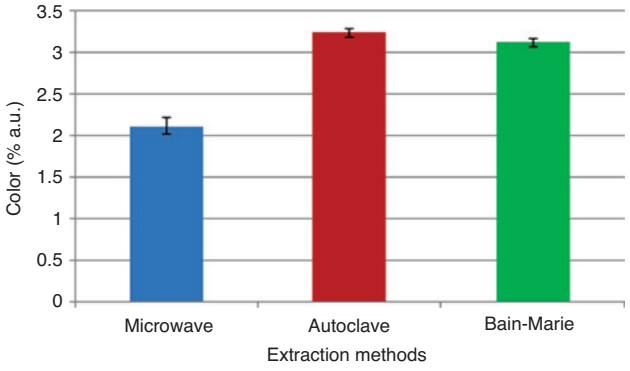

Figure 5: Color intensity of the saponin extracts obtained using three different extraction methods. Data are mean values of three replications.

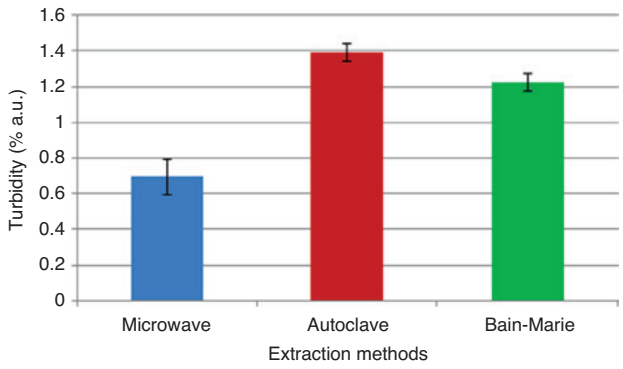

Figure 6: Turbidity of the saponin extracts obtained using three different extraction methods. Data are mean values of three replications.

methods are illustrated in Figure 6. As clearly observed in this figure, the turbidity values of the extracted samples by microwave, autoclave and Bain-Marie heating methods were 0.69% a.u., 1.39% a.u. and 1.22 % a.u., respectively. The statistical analysis indicated that the turbidity value of the saponin solution obtained by the autoclave heating method was significantly ($p < 0.05$) higher than that of other samples. This can be explained by the fact that in saponin extraction and other unwanted chemical and biochemical reactions, such as caramelization of the sugars and Maillard reactions, could be increased, leading to formation of brown and dark compounds such as melanoidins [30].

3.6 HPLC chromatogram of the extracted saponin

The HPLC profile of the extracted saponin from *Z. spina-christi* leaves using the three extraction methods is shown in Figure 7A–C. As observed in Figure 7A, there were three recorded peaks at the retention time range of 2–10 min for the case of the microwave method. However, Figure 7B and C shows five detected peaks in the HPLC chromatogram of autoclave and Bain-Marie methods (Figure 7C). The obtained results reconfirm the turbidity and color intensity analyses of the extracted saponin solutions through the three different methods. In fact, the higher turbidity and color intensity of the extracted saponin solutions using the autoclave and Bain-Marie methods, as compared to those obtained by microwave, could be related to other bioactive compounds being present in the extracted samples. The peak sharpness centered at 2 min (retention time) was related to the extracted saponin. As clearly observed in Figure 7A–C, the highest peak in the

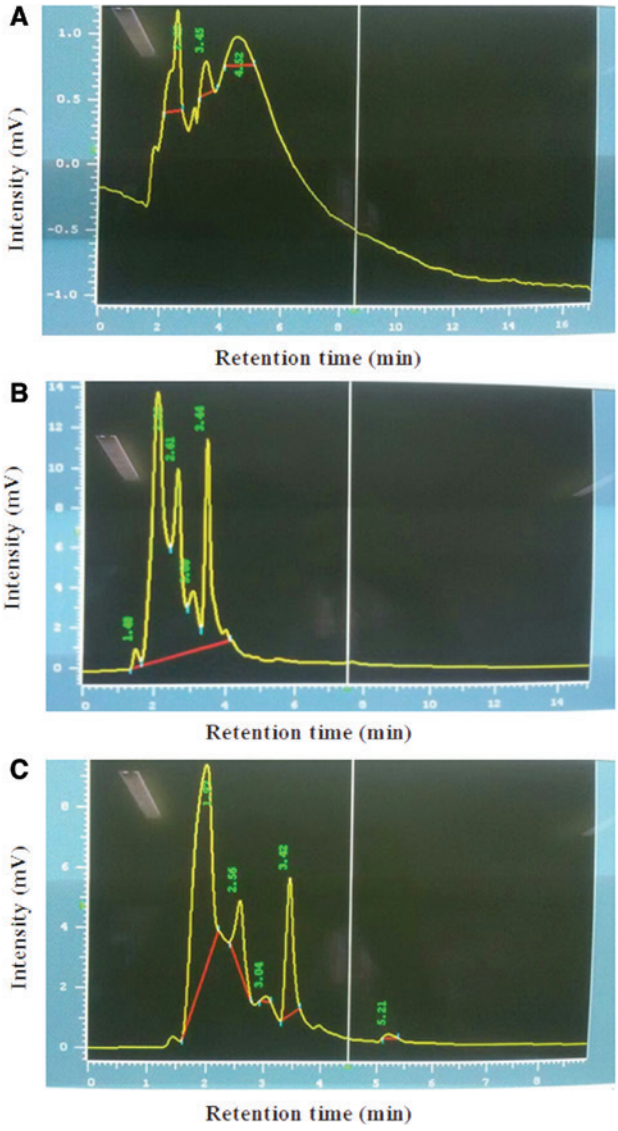

Figure 7: High performance liquid chromatography (HPLC) chromatograms of the saponin extracts obtained using (A) microwave, (B) autoclave and (C) Bain-Marie heating methods.

2 min retention time was related to the extract obtained by autoclave (14 intensity mV) and Bain-Marie (8.8 intensity mV) heating methods, respectively.

4 Conclusions

The green extraction of saponin from *Ziziphus spina-christi* leaves was studied using a series of simple and low energy consuming methods (microwave, autoclave and Bain-Marie) based on water as the extraction solvent. The results indicated saponin could be extracted efficiently by the hydrothermal methods. The HPLC results

revealed that the yield of saponin extraction using autoclave was higher than that obtained using microwave and Bain-Marie heating extraction methods. The achieved results can be easily developed and used to extract lipid-based bioactive compounds with high decomposition temperature from the plants and other natural resources.

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