Trends Phytochem. Res. 7(3) 2023 207-219

Trends in Phytochemical Research (TPR)

Journal Homepage: https://tpr.shahrood.iau.ir/

Original Research Article

Investigation of chemical characteristics of Eshnan (*Seidlitzia rosmarinus* Bunge ex Boiss) and quantitative determination of saponin ginsenosides by high-performance liquid chromatography

Mahnaz Davabi[1], Maryam Kolahi [2]✉*, Roya Azadi[1], And Nahid Pourreza[1]

[1]Department of Chemistry, Faculty of Science, Shahid Chamran University of Ahvaz, Ahvaz 61357-43169, Iran
[2]Department of Biology, Faculty of Science, Shahid Chamran University of Ahvaz, Ahvaz 61357-43169, Iran

ABSTRACT

Eshnan (*Seidlitzia rosmarinus* Bunge ex Boiss), a salt-tolerant and drought-resistant plant, is often found in salty desert areas. In the present report, Eshnan was subjected to extraction using methanol, ethanol, and water utilizing a Soxhlet extractor. A total of 51 compounds were identified in the plant inclusive of secondary metabolites such as phenols, polyphenols, phytosterol, amino acid, fatty acid, etc. utilizing GC/MS analysis. The methanolic extract had the highest phenolic content, whereas ethanolic extract had the highest antioxidant activity. Aqueous extract contained the highest levels of saponin, as well. CTAB represented a higher foaming capacity as compared to the plant extracts. HPLC analysis of the plant extract confirmed the presence of the saponin ginsenoside Rb1 for the first time. The results showed a rich source of saponins, polyphenols, and fatty acids in Eshnan. Due to the significant quantity of saponins within the plant, it can be used as an alternative natural surfactant to chemical surfactants that are currently utilized in industry.

ARTICLE HISTORY

Received: 01 May 2023
Revised: 11 September 2023
Accepted: 14 September 2023
ePublished: 28 September 2023

KEYWORDS

Antioxidant
Eshnan (*Seidlitzia rosmarinus* Bunge ex Boiss)
HPLC
Natural surfactant
Saponin ginsenosides

dor: 20.1001.1.25883623.2023.7.3.5.2

1. Introduction

Herbal remedies have been widely used in the healthcare system for centuries. The global market value of medicinal plant products is estimated to be more than $100 billion annually. Today, the use of medicinal plants in the fight against diseases, public health compliance, and special emphasis on current strategic approaches to disease prevention are taken into consideration. Medicinal plants play a vital role in preventing disease and their use in all prevention strategies is of paramount interest and importance. However, there is a need for conscious efforts to identify, recognize, and position medicinal plants in the design and implementation of these strategies. Recently, a large number of reports have been published on the potential use of medicinal plants and their compounds in the prevention of a wide range of diseases (Mohammadhosseini et al., 2021; Tanaka and Kashiwada, 2021; Thagriki and Ray, 2022). Natural products, especially the unique secondary metabolites from which herbal medicines are extracted, including those that have been used not only in traditional folk medicine but also in modern medical systems, offer potential molecules to produce new drugs and develop advanced therapeutic strategies (Mohammadhosseini et al., 2022). Eshnan (*Seidlitzia rosmarinus* Bunge ex Boiss), a salt-tolerant and drought-resistant shrub, can often be found in salty desert areas. The perennial shrub, Eshnan, belonging to the genus Seidlitzia and family Chenopodiaceae has white branches and can attain heights up to 1.5 m (Farahnejad et al., 2017). Clinical research has shown that hydro-alcoholic extracts of the plant exhibit therapeutic

✉Corresponding author: Maryam Kolahi
Tel: +98-613331056; Fax: +98-613331056
E-mail address: m.kolahi@scu.ac.ir, **doi: 10.30495/tpr.2023.1985127.1337**

activity on the mouse model of cutaneous leishmaniasis (Ahmadi et al., 2014). Eshnan also plays an important role in the preservation of soil in desert arid regions. The salinity of arable lands is globally challenging and affects a total of 955 million hectares of the world (7.0%) and surfaces. Iran is affected by mild and moderate salinity which equates to approximately 25.5 million hectares, whereas those severely affected are estimated at 8.5 million hectares. Utilizing salt-tolerant plants will give further insight into the mechanisms whereby these plants have the ability to tolerate high salt concentrations. Root tissues of *S. rosmarinus* Bunge ex Boiss have the capability of absorbing large quantities of alkali metals such as Na^+ and K^+ from the soil which are subsequently transferred to the shoots. The main mechanism of salt resistance in this plant is tolerance. It has been documented that large quantities of sodium accumulate in cell vacuoles (Mirheidari and Khadivi, 2022). The ash of the plant has been found to contain large quantities of sodium and potassium carbonate. Previously, the Eshnan plant was widely utilized for the washing of textiles. Currently, it is underutilized in countries such as Iran where the plant has origins or is endemic (Hadi, 2009). Phytochemicals are produced by various parts of the plants. These bioactive constituents include steroids, terpenoids, carotenoids, flavonoids, alkaloids, tannins, and glycosides. Most of these natural products are secondary metabolites with approximately 12,000 such products being isolated thus far. These metabolites serve as plant defence mechanisms against predation by microorganisms, insects, and herbivores. During the past two decades, the pharmaceutical industry has heavily invested in pharmacological and chemical research around the globe in efforts to discover more potent drugs, and not just a few novel drugs (Roy et al., 2022). Phytochemicals synthesized by plants as secondary metabolites exert various physiological effects on mammals including humans and are therefore referred to as the active principles and constituents of the plant material (Lalrinzuali et al., 2015). Saponins are basically secondary metabolites with a wide distribution range within the plant kingdom serving as a chemical barrier or shield in plant defense mechanisms to counterattack pathogens and herbivores. Saponins consist of an aglycone with carbohydrate moieties. The aglycone can be a triterpene or a steroid and can have a number of different substituents (-H, -COOH, $-CH_3$). The number and type of carbohydrate moieties result in a considerable structural diversity of the saponins. The biological activities of saponins are not limited to traditional use. Saponins have been found to exhibit various pharmaceutical properties, *e.g.*, hemolytic, molluscicidal, anti-inflammatory, antifungal or anti-yeast, antibacterial or antimicrobial, antiparasitic, antitumor, and antiviral activities. In the pharmaceutical industry, it may also be employed as the starting point for the semi-synthesis of steroidal drugs. Saponins are also known to possess chelating properties forming mineral complexes of iron, zinc, and calcium (Cheok et al., 2014). Scientists are actively seeking ways to combat environmental pollution and damage caused by industrial detergents. Extracts from the plant may be used in green chemistry as a surfactant. The plant (Eshnan) is widely distributed in Iran in areas facing environmental and dust pollution and its phytochemical investigation may lead to the discovery of valuable components with industrial applications.

Despite the importance and medicinal properties of *S. rosmarinus* Bunge ex Boiss, to the best of our knowledge, a relevant comprehensive phytochemical study has not been conducted so far. Regardless of its many traditional usages as pharmaceuticals and for detergency, the main secondary metabolites of the plant have not been thoroughly investigated. The current study evaluated the phytochemicals of *S. rosmarinus* Bunge ex Boiss, specifically phenolic and saponins, using different extraction solvents. This research provides information on the potential application of extracts of the plant (*S. rosmarinus* Bunge ex Boiss) for potential use as a natural detergent. Due to its widespread distribution, the plant may be considered for commercial applications.

2. Experimental

2.1. Collection and identification of plant sample

The aerial parts of Eshnan plant were collected in 2018 from 20th km Ahvaz-Khorramshahr road and identified by the Department of Botany, Faculty of Science, Shahid Chamran University of Ahvaz. The sample was washed with distilled water, dried at shade and room temperature, and milled.

2.2. Extract preparation

Extraction of the milled Eshnan was facilitated by the use of a Soxhlet extractor using ethanol, methanol, and water. Accordingly, the powdered sample (10 g) was transferred into a thimble and placed in the Soxhlet extractor. Solvent (120 mL) was then added and the extraction was allowed to proceed for 7 h. The solvent was removed in vacuo, and the resulting extract was dried at 60 °C to complete dryness. The extract was stored in airtight containers and refrigerated until required (Redfern et al., 2014).

The extraction yield was determined utilizing Eqn. 1:

$$EY = DE/DS \times 100 \quad \text{(Eqn. 1)}$$

Where EY (% by dry weight), DE (g), and DS (g) respectively account for the extraction yield, the weight of dry extract, and the dry weight of the sample before extraction.

2.3. Determination of phenolic content

Total phenolic content was determined by the Folin-Ciocalteu reagent method with some modifications (Hossain, 2013). Briefly, solutions were prepared from the different extracts (ethanol, methanol, and water) to a final concentration of 1000 µg/mL. To the different extracts (200 µL), Folin-Ciocalteau reagent (10%, 1.5 mL) was added. After 5 min, sodium carbonate (5%, 1.5 mL) was added and the mixture was incubated for 45 min on a shaker. The absorbance was measured using a UV/Vis. spectrophotometer at 760 nm. A calibration curve was plotted using gallic acid as a standard and

the total phenolic content was expressed as µg/mL.

2.4. Determination of flavonoid content

Total flavonoid content was determined utilizing the aluminum chloride colorimetric assay (Zhishen et al., 1999). In this relation, to the extracts (1000 µg/mL, 1 mL), deionized water (4 mL), and sodium nitrite solution (5%, 0.3 mL) was added. After 5 min, aluminum chloride (10%, 0.3 mL) was added followed by the addition of NaOH (1 M, 2 mL). Deionized water was added to a final volume of 10 mL. The solutions were shaken vigorously, and the absorbance was measured utilizing a UV/Vis. spectrophotometer at 510 nm. A calibration curve was plotted using quercetin as standard. Total flavonoid content was calculated as µg/mL.

2.5. Determination of flavonol content

The flavonol content of the extracts was determined utilizing the aluminum chloride colorimetric assay (Kumaran and Karunakaran, 2007). Accordingly, extract solutions with a concentration of 1000 µg/mL were prepared. To the various extracts (2 mL), aluminum chloride solution in ethanol (2%, 2 mL), and an aqueous solution of sodium acetate (50 g/L, 3 mL) were added. The solutions were shaken vigorously, and incubated for 150 min and the absorbance was measured at 440 nm utilizing a UV/Vis. spectrophotometer. A calibration curve was plotted using quercetin as standard. Total flavonoid content was calculated as mg/mL.

2.6. DPPH radical scavenging activity

The free radical inhibitory activity of the extracts was determined using the method of Norhaiza et al. (2009) with some modifications. In this sense, different concentrations of the extracts were prepared (500, 1500, 1000, and 2000 µg/mL). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) solution (0.1 mM) in methanol was prepared and 1 mL of this solution was added to the extracts (1 mL). The mixture was incubated in the dark on a shaker for 30 min at room temperature. Control samples were also prepared with the extract being replaced with solvent. The absorbance was measured at 517 nm using a spectrophotometer. The free radical inhibitory activity of DPPH was calculated using Eqn. 2.

DPPH• scavenging effect (% of inhibition) = $(A_0\text{-}A_1) \times 100/A_0$ (Eqn. 2)

Where the terms A_0 and A_1 respectively account for absorbance of the control and absorbance of sample extracts. The inhibitory activity (IC_{50}) is the concentration of sample that can inhibit 50% of free radicals and was expressed as µg/mL.

2.7. Determination of saponin content

The saponin content of the extracts was determined based on the vanillin-sulfuric acid colorimetric method with some modifications (Jeong and Park, 2006). Accordingly, vanillin solution (8%, 0.5 mL) and sulfuric acid (77%, 5 mL) were added to each sample and the mixture vortexed. The reaction mixture was incubated in a hot water bath (60 °C, 20 min) followed by placing in an ice bath (10 min). The absorbance of the mixture was finally measured at 550 nm using a spectrophotometer. A calibration curve was plotted using ginsenoside Rb_1 as standard. Total saponin content was finally calculated as µg/mL (Jeong and Park 2006).

2.8. Foaming capacity

To measure foam strength, different concentrations of the extracts (1000, 2000, 3000, and 4000 µg/mL) along with CTAB surfactants were prepared with distilled water and poured into a test tube (5 mL) and each test tube was subsequently vortexed for 5 s. The height of the foam was measured after 1 min and the mean height of the foam was plotted as a function of concentration (İbanoğlu and İbanoğlu, 2000).

2.9. Determination of crude saponin content

Saponins were extracted based on the Kwon method with some modifications (Kwon et al., 2003). Powdered samples of the Eshnan plant (5.0 g) were extracted with methanol (80%) utilizing a Soxhlet extractor (6 h). The solvent was evaporated and the resulting sample was dried in an oven (100 °C) and cooled and the weight of the extract was recorded. The extract was dissolved in distilled water (50 mL) and extracted with diethyl ether (50 mL) to remove fat. Separation was facilitated by the use of a separator funnel. The aqueous layer was further extracted with *n*-butanol (50 mL) saturated with water. The butanol solution was washed with distilled water (30 mL) to remove impurities. After the complete separation of the two phases (approx. 8 h), the remaining butanol solution was concentrated with a rotary evaporator. The resulting product was the crude saponin extract for which the percentage of saponin was calculated gravimetrically.

2.10. Analysis of compounds in saponin by HPLC

2.10.1. Sample preparation for HPLC analysis

Powdered samples of the plant (5.0 g) were extracted with water (120 mL, 7 h) using the maceration method. The resulting extract was filtered, and the sample was concentrated in vacuo utilizing a rotary evaporator.

2.10.2. HPLC analysis

The standard ginsenoside Rb1 (1000 µg/mL) and aqueous extracts of Eshnan were prepared in methanol solution (30%) and injected into the HPLC system. Chromatographic analysis was performed using a C18 column at 40 °C. Run conditions were as follows: injection volume: 10 µL; mobile phase: A: water, B: ACN, gradient: 0 min, 19% B; 35 min, 19% B; 55 min, 29% B; 70 min, 29% B; 100 min, 40% B; flow rate: 1 mL/min; and detection wavelength: 259 nm.

2.11. Analysis of compounds in saponin by GC-MS

Components in the methanolic extracts of Eshnan were

detected using the Perkin-Elmer Clarus 680 system (Perkin-Elmer, Inc. U.S.A) equipped with a fused silica column, packed with an Elite-5MS capillary column (30 m in length × 250 µm in diameter × 0.25 µm in thickness). Pure helium gas (99.99%) was used as the carrier gas at a constant flow rate of 1 mL/min. For GC-MS spectral detection, an electron ionization energy method was adopted with high ionization energy of 70 eV with a scan time of 0.2 s and fragments ranging from 40 to 600 m/z. The injection quantity of 1 µL was used (split ratio 10:1), and the injector temperature was maintained at 280 °C (constant). The spectral data were recorded (Fig. 1). Compounds were identified based on retention time or mass spectra, and NIST, PubChem libraries were used to identify compounds.

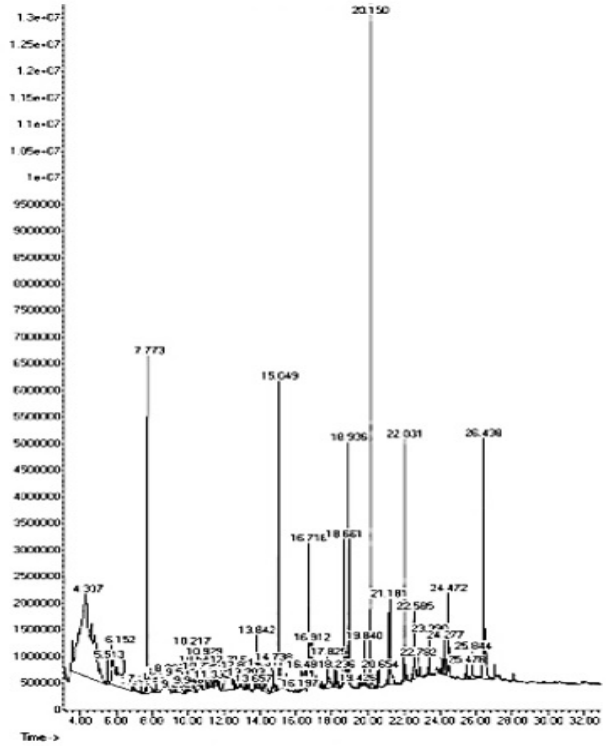

Fig. 1. GC-MS chromatogram of methanolic extract of Eshnan.

2.12. Calf thymus DNA (CT-DNA) cleavage

A gel electrophoresis mobility shift assay was performed on the different extracts (ethanol, methanol). A stock of the different extracts was prepared in tris buffer (10 mM) and NaCl (10 mM) at pH 7.2. A stock solution of the free extract was prepared by dissolving the components in a solution of dimethyl sulfoxide/Dulbecco's modified eagle's medium (DMSO/DMEM) and then suitably diluting with the corresponding buffer to the required concentration. The final DMSO concentration did not exceed 1.0 v/v%. The DNA sample (5.0 L) was prepared by incubating DNA with the extracts in a buffer solution (10 mM, pH 7.0) for 4 hours at an incubator temperature of 37 °C. After mixing with a loading buffer containing 0.25% bromophenol blue, 0.25% xylene cyanol, and 30% glycerol, the solution was loaded on a 1.0% agarose gel electrophoresis at a constant voltage (80 V) until the bromophenol blue reached 3/4 of the gel length. The bands were visualized with UV light and photographed.

2.13. Anatomic study

Paraffin sections were prepared to study the anatomic structure of the plant stalk of Eshnan. Plant stalks were placed in formalin acetic acid (FAA) solution for fixation (overnight, 4 °C). The histochemical tests conducted were as follows: Carmine and methylene blue for the determination of pectocellulose and lignified cell walls lugol's iodine for cellulose determination. The sections were studied and images were examined utilizing an Olympus light microscope (Olympus Inc., Tokyo, Japan) (Bryan, 1955).

2.14. Statistical analysis

Data analyses were performed using the SPSS 20.0 software package (SPSS Inc., Chicago,IL, USA). All experimental data were presented as the mean ± SD. One-way ANOVA was used to test differences between various means followed by the post hoc Tukey test. The level of significance was set at $p < 0.05$ for all tests.

3. Results and Discussion

3.1. Yield of extracts

This study was conducted in order to investigate the phytochemical properties, especially the antioxidant property as well as to identify, extract and determine the amount of saponins due to their use as a detergent from Eshnan plant. Providing basic information for the purpose of designing and producing natural detergents, due to the wide distribution of this plant, it can be used in an industrial scale as well as for the promotion of the relevant knowledge-based companies. The yield of crude extracts of the Eshnan plant utilizing different solvents viz. water, ethanol, and methanol is illustrated in Fig. 2. As can be seen in this figure, water extracts gave the highest yield (32.80% ± 1.67). However, the yields from the methanolic and ethanolic extracts were not significantly different. The results show that the more polar solvent had the highest extraction efficiency suggesting that most of the compounds within the plant are most likely polar or attached to sugars or other polar groups. Extraction is the main step to recover and isolate chemical and phytochemical compounds from the plant. Extraction efficiency depends on the type of solvent, extraction method, sample particle size, pH, temperature, extraction time and sample composition. According to the obtained results, the type of solvent is an effective factor on the extraction efficiency. It should be noted that the higher yield of extraction alone cannot mean the presence of phenolic compounds with antioxidant properties. This shows that probably most of the compounds in this plant are very polar or that they are attached to sugar or other polar groups.

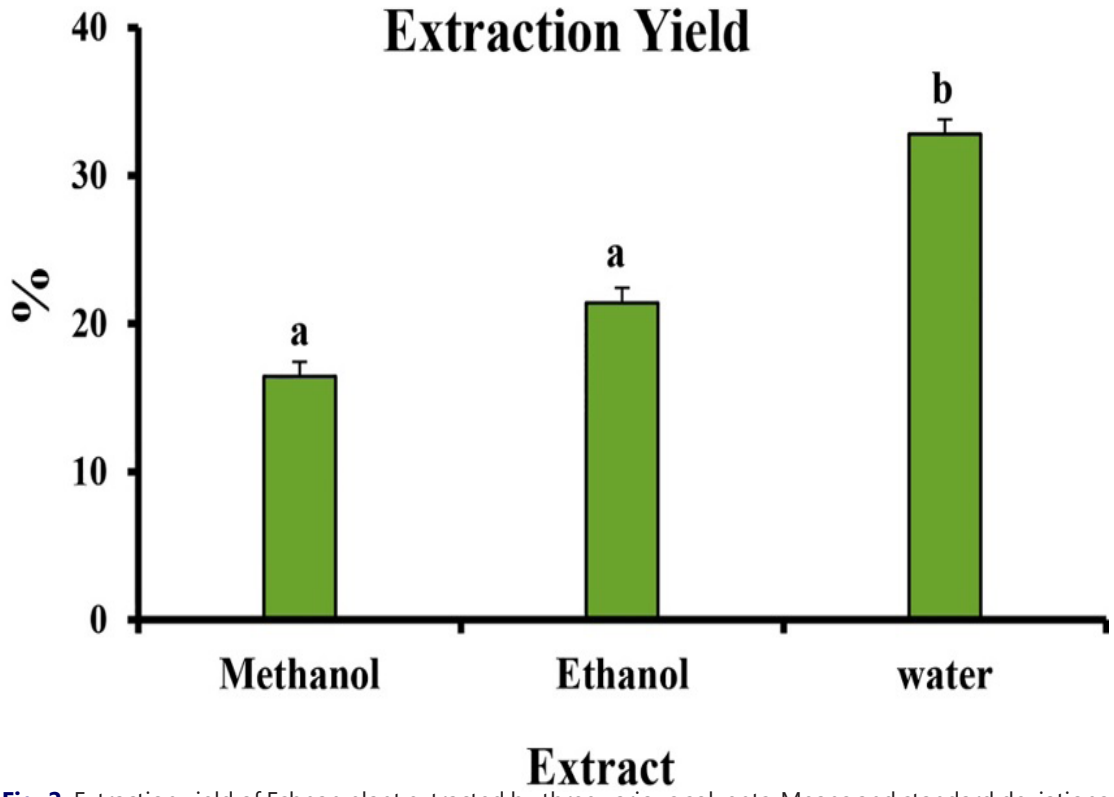

Fig. 2. Extraction yield of Eshnan plant extracted by three various solvents. Means and standard deviations were of triplicate. Different letters (a, b, c) within the columns denotes a significant difference between different ($p < 0.05$).

3.2. Total phenol, flavonoid and flavonol contents and antioxidant activity

The total phenolic content of Eshnan plant extracts was determined utilizing the Folin-Ciocalteu assay (Fig. 3A). Of the three extracts, the methanol extract contained the highest levels of phenolics (52.38 ± 2.67 µg/mL), and aqueous extracts (23.55 ± 0.98 µg/mL) involved the lowest quantities. The total flavonoid and flavonol content of the various extracts was also determined (Fig. 3B, 3C) in which the methanolic extracts contained the highest levels of flavonoids (238.67 ± 2.42 µg/mL), and aqueous extracts had the lowest levels (50.99 ± 3.88 µg/mL). Methanolic and ethanolic extracts contained the highest levels of flavonol compounds, and aqueous extracts were found to contain the lowest quantities (37.02 ± 0.36 µg/mL). The free radical scavenging activity (FRSA) of Eshnan plant extracts was determined based on the DPPH assay (Fig. 3D). Of the three extracts, ethanolic extracts exhibited the lowest IC_{50} value (599.75 ± 49.62 µg/mL), whereas aqueous extracts were found to have the highest IC_{50} (1751.49 ± 64.29 µg/mL).

A comparison of Eshnan extracts in terms of phenolic, flavonols, and flavonoid content showed higher levels of phenolic and polyphenolic compounds in ethanolic and methanolic extracts as compared to the water extracts. Phenolic compounds are one of the best sources of natural antioxidants which often have a polar structure, but in some instances, due to the binding of non-polar groups to the molecule, their polarity is slightly reduced. To separate them from within plant tissue, the use of solvents with different polarities is very effective (Goli et al., 2012). The results of the study showed that the highest quantities of phenolic compounds were obtained from the methanolic extract (Fig. 3A). Higher levels of phenolic compounds in the ethanolic and methanolic extracts as compared to water extracts are due to the lower polarities of methanol and ethanol having *vs* water. These solvents have a greater ability to release phenolic compounds by acting on the cell wall and disrupting the relevant tissue structure (Tiwari et al., 2011). Also, the results of the study showed that the amount of flavonoids in different extracts, including ethanolic and methanolic extracts, were significantly different, and the ethanolic extract had the highest level of flavonol compounds. Solvent polarity has been used to extract flavonoid and flavonol compounds. The lower the solubility of the solvent, the more suitable it is for the extraction of flavonoids and flavonols. Antimicrobial properties of the plant showed that the high flavonoid and flavonol content of the alcoholic extract as compared to aqueous extracts is related to the greater penetration of the alcoholic solvent into the cell wall extracting intracellular material within the plant (Bashiri Rezaei et al., 2017). Almost all of the compounds exhibiting antimicrobial properties were aromatic compounds extracted with ethanol or methanol. In the case of two solvents, methanol is more polar than

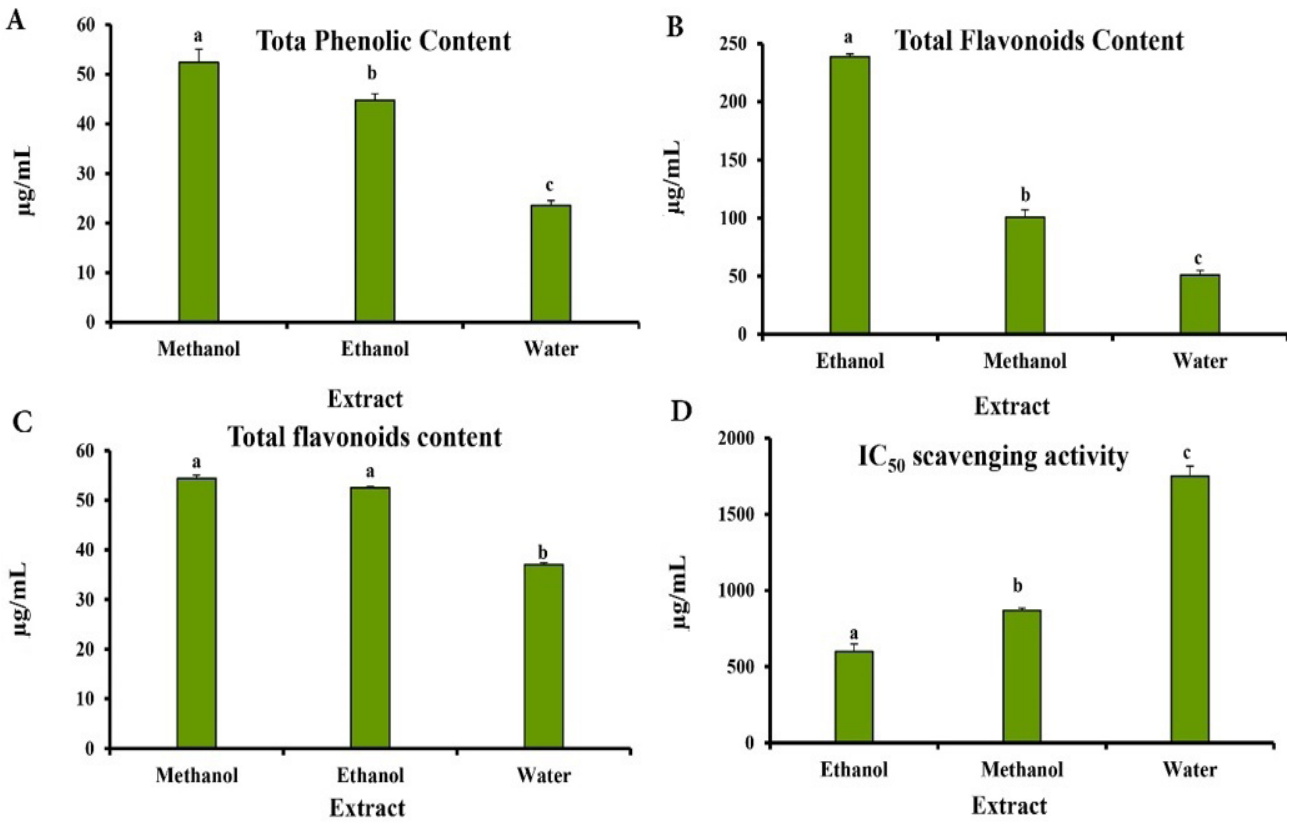

Fig. 3. Total phenolic, flavonoids, flavonols content, and antioxidant activity content of Eshnan plant extracted by three various solvents. Means and standard deviations were triplicate. Different letters (a, b, c) within the columns denote a significant difference between different ($p < 0.05$).

ethanol, but due to its cellular toxicity, it is not suitable for extraction in certain types of studies and in most studies, ethanol is the solvent of choice (Wang et al., 2010). Phytochemical examination of S. *rosmarinus* Bunge ex Boiss brought about the separation of two cinnamic corrosive subsidiaries and a benzaldehyde subsidiary as the most phenolic compounds of airborne parts of the plant. Utilizing comprehensive spectroscopic strategies, 1D and 2D NMR and MS, the chemical structure of the separated compounds was decided as *N-cis*-feruloyltyramine, *N-cis*-caffeoyltyramine, and *p*-hydroxyacetophenone (piceol), separately (Zolfaghari et al., 2017). Determination of the IC_{50} of the various extracts from the Eshnan plant using the DPPH assay revealed that the type of solvent used for the extraction had a significant effect on the antioxidant activity of the various extracts. The ethanolic extract had the highest antioxidant activity and was significantly different from that of the water extract which had the lowest levels of antioxidant activity. DPPH assay is one of the simplest, most cost-effective, and most accurate ways to evaluate the antioxidant activity of plants extracts. Ethanolic extracts of Eshnan plants had higher phenolic and flavonoid contents than aqueous extracts. The determined free radical scavenging activity utilizing the DPPH and ferric reducing ability of plasma (FRAP) assays. Ethanolic extracts had a higher percentage of free radical scavenging activity as compared to the aqueous extracts. The results of their study complement the results from the current study (Azizian et al., 2014). It has been shown that Eshnan prevents the accumulation of proteins and diseases such as Parkinson's and Alzheimer's, due to its high antioxidant power.

3.3. GC-MS analysis

GC-MS analysis of the methanolic extract of *Seidlitzia rosmarinus* Bunge ex Boiss showed 51 peaks indicating the presence of chemical and phytochemical constituents in the plant. Some of the major phytochemicals present in the *Seidlitzia rosmarinus* Bunge ex Boiss plant have been listed in Table 1. Analysis of the spectral data showed that the compound having the highest percentage composition was *N*,*N*-dimethyl glycine, which is derived from the amino acid glycine. Glycine is one of the 20 amino acids that is involved in the synthesis of proteins in the body of living organisms. The results also revealed that the major constituents of the plant were phenolic compounds and fatty acids. Other secondary metabolites identified included vitamin E, phytosterol, alkaloids, and glucose. The absence of saponins in the GC/MS spectral data can be attributed to the high polarity of these compounds or their high molecular weights. The compounds introduced by GC-MS could be the real compounds of the plant or they

Table 1
Identified compound in the methanolic extract of *Seidlitzia rosmarinus* by GC/MS.

Sr. Num.	RT	Name of the compound	Molecular formula	Peak area (%)	Nature of compound
1	4.334	*N*,*N*-Dimethyl glycine	$C_4H_9NO_2$	26.05	Amino acid
2	7.773	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	5.32	Phenol
3	8.551	2-[*p*-Chlorobenzyl] piperidine	$C_{12}H_{16}ClN$	0.41	Alkaloid
4	8.866	Vanillin	$C_8H_8O_3$	0.77	Phenolic glycosides
5	9.947	1-(4-Hydroxy-3-methoxyphenyl)ethanone	$C_9H_{10}O_3$	0.16	Phenol
6	10.216	3,4-Altrosan	$C_6H_{10}O_5$	1.63	Monosaccharide
7	10.542	Homo vanillyn alcohol	$C_9H_{12}O_3$	0.85	Phenol
8	14.319	3,7-Dihydro-3,7-dimethyl-1H-purine-2,6-dione	$C_7H_8N_4O_2$	0.71	Alkaloid
9	15.051	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	5.6	Fatty acid
10	16.716	(9*Z*)-9,17-Octadecadienal	$C_{18}H_{32}O$	4.74	Fatty acid
11	20.15	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	7.79	Fatty acid esters
12	24.275	Stigmastan-3,5-diene	$C_{29}H_{48}$	0.7	Phytosterol
13	24.47	Vitamin E	$C_{29}H_{50}O_2$	1.68	Vitamin
14	26.438	β-Sitosterol	$C_{29}H_{50}O$	7.63	Phytosterol

are the result of the destruction of other compounds due to the high temperature of the experiment or the plant contamination, which are introduced as the compounds of the extract. The presence of 2-[*p*-chlorobenzyl] piperidine and the anhydrosugar 3,4-altrosan compounds in Eshnan extract can be due to the destruction of other compounds under high temperatures during GC-MS. However, according to previous studies, it seems that the presence of 1,2-benzenedicarboxylic acid, diisooctyl ester in the GC report of Eshnan extract could be regarded as the contamination of the plant with phthalates, and it seems unlikely that these compounds are the real phytochemicals of the plant (Bianco, et al., 2014; Venditti, 2020). A phytochemical study of the aerial parts of the Eshnan plant led to the isolation and identification of alkaloid compounds such as metformin. Interpretation of the ^{13}C NMR spectrum revealed the presence of a new natural compound *N*-(4-hydroxyphenyl ethyl)-α-chlorofluorolamide as a mixture of *E* and *Z* isomers and several other secondary metabolites (Hassan et al., 2017). Quantitative and qualitative analyses of Eshnan extracts revealed that this salt-resistant plant is suitable for the production and extraction of plant oils. Oil extracts from the Eshnan plant were obtained with a yield of 5.37% and were analyzed by HPLC (Mahdavi et al., 2018). The plant contained 16 fatty acids, which is consistent with the results of this study. Saturated (butyric acid, palmitic acid) and unsaturated (linoleic acid, oleic acid) fatty acids in the plant were identified. Due to the level of unsaturated fatty acids in the plant, the oil can be used to improve the fatty acid profile of many oil products and can play an important role in improving the nutrition of poor communities.

3.4. Extraction, foaming properties, and total saponin content

The total saponin content of the various extracts was determined (Fig. 4). Of the three extracts, the aqueous extracts contained the highest (760.62 ± 10.03 µg/mL) levels of saponins, while the methanolic extracts (440.65 ± 3.42 µg/mL) contained the lowest contents of saponins. Furthermore, the aqueous extracts exhibited the highest levels of foaming and methanolic extracts had the lowest amounts (Fig. 5A, Fig. 5B). The percentage yield of saponin from dried Eshnan powder was found to be 13.11 ± 0.04 %.
The choice of solvent significantly affected the rate of adsorption of bioactive compounds. The aqueous extract was the most effective medium for obtaining the high levels of saponins from the Eshnan plant. In addition, the methanol extracts contained the lowest quantity of saponins that were significantly different. In addition to potential use for washing, the foaming power breaks down the bond between stains and fibers and removes the contamination from the surface. After cleaning with surface-active substances, the foam suspension prevents covering the surface of the fibers again. Foaming power though not directly related to the washing power of the detergent, plays a role in the performance of detergents which is noteworthy (İbanoğlu and İbanoğlu, 2000). The use of chemical surfactants in various industries can lead to environmental pollution and create serious problems with regard to the treatment of plant effluent. Increased environmental hazards and risks associated with

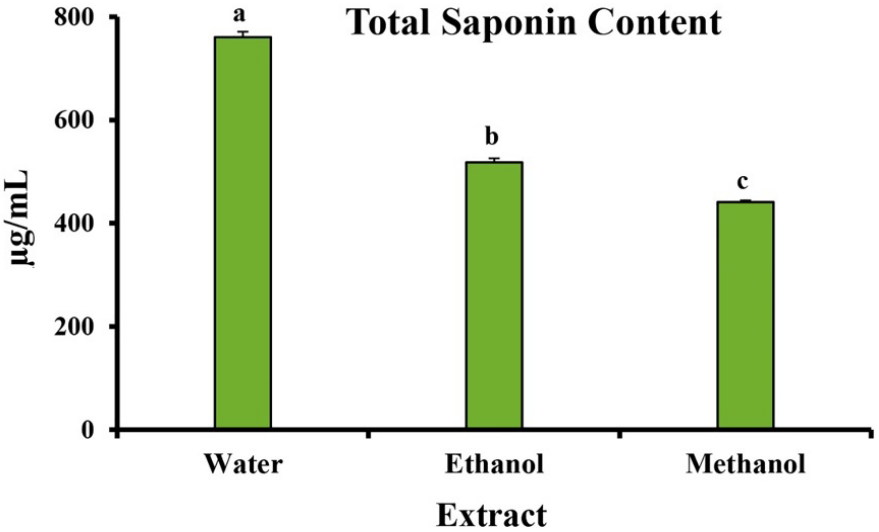

Fig. 4. Total saponin content of Eshnan plant extracted by three various solvents. Means and standard deviations were triplicate. Different letters (a, b, c) within the columns denote a significant difference between different ($p < 0.05$).

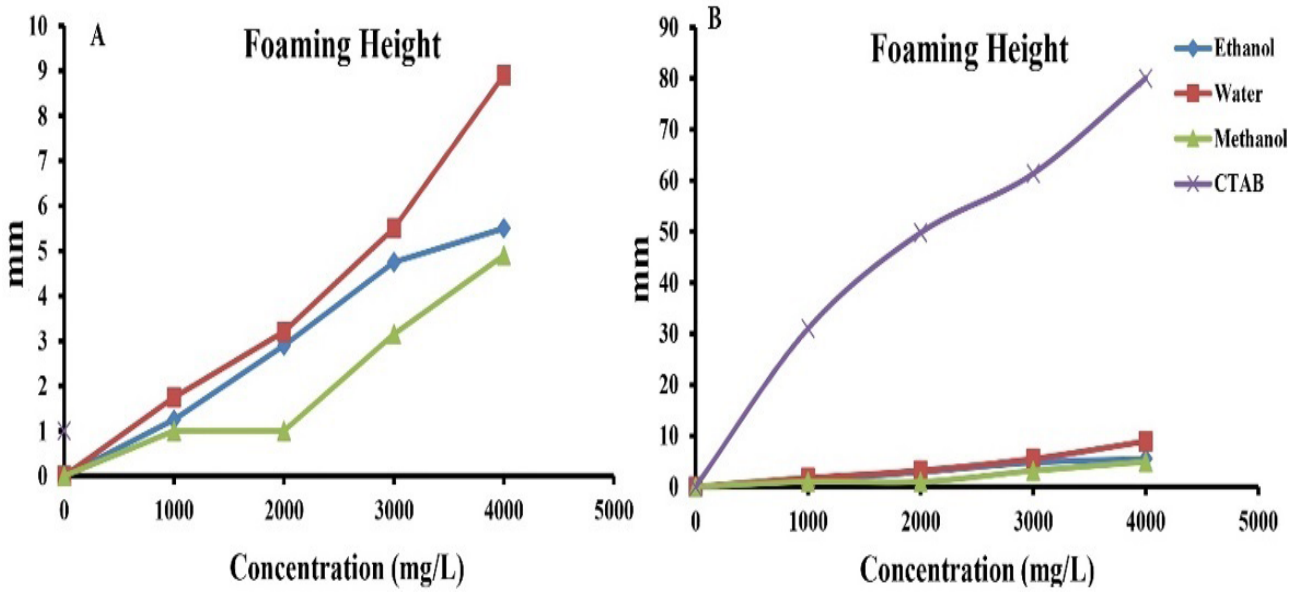

Fig. 5. Foaming height of Eshnan plant extracted by three various solvents and CTAB chemical surfactant.

chemical surfactants are considered logical reasons to convince researchers to replace these surfactants with plant-based surfactants (Crawford and Zirwas, 2014). A comparison of plant extracts with chemical based surfactants revealed that chemical surfactants had significantly more foaming properties as compared to plant extracts. Plant surfactants are environmentally friendly compounds produced by some plants. The use of plant surfactants reduces the emission of greenhouse gases and also due to their low toxicity, biodegradation, and relative stability in a wide range of physicochemical environments, they are called "green surfactants". Although they have fewer foaming properties than chemical surfactants, they can be a good alternative due to the aforementioned advantages (Deymeh et al., 2012). A natural cationic surfactant extracted from the plant *Seidlitzia rosmarinus* Bunge ex Boiss, which can be used as a suitable alternative to artificial surfactants for the recovery of chemical oils (Deymeh et al., 2012). In this study, the effects of herbal and synthetic surfactants on oil-water interfacial tension in working order for chemically enhanced oil recovery were compared. The results showed that surfactants derived from *S. rosmarinus* Bunge ex Boiss reduced

interfacial tension more than surfactant solutions of amphosol CG, formation, enordet, stepantan, CS 1045, CS 1040, stepantan and formatron, and *Zyziphus spina* Christi. The leaves of *S. rosmarinus* Bunge ex Boiss accumulate a large number of saponins which can be used in several industries for the making of soaps and detergents, pottery, ceramics, sugar factories (sugar crystallization), copper bleaching, etc.

The cytotoxic effect of various Eshnan extracts on two types of liver and uterine cancer cells has been also investigated. Taking into account the obtained results, the methanol and chloroform extract significantly reduced the survival of cancer cells. The most cytotoxic effect was related to the methanolic and chloroform extracts in both of the cancer cell lines. Various studies have shown that the Chenopodiaceae family exhibits anti-cancer properties (Zolfaghari et al., 2018). Two saponins (novel 30-nortriterpenoid glycosides) with anti-cancer properties have been reported from this family. The study introduces the anti-cancer properties of the Eshnan plant in relation to saponin compounds. Identification of piceol from the aerial parts of *S. rosmarinus* Bunge ex Boiss could be used to explain some of the biological and medicinal activities reported for the plant and make it a candidate for evaluating various pharmacological effects (Zolfaghari et al., 2017). The utilization of *S. rosmarinus* Bunge ex Boiss leaf extract (SRLSE), a novel bio-based surfactant, to inhibit shale hydration has been assessed for the first time through extensive experimentation (Aghdam et al., 2019). Detergents have found widespread application around the world due to their many beneficial properties. They however contribute to environmental pollution. Their detergency and cleansing properties are a result of surface activity. Unfortunately, detergents are unable to degrade easily in the environment. Most commercial surfactants used in detergents are from oil derivatives which with long-term use can result in significant damage to the environment. However, surfactants of natural origin have a negligible detrimental effect on the environment due to their purity, safety, color, and odor. Due to environmental pollution and the problems resulting from the use of industrial detergents, natural alternatives are being sought.

3.5. HPLC analysis

HPLC analysis was used to obtain more accurate results and identify saponins in the plant. The presence of saponin ginsenoside Rb1 within extracts of the plants was confirmed by HPLC (Fig. 6A, Fig. 6B). HPLC analysis revealed a sharp peak with a retention time of 2.7 min which correlates with the presence of ginsenoside Rb1 in the extract.

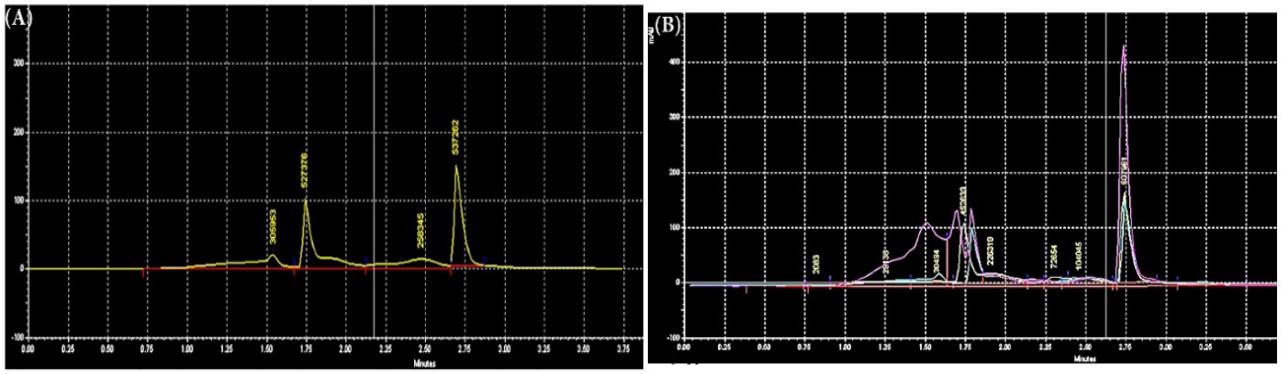

Fig. 6. (**A**) Chromatogram of the standard of saponin and (**B**) extracted saponins of Eshnan plant. The presence of saponin ginsenoside Rb1 within extracts of the plants was confirmed by HPLC.

The concentration of saponin in the extract was 8837 µg/mL. A comparison of herbal extracts with chemical surfactants (Fig. 5B) showed that chemical surfactants had the highest level of foaming compared to herbal extracts. Ginsenoside Rb1 was detected for the first time in the plant utilizing the HPLC method as one of the principal bioactive compounds that can be of great value in the medicinal use of this plant. This compound exhibits a wide range of biological functions such as antioxidative, anti-inflammatory, and various neuroprotective effects in the treatment of eye diseases such as diabetic keratopathy that have been reported for Rb1. HPLC tandem mass spectrometry separates different types of ginsenosides from various ginseng species. 8 Ginsenosides (Rb1, Rb2, Rc, Rd, Re, Rf, and Rg1) were determined by reversed-phase HPLC-MS/MS employing a quadrupole-ion trap mass spectrometer in Panax ginseng (Korean or Chinese ginseng) and P. quinquefolius (American ginseng), ginsenoside Rb1 with a retention time of 30.4 s also recognized (Ji et al., 2001). They utilized HPLC-UV method to separate ginsenoside from *Panax quinquefolius* L (commercial ginseng species). The ginseng plant contains about thirty saponins, which are the most abundant ginsenoside of the R family in the root of the plant, including Rb1, Rb2, Rc, Rd, Re, and Rg1. Rb1 acts as a phytoestrogen in cancer by activating the pectoral receptor clips of the breast (Corbit et al., 2005). Microwave methods were used to separate ginsenoside from commercial ginseng species (Kwon et al., 2003). Nine ginsenosides were identified, including Rb1, in four types of commercial ginseng utilizing RP-HPLC (Gao et

al., 2012). In recent years, ginsenoside analyses have primarily been conducted utilizing HPLC for saponin characterization. HPLC analysis was used to extract the non-saponins from ginseng (Wan et al., 2006). Another study investigated three ginseng plants and noted pharmacological differences, despite their morphologic similarities, which were related to the differences in their chemical composition and the type of ginsenoside present. In the study, 12 different saponins, including Rb1, were identified using HPLC-ELSD (Wan et al., 2007).

3.6. DNA-Binding

Investigation of the interaction of ethanolic and methanolic extracts of the plant with DNA showed that both extracts affected the structure and quality of DNA. A more accurate evaluation of the results revealed that the methanolic extract had a higher potential for DNA fragmentation and interaction. The ethanolic extract also partially destroyed the DNA in proximity to the well. The difference in the interaction of the extracts with DNA is dependent on the composition of the ethanolic and methanolic extracts (Fig. 7).

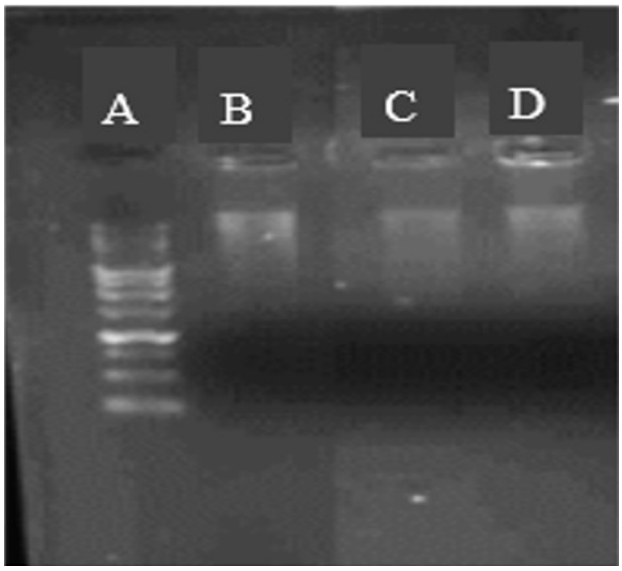

Fig. 7. DNA-binding test to show the pattern of DNA fragmentation using Eshnan extracts (**A**. Molecular markers; **B**. Pure DNA; **C**. Methanol extract; **D**. Ethanol extract.)

3.7. Anatomic study

A transverse incision of the Eshnan plant stalk, external to the epidermal tissue, revealed the cortex and vascular cylinder. Within the vascular cylinder, the xylem accounted for most of the structure and had a large spread. The central part of the stem was filled with parenchymal tissue with drainage tissue observed around the purple xylem. Fig. 8A shows the color ranging from yellow to brown depending on the cellulose content of the tissue. In Fig. 8B, the cellulose tissue is purple and the xylem tissue is green.

Today, the demand for natural products instead of synthetic ones is growing rapidly for food, cosmetics, and pharmaceutical disciplines. In recent years, natural herbal products have been used in medicine, cosmetics, nutrition, and flavorings with limited or no side effects. Some secondary metabolites in plants are good alternatives to synthetic chemicals utilized in the industry (Wong-Paz et al., 2015). The results of the current study showed that the Eshnan plant has potential applications in the health and pharmaceutical industries due to the presence of various secondary metabolites such as saponins and phenolic compounds. In addition to the medicinal and health properties exhibited by the Eshnan plant, it also possesses valuable environmental properties. The phenomenon of fine dust is one of the environmental problems that has increased in recent years following human activities. Over three hundred thousand hectares of Khuzestan plain area in southern Iran is the source of fine dust production. Eshnan, a native plant of Khuzestan is one of the plants compatible with the stressful and low water conditions of the region. This vegetation prevents water from evaporating from the soil surface, keeping it moist. Eshnan shrub or shrub plant, with its fleshy limbs and osmotic pressure regulation, is a suitable and recommended species for cultivation in Khuzestan (Dinarvand et al., 2018). Identification of chemical components involved in the environmental adaptations of this plant can be very important for basic studies as well as the management of environmental problems. Despite its importance, little phytochemical studies have been performed on this plant up to the present.

4. Concluding remarks

In general, the results of this study showed that in addition to having various compounds, such as fatty acids, and being a rich source of sapiens and polyphenols, Eshnan exhibits remarkable antioxidant properties that permit its usage as a suitable raw material in various food, pharmaceutical and cosmetic industries. Eshnan can be considered a good candidate for treating diseases caused by oxidative stress or pathogenic microbes. Ginsenoside Rb1 was detected for the first time in the plant and is one of its principal bioactive agents which can be of great use from a medicinal point of view. The results also showed that due to the abundance of these plants in areas with saline soils such as the Khuzestan province and convenient access to their extract in addition to their traditional use as a detergent and for the glazing of dishes, they may be recommended for use in health and food industries as a preservative, as well as for the pharmaceutical and cosmetics industries. Due to the significant amounts of sapiens within the plant, it can be used as a natural surfactant and a proper alternative to the chemical surfactants being utilized in industry. Cultivation and exploitation of halophyte plants in salt marshes, where it is not possible to cultivate crops while protecting itself, helps to prevent the intensification of the desertification process and can be a suitable option to be employed for the production and extraction of vegetable oils from plants. However, more research is still required in the field to gain more knowledge about the various types of oily halophytes. To manage

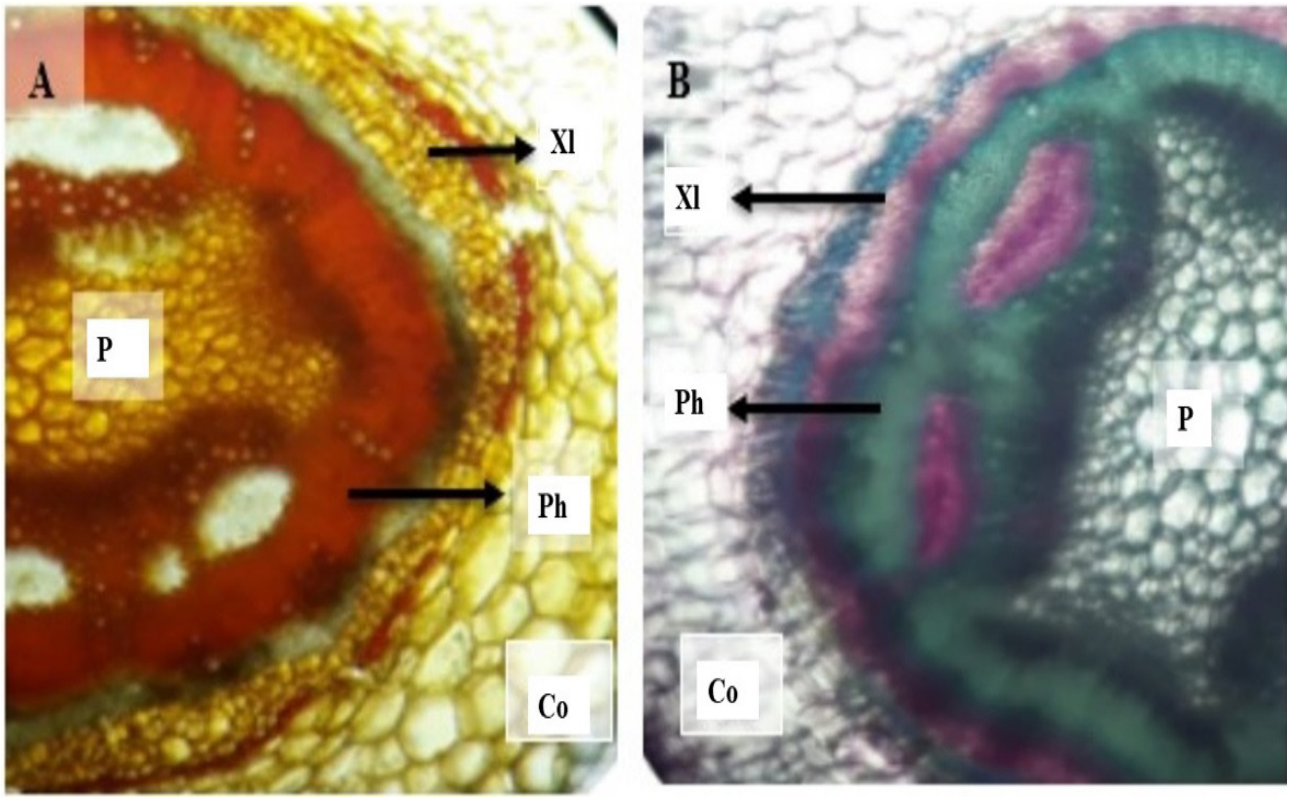

Fig. 8. Anatomy of the Eshnan stem stained with (**A**) Lugol's Iodine and (**B**) Methylene blue and Carmine. Co: Cortex, Ph: Phloem, Xl: Xylem vessels, P: Pith.

ecosystems in saline areas, improve pollutant problems, as well as to reduce the dust crisis in these areas, biotechnological applications of the plant should also be considered.

Acknowledgment

This project was completed through a common project which is funded by a grant from the Shahid Chamran University of Ahvaz Research Council. Last but not least, an emotional, heartfelt thank you to my late uncle Dr. Reza Oboodi for his unending support and guidance to the author (Dr. Maryam Kolahi). I hope he is somehow watching this from somewhere with a satisfied smile. I would like to appreciate the late Mr. Abbas Nejad Fathi for his knowledge and input on Eshnan, he was of great help help and very knowledgeable on the history of Khuzestan.

Funding

Not applicable' for that section.

Abbreviation

CTAB: Cetrimonium bromide; DE: The weight of the dry extract; D.M.S.O/D.M.E.M: Dimethyl sulfoxide/ Dulbecco's modified eagle's medium; DPPH: 2,2-Diphenyl-1-picrylhydrazyl; DS: Dry weight of the sample before extraction; EY: The extraction yield; FAA: Formalin acetic acid; FRSA: Free radical scavenging activity; GC/MS: Gas Chromatography-Mass Spectrometry; HPLC: High performance liquid chromatography; IC_{50}: Concentration of the sample that can inhibit; NMR: Nuclear magnetic resonance.

Author contribution statement

Conceptualization and literature search were performed by Maryam Kolahi and Roya Azadi. The first draft of the manuscript was prepared by Maryam Kolahi. Mahnaz Davabi and Nahid Pourreza critically analyzed and gave suggestions to finalize the manuscript. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

References

Aghdam, S.K.Y., Moslemizadeh, A., Madani, M., Ghasemi, M., Shahbazi, K., Moraveji, M. K., 2019. Mechanistic assessment of *Seidlitzia rosmarinus*-derived surfactant for restraining shale hydration: A comprehensive experimental investigation. Chem. Eng. Res. Des. 147, 570-578.

Ahmadi, M., Fata, A., Khamesipour, A., Rakhshandeh, H., Miramin Mohammadi, A., Salehi, G., Monavari, H., 2014. The efficacy of hydro alcoholic extract of *Seidlitzia rosmarinus* on experimental zoonotic cutaneous leishmaniasis lesions in murine model. Avicenna J. Med.

4(6), 385-391.
Azizian-Shermeh, O., Taherizadeh, M., 2014. Phytochemical Investigation, Antioxidant and Antimicrobial Activities of *Seidlitzia rosmarinus* L. from Sistan and Baluchestan. Iran. Chem. Congress. pp. 17-97.
Bashiri Rezaie, A., Montazer, M., Rad, M.M., 2017. Biosynthesis of nano cupric oxide on cotton using *Seidlitzia rosmarinus* ashes utilizing bio, photo, acid sensing and leaching properties. Car. Polym. 177, 1-12.
Bianco, A., Venditti, A., Foddai, S., Toniolo, C., Nicoletti, M., 2014. A new problem. Contamination of botanicals by phthalates. Rapid detection tests. Nat. Prod. Res. 28(2),134-137.
Bryan, H.D. 1955. Differential staining with a mixture of safranin and fast green FCF. Stain. Technol. 30, 25-31.
Cheok, C.Y., Salman, H.A.K., Sulaiman, R., 2014. Extraction and quantification of saponins: A review Int. Food Res. J. 59, 16-40.
Corbit, R.M., Ferreira, J.F., Ebbs, S.D., Murphy, L.L., 2005. Simplified extraction of ginsenosides from American ginseng (*Panax quinquefolius* L.) for high-performance liquid chromatography–ultraviolet analysis. J. Agric. Food Chem. 53(26), 9867-9873.
Crawford, C., Zirwas, M.J., 2014. Laundry detergents and skin irritancy-A comprehensive review. Skinmed 12(1), 23-31.
De, S., Malik, S., Ghosh, A., Saha, R., Saha, B., 2015. A review on natural surfactants. RSC Adv. 5(81), 65757-65767.
Deymeh, H., Shadizadeh, S., Motafakkerfard, R., 2012. Experimental investigation of *Seidlitzia rosmarinus* effect on oil-water interfacial tension: Usable for chemical enhanced oil recovery. Sci. Iran. 19(6), 1661-1664.
Dinarvand, M., Keneshloo, H., Fayaz, M., 2018. Vegetation of dust sources in Khuzestan Province. Iran Nature 3(3), 32-42.
Farahnejad, Z., Izadpanah, M., Ghasemian, A., Nojoomi., F., 2017. Antibacterial effect of *Seidlitzia rosmarinus* extract and silver nanoparticles on *Staphylococcus aureus* and *Klebsiella pneumoniae* isolated from urinary tract infections. Ann. Med. Health Sci. Res. 15(3), 1-4.
Gao, Y., Zang, P., Hao, J., Li, P., Li, X., Zhang, P.J., Zhang, L., 2012. The evaluation of contents of nine ginsenoside monomers in four commercial ginseng by reverse phase high performance liquid chromatography (RP-HPLC). J. Med. Plant Res. 6, 3030-3036.
Goli, S.A.H., Mokhtari, F., Rahimmalek, M., 2012. Phenolic compounds and antioxidant activity from saffron (*Crocus sativus* L.) petal. J. Agric. Sci. 4(10), 175-181.
Hadi, M.R., 2009. Biotechnological potentials of *Seidlitzia rosmarinus*: A mini review. Afr. J. Biotechnol. 8(11), 2429-2431.
Hassan, A.R., El-Kousy, S.M., El-Toumy, S.A., Frydenvang, K., Tung, T.T., Olsen, J., Christensen, S.B., 2017. Metformin, an anthropogenic contaminant of *Seidlitzia rosmarinus* collected in a desert region near the Gulf of Aqaba, Sinai Peninsula. J. Nat. Prod. 80(10), 2830-2834.
Hossain, M.A., AL-Raqmi, K.A.S., AL-Mijizy, Z.H., Weli, A.M., Al-Riyami, Q., 2013. Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown *Thymus vulgaris*. Asian Pac. J. Trop. Biomed. 3(9), 705-710.
İbanoğlu, E. and İbanoğlu, Ş., 2000. Foaming behavior of liquor ice (*Glycyrrhiza glabra*) extract. Food Chem. 70(3), 333-336.
Jeong, G.T.A., Park, D.H., 2006. Enhanced secondary metabolite biosynthesis by elicitation in transformed plant root system. Appl. Biochem. Biotechnol. 130(1-3), 436-446.
Ji, Q.C., Harkey, M.R., Henderson, G.L., Eric Gershwin, M., Stern, J.S., Hackman, R.M., 2001. Quantitative determination of ginsenosides by high-performance liquid chromatography-tandem mass spectrometry. Phytochem. Anal. 12(5), 320-326.
Kumaran, A., Karunakaran, R.J., 2007. *In vitro* antioxidant activities of methanol extracts of five *Phyllanthus* species from India. LWT-Food Sci Technol. 40(2), 344-352.
Kwon, J.H., Belanger, J.M., Pare, J.J., Yaylayan, V.A., 2003. Application of the microwave-assisted process (MAP™) to the fast extraction of ginseng saponins. Food Res. Int. 36(5), 491-498.
Lalrinzuali, K., Vabeiryureilai, M., Jagetia, G.C., 2015. Phytochemical and TLC profiling of *Oroxylum indicum* and *Milletia pachycarpa*. J. Plant. Biochem. Phys. 3(3), 1-8.
Mahdavi, M.J., Ranjbar, A., Zandi Esfahan, E., Dehghani Bidgoli, R., 2018. Potential of halophytes as source of edible oil. Iranian J. Range Des. Res. 24(4), 920-927.
Mirheidari, F., Khadivi, A., 2022. Multivariate analysis of eshnan (*Seidlitzia rosmarinus* Boiss.) based on morphological characterizations. Food Sci. Nutr. 10(11), 3662–3671.
Mohammadhosseini, M., Frezza, C., Venditti, A., Mahdavi, B. 2022. An overview of the genus *Aloysia* Paláu (Verbenaceae): Essential oil composition, ethnobotany and biological activities. Nat. Prod. Res. 36(19), 5091-5107.
Mohammadhosseini, M., Frezza, C., Venditti, A., Sarker, S.D., 2021. A systematic review on phytochemistry, ethnobotany and biological activities of the genus *Bunium* L. Chem. Biodivers. 18, e2100317.
Norhaiza, M., Maziah, M., Hakiman, M., 2009. Antioxidative properties of leaf extracts of a popular Malaysian herb, *Labisia pumila*. J. Med. Plant Res. 3(4), 217-223.
Redfern, J., Kinninmonth, M., Burdass, D., Verran, J., 2014. Using Soxhlet ethanol extraction to produce and test plant material (essential oils) for their antimicrobial properties. J. Microbiol. Biol. Educ. 15(1), 45-46.
Roy, A., Khan, A., Ahmad, I., Alghamdi, S., Rajab, B.S., Babalghith, A.O., Alshahrani, M.Y., Islam, S., Islam, M.R., 2022. Flavonoids a bioactive compound from medicinal plants and its therapeutic applications. Biomed. Res. Int. 6 (2022), 5445291.
Tanaka, N., Kashiwada, Y., 2021. Phytochemical studies on traditional herbal medicines based on the ethnopharmacological information obtained by field studies. J. Nat. Med. 75(4), 762-783.
Thagriki, D., Ray, U., 2022. An overview of traditional medicinal plants as dengue virus inhibitors. Trends Phytochem. Res. 6(2), 116-136.
Tiwari, P., Kumar, B., Kaur, M., Kaur, G., Kaur, H., 2011. Phytochemical screening and extraction: A review. Int. Pharm. Sci. 1(1), 98-106.

Towhidi, A., Zhandi, M., 2007. Chemical composition, *in vitro* digestibility and palatability of nine plant species for dromedary camels in the province of Semnan, Iran. Egypt. J. Biol. 9(1), 47-52.
Venditti, A., 2020. What is and what should never be: Artifacts, improbable phytochemicals, contaminants and natural products. Nat. Prod. Res. 34(7), 1014-1031.
Wan, J.B., Lai, C.M., Li, S.P., Lee, M.Y., Kong, L.Y., Wang, Y.T., 2006. Simultaneous determination of nine saponins from *Panax notoginseng* using HPLC and pressurized liquid extraction. J. Pharm. Biomed. Anal. 41(1), 274-279.
Wan, J.B., Li, S.P., Chen, J.M., Wang, Y.T., 2007. Chemical characteristics of three medicinal plants of the *Panax* genus determined by HPLC-ELSD. J. Sep. Sci. 30(6), 825-832.
Wang, G.X., Zhou, Z., Jiang, D.X., Han, J., Wang, J.F., Zhao, L.W., Li, J., 2010. *In vivo* anthelmintic activity of five alkaloids from *Macleaya microcarpa* (Maxim) Fedde against *Dactylogyrus intermedius* in *Carassius auratus*. Vet. Parasitol. 171(3-4), 305-313.
Wong-Paz, J.E., Contreras-Esquivel, J.C., Rodríguez-Herrera, R., Carrillo-Inungaray, M.L., López, L.I., Nevárez-Moorillón, G.V., et al., 2015. Total phenolic content, *in vitro* antioxidant activity and chemical composition of plant extracts from semiarid Mexican region. Asian Pac. J. Trop. Med. 8(2), 104-111.
Zhishen, J., Mengcheng, T., Jianming, W., 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 64(4), 555-559.
Zolfaghari, B., Jafarian, A., Rezaei, M., 2018. Evaluation of cytotoxic effect of different extracts of *Seidlitzia rosmarinus* on HeLa and HepG2 cell lines. Adv. Biomed. Res. 7(132), 1-5.
Zolfaghari, B., Mazaheri, M., Dinani, M.S., 2017. Isolation and identification of cinnamic acid derivatives from the aerial parts of *Seidlitzia rosmarinus* Ehrenb. Ex Boiss. J. Rep. Pharm. Sci. 6(1), 77-83.