ICC
Iranian Chemical Communication
Original Research Article
Payame Noor University http://icc.journals.pnu.ac.ir

Physico-chemical features of aqueous extract of *Acanthophyllum laxiusculum* roots from natural steppe habitats of Iran: evaluating surface activity and thermal behavior of partially purified extract

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Received: 20 May 2015, Accepted: 1 December 2015, Published: 1 December 2015

Abstract

Acanthophyllum laxiusculum is one of the most widely distributed species of the genus in Iran that flourishes in steppe and mountainous regions of the country. In the present study, water-soluble content of *A.laxiusculum* roots was extracted by boiling water and further successively purified partially by a defined solvent system. Surface tension measurements revealed the ability of plant extract to decrease the surface tension of water from 72 to 38mN/m with a critical micelle concentration (CMC) of 87.3 mg/l. The partially purified natural extract (PPNE) exhibited 65% emulsification activity (E24) on kerosene. A combination of UV–VIS spectroscopy and Fourier transform infrared spectroscopy (FTIR) demonstrated the presence of saponin compounds in PPNE. Moreover, thermostability of PPNE was evaluated by thermal gravimetric analysis (TG) and differential thermal analysis (DTA). TG-DTG analysis showed a complex three-stage thermal degradation mechanism and this conclusion was also supported by the DTA spectrum.

Keywords: *Acanthophyllum laxiusculum*; saponin; plant; critical micelle concentration.

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Introduction

Surface active agents are currently used throughout the world and offer a diverse range of industrial applications [1–4]. Recently, due to increased environmental concerns, extensive research has been carried out to find natural substitutes for chemically-synthesized surfactants. A number of studies have focused on surfactants produced by microorganisms known as biosurfactants. However, low production rates and difficulties with operational control at large volumes are the main drawbacks in attaining the mass production of biosurfactants at a commercial level. The most promising source of biosurfactants to meet both environmental and production standards are plant species. Surface active compounds known as saponins occur in many plant species.

Saponins are glycosides of triterpenes, steroids, and sometimes alkaloids grouped in amphipathic molecules with both hydrophilic and lipophilic moieties. A diverse range of foaming and emulsifying properties, as well as medicinal and antimicrobial properties of saponins, provide a range of applications in food, cosmetic and pharmaceutical industries as well as in bioremediation and nanotechnology [5]. Furthermore, saponins are highly soluble in water [6] and can easily be extracted by aqueous system without need for organic solvents, which are used in microbial biosurfactant extraction. Therefore, lack of organic solvents in extraction procedure of plant biosurfactants made them more preferred in comparison to microbial biosurfactants due to improvement in environmental safety and overall cost considerations.

A number of studies have shown that genus *Acanthophyllum* is rich in saponins [7–10]. Among the *Acanthophyllum* genus, *A. laxiusculum*is a very common plant species found in most mountainous and steppe regions of Iran, particularly in eastern and western parts of the country (Schiman-Czeika 1988). The roots of genus *Acanthophyllum* have been known as the "*chubak*" among Iranians. These roots were commonly used as the cleansing agent in the distant past, due to soap-like foam they produce when shaken in aqueous solutions. The root powder is capable of emulsifying and stabilizing oil-water systems and therefore is used as the stabilizer in preparation of local desserts such as "*Halva Ardeh*". Indeed, several researches have suggested that the saponins of some species of *Acanthophyllum* would be a better

alternative for synthetic surfactants in shampoo [1, 7].

In the present work, aqueous extract of *Acanthophyllum laxiusculum* Schiman-Czeika was prepared and physico-chemical analysis were performed on it to elucidate surfactant characteristics and thermal stability of the extract. The potentiality of extract for substituting the synthetic surfactants is enlightened through this research process.

Experiments

Materials

Normal buthanol (n-BuOH), methanol (MeOH), diethyl ether (Et_2O) and other chemicals used in the experiments were of analytical grade and purchased from Merck Company. They were used without further purification. Double-distilled water was used for preparing solutions. *Acanthophyllum laxiusculum* Schiman-Czeika roots were collected from steppe regions in Iran near the Qom Province, in June 2013 and were stored at the Iranian Biological Resource Center (voucher number IBRC-1275).

Aqueous extraction and partial purification

The roots were washed with deionised water and allowed to air-dry for 7 days at room temperature (20 to 25°C). Approximately 100g was weighted and soaked in 2000 mL of distilled water overnight to facilitate the peeling process. After being peeled, they were then grated and mixed with the soaking water. The mixture was boiled for 4 h and filtered through filter papers (Whatman No. 1) mediated by a Büchner funnel and vacuum pump to separate out the solid portion. The filtrate was further clarified by centrifugation at 9391×g for 20 min to remove any residue and then concentrated by boiling and being put through a rotary evaporator in order to attain a brownish, honey-like compound. A 7% solution of this crude natural extract (CNEx) in double-distilled water was extracted 2x with H_2O saturated n-BuOH, and then solvent was removed by rotary evaporator. The solid residue was dissolved in a few mL of MeOH and precipitated by the addition of 5 vols of Et_2O. Precipitates were collected by centrifugation and dissolved in a little MeOH to repeat the last step and obtain a pale yellow powder called Partially Purified Natural Extract or PPNE [11].

Determination of critical micelle concentration

Surface tension measurements were performed on different dilutions of PPNE in deionized water through the Du Nuoy ring method using an automatic tensiometer at room temperature (Sigma 700, Finland) [12].

Critical micelle concentration (CMC) is the breakpoint when surface tensions were correlated with concentrations in a plot. This procedure was repeated for crude natural extract (CNEx) without purification.

Determination of emulsification index

The emulsification capability, as one of the surface activity measures, was determined for PPNE according to the Cooper and Goldenberg procedure [13]. Aqueous PPNE solution (80 mg/l) and kerosene were vigorously mixed at a 1:1 ratio, for 2 min, and left standing at 25 °C for 24 h. The emulsifying index (E24) was calculated as the ratio of emulsified layer height to total height of the liquid column by equation

$$E24 = \left(\frac{height\ of\ the\ emulsion\ layer}{total\ height\ of\ the\ liquid}\right)_{24h} * 100$$

UV-Vis and Fourier Transform Infrared Spectroscopy (FTIR)

Aqueous solutions of crude and partially purified extracts at concentrations of 0.05% were scanned in the range from 190 to 1000 nm using a UV–VIS spectrometer (T80+, PG Instruments Ltd.).

Moreover, FTIR spectrum was recorded for PPNE using a transform infrared spectrophotometer (Equinox, Bruker, Germany) with a KBr disk in the frequency range 4000–400 cm^{-1}.

Thermal analysis

Thermal degradation behavior of PPNE was investigated by using the Perkin Elmer Pyris Diamond TG/DTA simultaneous TGA/DSC with 10 ± 0.1 mg of specimen in a platinum sample pan. Analysis was conducted under nitrogen atmosphere (50 ml min^{-1}) and the temperature was ramped from 25 °C to 600 °C at a rate of 10 °C min^{-1}.

Results and discussion

Preparation, extraction and partial purification

Consecutive preparation steps for PPNE such as (1) pre-treatment of the roots including peeling and grating, (2) aqueous extraction including boiling, vacuum filtration and clarifying centrifugation, and (3) solvent purification including multi-step precipitation are shown in supplementary figure.

Furthermore, 100g roots lead to specified residues during the sequential steps of extraction and purification, as follows:

100 g roots → 5.5 g n – BuOH – purified extract

→ 3.5g PPNE Et2O – purified extract

Considering this, production yield of PPNE was calculated as Y_{PPNE}=3.5%.

Surface activity characteristics

Lowering of surface tension and/or interfacial tension has been proposed as the intrinsic feature of all surfactants. The surfactant power and/or effectiveness are characterized by the capability for maximum reduction of surface tension defined in terms of the critical micelle concentration. The critical micelle concentration (CMC) is defined as the concentration above which any added surfactant molecules have a tendency to display aggregate structures like micelles due to thermodynamic considerations [14].

To determine the CMC for CNEx and PPNE, surface tensions of aqueous solutions were plotted against the logarithm of corresponding concentrations in mg/l. The intersection of two regression lines in each diagram estimated the CMC (Figure 1). CMCs were estimated by the regression method as 662 mg/l and 87.3 mg/l for CNEx and PPNE, respectively. Partial purification expedited the micelle formation and improved the surfactant effectiveness due to the removal of non-surface active impurities.

Both CNEx and PPNE caused the surface tension of aqueous solution to be reduced from 72. ±0.2 mN/m (for distilled water) to γ≃38.0±0.1 mN/m. Minimum surface tension recorded for PPNE was comparable to that estimated for microbial surfactants such as rhamnolipids (27.89 mN/m) [15] and surfactin (29.3 mN/m) [16].

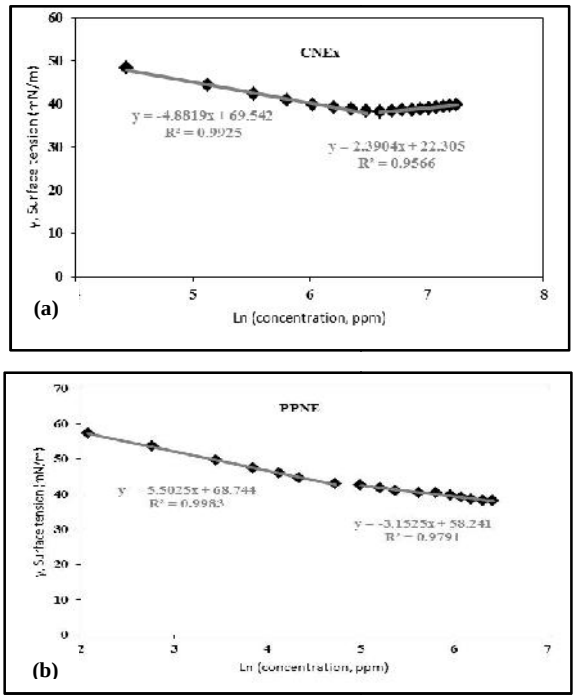

Figure 1. Determination of CMC value for crude extract (a) and partial purified extract (b) of *Acanthophyllum laxiusculum* roots

CMC is the intersection of two trend lines.

To further investigate the surface properties, emulsification activity was determined for partially purified extract. PPNE proved an efficient emulsifier against kerosene with an emulsifying index (E24) of 65 % at a concentration of 80 mg/l. An emulsifying index (E24) of approximately 65% is comparable to that estimated for microbial surfactants [17–19].The surface characteristics such as minimum surface tension, critical micelle concentration and

emulsifying activity (E24) exhibited by PPNE render it a good potential substitute for microbial surfactants.

Chemical analyses

The UV spectrum revealed the maximum absorption peak at 245 nm and 247 nm (Figure 2) for crude and partially purified extract which confirmed the presence of saponin compounds in these extracts [20].

Functional groups of PPNE were examined by FTIR spectroscopy in the 400 to 4000 cm^{-1} region (Figure 3). The FTIR spectrum exhibited a broad stretching peak at 3421.1 cm^{-1} assigned to the hydroxyl group. A sharp peak at 2921.1 cm^{-1} indicated the C–H stretching and bending vibrations. An absorption band appeared at 1722.1 cm^{-1} in the region of carbonyl stretching (C=O). Moreover, an intense peak at 1074.1 cm^{-1} displayed the presence of a glycosidic bond (C-O-C) explaining oligosaccharide linkage absorption to sapogenins [21]. It can thus be concluded that the FTIR spectrum of PPNE revealed the characteristics of triterpenoid saponin absorptions.

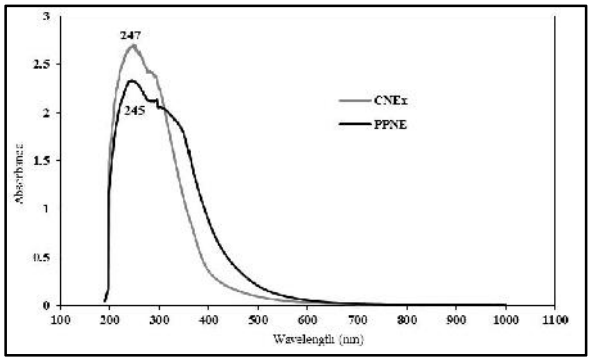

Figure 2. UV spectrum for crude (CNEx) and partial purified natural extracts (PPNE) of *Acanthophyllum laxiusculum* roots

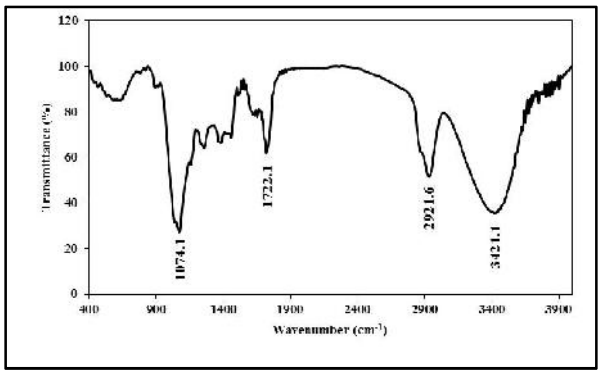

Figure 3. FTIR spectrum for partial purified natural extract (PPNE) of *Acanthophyllum laxiusculum* roots

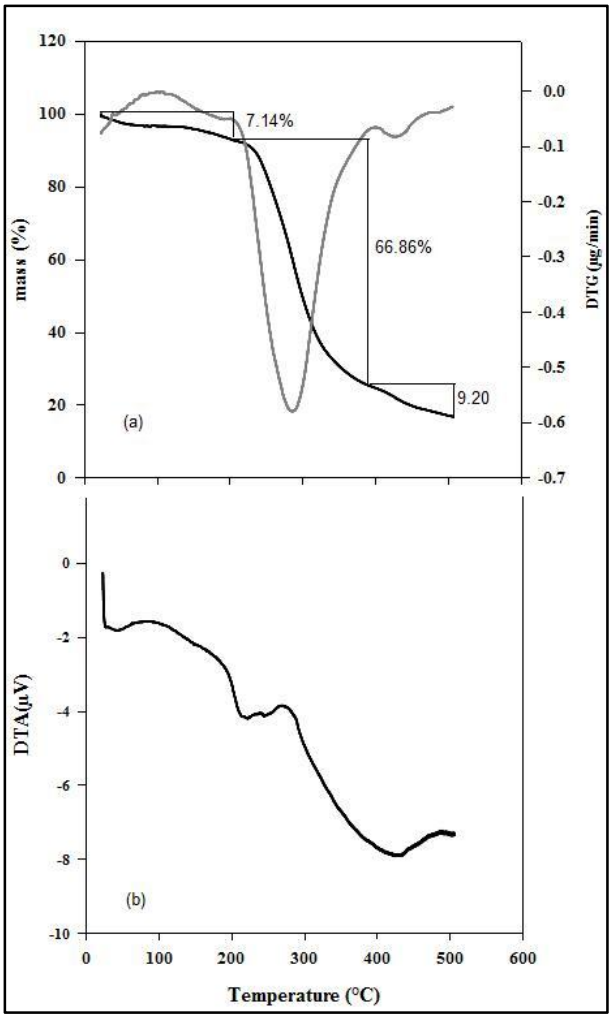

Figure 4. Thermogravimetric (a) and differential thermal (b) profiles of PPNE

Thermal gravity (TG)

Thermal stability is one of the most important properties for each compound used in industrial processes facing extreme temperatures or temperature fluctuations. Thermogravimetric analysis was used to determine any weight changes or thermal events in PPNE during the thermal treatments.

TG profile exhibited negligible or minor mass reduction (7.14 %) before 287 °C due to loss of moisture or alcohol present in the PPNE (Figure 4a.). The maximum degradation occurred at 287 °C resulting in a 66.86% loss of mass. Similarly, beyond 430 °C, 9.20% of PPNE was decomposed and disintegrated. The thermal profile for PPNE is consistent with that reported for saponin extracted from the tea seeds of *Camellia oleifera*by Jian *et al* [5]. Furthermore, endothermic as well as exothermic events are shown as the three concavities and one convexity along the differential thermal analysis (DTA) profile (Figure 4b.) correlated well with extremuma on the derivative thermogravimetric (DTG) curve.

Conclusion

The present study has *concluded* that an aqueous extract of *Acanthophyllum laxiusculum* exhibited saponin chemistry. The extract demonstrated notable surfactant

features such as high capability for reducing surface tension and emulsifying the two immiseable phases. Thermograms showed thermostability of this extract at extreme temperatures which is highly desirable in some practical procedures such as enhanced oil recovery. Due to the abundance and high availability of the *Acanthophyllum* species and the relative simplicity of extraction technology, its extract proves very cost effective. These economical advantages along with its environmental compatibility suggest *Acanthophyllumlaxiusculum* extract as a potent substitute for chemically-synthesized surfactants used in large scale industries such as food, pharmaceuticals, personal care, health care, cosmetics and oil drilling.

Acknowledgements

The authors gratefully acknowledge the financial support of Nastooh Commercial Engineering Co. for this research.

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