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SURFACTANT CHARACTERIZATION OF CRUDE SAPONIN EXTRACTS OF GUAIACUM OFFICINALE LEAF AND STEM

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ABSTRACT

Saponins are secondary metabolites that are produced by plants and some lower marine invertebrates. Because of their amphiphilic chemical structure, saponins exhibit surface activity similar to that of surfactants and are, therefore, known as natural detergents. Over 100 families of plants include species that are known to contain saponins. Many plant-derived saponins are currently used as emulsifiers in food and beverage and cosmeceutical industries. In the present article, crude saponin extracts of the leaf and stem of *Guaiacum officinale* (Zygophyllaceae) were characterized for their potential surfactant activity. The leaf and stem extracts reduced the surface tension of water from 72.8 mN m^{-1} to 49.584 mN m^{-1} and 49.875 mN m^{-1}, respectively. Additionally, the critical micelle concentration for the leaf and stem extracts was identical (0.037 g L^{-1}). The surface density of micelles for leaf and stem extracts was 0.119 nmol $cm^{-2}$and 0.121nmol cm^{-2}, respectively. The areas per head group were 139.75 $Å^2$ and 137.49 $Å^2$ for the leaf and stem extracts, respectively. The micellar hydrodynamic radii for the leaf and stem extracts were 18.62 nm and 18.98 nm, respectively. The zeta potential values of the leaf and stem extracts were −15.83 mV and −17.53 mV, respectively. Based on these results, we conclude that crude saponin extracted from *Guaiacum officinale* leaves and stems have potential application as biosurfactants.

KEYWORDS: *Guaiacum*, saponin, surfactant, biosurfactant.

INTRODUCTION

Saponins are glycosidal triterpenoids with characteristic surfactant activity. This property of saponins causes the formation of aqueous colloidal solutions that foam on shaking. The Latin term *sapo* literally translates to 'soap'. It is because of this property that saponin-containing plants are suitable candidates for use as household detergents.[1]

Many saponin-containing plants have been given common names which indicate their soap-like foaming property, for example, soapwort (*Saponaria officinalis* L.), soapberry (*Sapindus saponaria* L.) and soapbark (*Quillaja saponaria* Molina). It has been estimated that over 100 plant families include species that produce saponins.[2] Because of their surfactant properties, saponins have been widely investigated for use as biosurfactants.[3]

The surface activity of surfactants is attributed to their amphiphilic nature, i.e., they are molecules that contain both water soluble and water insoluble moieties. The polar hydrophilic sugar group is responsible for the water solubility of surfactants, while the non-polar lipophilic portion concentrates at the air–water interface, reducing the surface tension of water. The characterization of surfactants is based on parameters such as critical micelle concentration (CMC), hydrophilic–lipophilic balance, their chemical structure and their source properties. Saponins initially exist as monomers which assemble at the air–water interface in the solution. However, once the CMC has been reached, there is a high probability of monomers aggregating to form micelles with a hydrophobic or lipophilic center.

In addition to their detergent capacity, biosurfactants are also used in industrial processes for solubilization, emulsification, dispersion, wetting and foaming. Furthermore, some biosurfactants also act as antimicrobial agents. Recent applications have also seen biosurfactants, particularly saponins, being used for the bioremediation of soils contaminated with chemicals which have low water solubility.[3] Because of the multitude of applications that biosurfactants have, there is a continual interest in the discovery of new biosurfactants. In the present article, surfactant characterization of crude saponin extracts of the leaf and stem of *Guaiacum officinale* (Zygophyllaceae) was performed.

MATERIALS AND METHODS

Crude Saponin Extract

Extracts were prepared by refluxing 2 g of *Guaiacum officinale* L. leaf and stem powder with 10 mL 70% ethanol for 10 min. After cooling, the mixture was filtered and the filtrate was evaporated to dryness. The residue was suspended in 10 mL distilled water. The water suspension was then shaken several times with 10 mL water-saturated n-butanol. The n-butanol phase was collected, filtered over anhydrous sodium sulphate and evaporated to dryness. The n-butanol phase containing crude saponin residues was reconstituted using distilled water. The reconstituted extracts were then filtered using a 0.22-µm membrane filter. The membrane filtered extracts were evaporated to dryness. The residue was reconstituted in distilled water to obtain a 100 g L^{-1} stock solution.

Surface Tension and Critical Micelle Concentration

The ability of the plant extracts to reduce the surface tension of water was assessed using a tensiometer (v3.01, GBX Instrumentation Scientifique) by the Wilhelmy's plate method and the accompanying Balance 3S computer software. The extract concentration varied from 0.001 to 1.00 g L^{-1}. The CMC was calculated by plotting a graph of the surface tension values versus the natural logarithm of extract concentration. All readings were taken at 25 ºC

Surface Density and Area Per Head Group

The surfactant number density Γ is the number of surfactant molecules that occupy a unit area at the air–water interface. It was calculated using Gibbs adsorption equation[4]

Equation 1:

$$\Gamma = -\left(\frac{1}{RT}\right)\left(\frac{d\gamma}{dlnc}\right)$$

where,

R = gas constant (8.314 J mol^{-1} K)

T = absolute temperature (K)

γ = surface tension (mN m^{-1})

lnc = natural logarithm of surfactant concentration

Using the surface density data, the area per head group of a micelle can be determined using the formula given below[4]

Equation 2:

$$A = \frac{1}{\Gamma N_{AV}}$$

where,

Γ = surface density (nmol cm^{-2})

N_{AV}= Avogardo's constant (6.023×10^{23} mol^{-1})

Micelle size and zeta potential

Zeta potential is a measure of the stability of the dispersed micelles against aggregation. The magnitude of the zeta potential indicates the degree of electrostatic repulsion between the micelles. The larger the zeta potential, the greater are the charge on micelles and the stability of the micellar solution. The hydrodynamic radii of the saponin aggregates were determined using dynamic light scattering (DLS). The DLS and zeta potential measurements were taken using a Zetasizer Nano ZS90 (Malvern Instruments, Worcestershire, UK). The concentration of the solutions used to determine the aggregate size and zeta potential was ten times CMC. All readings were taken at 25±2 ºC.

RESULTS

The reductions in surface tension of water due to *Guaiacum officinale* leaf and stem saponin extracts, respectively are shown in Figures 5.3.1 and 5.3.2. Based on the Wilhelmy's plate readings, the surface tension of water reduced from 72.667 mN m^{-1} to 49.584 mN m^{-1} in case of leaf extract, and to 49.875 mN m^{-1} in case of stem extract.

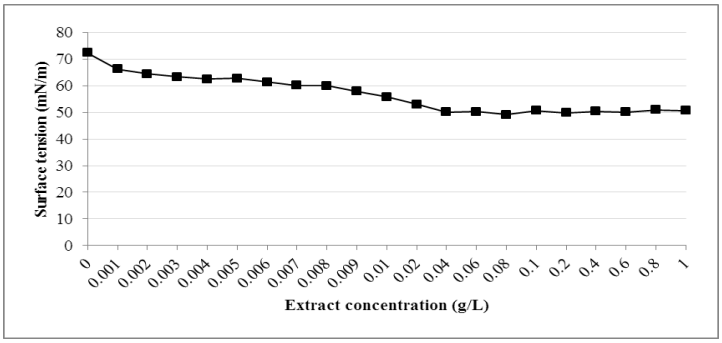

Figure 1: Effect of *Guaiacum officinale* leaf extract on surface tension of water.

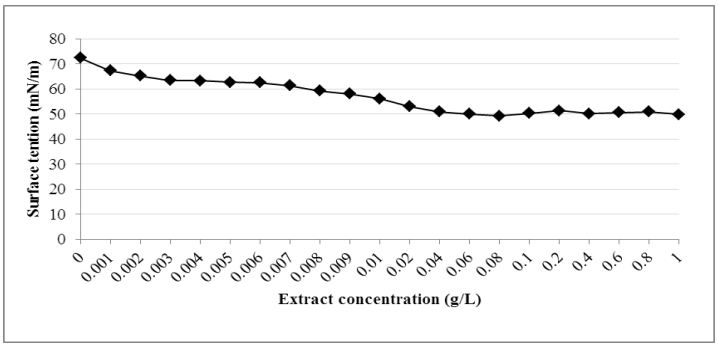

Figure 2: Effect of *Guaiacum officinale* stem extract on surface tension of water.

Based on the surface tension results, the CMC was determined as the concentration at which the curve of surface tension versus concentration shows a change of slope after which the slope remains constant with a subsequent increase in concentration. The CMC value for both the leaf and stem extracts was same, i.e. 0.037 g L^{-1}. Using Equations 1 and 2 respectively, the surface density and area per head group were determined. In case of the leaf extract, surface density was 0.119 nmol $cm^{-2}$and the area per head group was 139.75 $Å^2$. For the stem extract, the surface density was 0.121 nmol $cm^{-2}$and the area per head group was 137.49 $Å^2$.

The aggregate micelle size and zeta potential were determined using extracts of concentration 0.4 g L^{-1} (ten times CMC value). The DLS analysis for the aggregate micelle size of leaf and stem extracts revealed aggregates with Z-average diameters of 18.62 nm and 18.98 nm, respectively. The zeta potential of the micelles of leaf extract was −15.83 mV and that of stem extract was−17.53 mV, indicating a moderate stability of the dispersion.

Table 1: Surfactant characteristics of *Guaiacum officinale* leaf and stem saponin extracts.

Parameter	Leaf	Stem
Minimum attainable surface tension of water (mN m^{-1})	49.584	49.875
Critical micelle concentration (g L^{-1})	0.037	0.037
Surface density (nmol cm^{-2})	0.119	0.121
Area per head group ($Å^2$)	139.75	137.49
Hydrodynamic radii (nm)	18.62	18.98
Zeta potential (mV)	−15.83	−17.53

DISCUSSION

Biosurfactants are fast gaining importance because of their biodegradability and environment-friendly nature. Among the plant-produced secondary metabolites, saponins are known to have surfactant activity because of their unique amphiphilic nature.[5] Many plant saponins have been approved for use in commercial applications such as emulsifiers and surfactants for food and cosmetics. Of these, *Quillaja saponaria* (soap bark) and *Yucca schidigera* (Mohave yucca) saponins are the most widely used saponins. Other plants used commercially as sources of saponins include *Aesculus hippocastanum* (chestnut), *Glycyrrhiza glabra* (liquorice), *Gypsophylla paniculata* and *Trigonella foenum-graecum* (fenugreek).

The characterization of a surfactant is typically based on properties such as surface activity, critical micelle concentration, zeta potential and emulsion stability. The ability of *Guaiacum officinale* leaf and stem saponin extracts to reduce the surface tension of water was

determined using the Wilhelmy's plate method. The surface tension of water reduced from 72.667 mN $m^{-1}$to 49.584 mN m^{-1} in case of leaf extract, and to 49.875 mN m^{-1} in case of stem extract. Canto *et al*. (2010) reported that the saponin fraction of *Ilex paraguariensis* (mate)reduced the surface tension of water to 52.9 mN m^{-1}.[6] Similarly, a crude extract of *Sapindus mukorossi* reduced the surface tension of water to 51.7 mN m^{-1}.[7] *Glycyrrhiza glabra* saponin extract reduced the surface tension of water to approximately 46 mN m^{-1}.[8] Mitra and Dugan (1997), however, have reported that commercially available *Quillaja* saponin was capable of reducing the surface tension of water to approximately 36 mN m^{-1}.[4] The water surface tension reducing ability of *Quillaja* saponin was similar to that of SDS (37 mN m^{-1}) and SLS (35.6 mN m^{-1}).[6,7] The high activity of *Quillaja* saponin could be attributed to the fact that the extract used by Mitra and Dugan (1997) was that of a commercially available purified powder, while the extracts of *Guaiacum* used in the present study as well as the extracts of the other mentioned plants are enriched extracts prepared by phase fractioning. The high saponin content in the commercial sample is likely to produce better results because of higher concentrations of saponin in the extract.

CMC is defined as the concentration above which added surfactant molecules have a higher probability of appearing as aggregated micelles.[9] The CMC value for *Guaiacum* leaf and stem extract was 0.037 g L^{-1}; while the reported CMC values for the *Ilex* fraction and *Glycyrrhiza* saponin extract were 0.15 g $L^{-1}$and 0.53 g L^{-1}, respectively. In contrast, *Quillaja* saponins showed a CMC range of 0.5–0.8 g L^{-1}. The area per head group of the micellar aggregates for *Guaiacum* extract and *Quillaja* saponins were different from each other. While the area per head group of *Quillaja* saponins is 83 $Å^2$, those of *Guaiacum* leaf and stem extracts were 139.75 $Å^2$ and 137.49 $Å^2$, respectively.[4] The hydrodynamic radii of *Guaiacum* leaf and stem saponin extracts were 18.62 nm and 18.98 nm, respectively. These radii are much larger than that of *Quillaja* saponin micelles, which have been reported to attain a hydrodynamic radius of 3.6 nm at 25 ºC. The large micellar size of *Guaiacum* saponins potentially permits trapping of hydrophobic molecules within the centre of the micelle, indicating improved solubilization capacity.

The zeta potential value of *Guaiacum officinale* leaf extract was −15.83 mV, while that of the stem extract was −17.53 mV. Canto *et al*. (2010) reported a zeta potential value of −23.3 mV for the *Ilex paraguariensis* saponin fraction.[6] Zeta potential indicates the ability of a colloidal solution to remain a stable dispersed suspension. A high zeta potential value confers

a higher electrokinetic potential on the particles present in the colloidal suspension, engendering greater repulsion of like molecules, thereby preventing them from aggregating. However, a lower zeta potential indicates that the solution is less stable and exhibits flocculation or coagulation. A zeta potential of +/−30 mV generally suggests that the solution is likely to be a stable colloidal solution, whereas solutions with zeta potentials between +/−10 and +/−30 mV are incipiently stable. Solutions with zeta potentials below +/−10 mV are highly susceptible to rapid coagulation and breaking out of the colloidal solution. Because saponin extracts of *Guaiacum* leaf and stem have zeta potential values between −15 and −17 mV, the colloidal solution has incipient stability; it may not remain in colloidal form for long durations.

CONCLUSIONS

In conclusion, *Guaiacum* saponin extracts from leaf and stem exhibit potential for application as biosurfactants. The poorly understood *Guaiacum* saponins could potentially lend themselves to commercial application because of their low CMC value and relatively large micellar size. Studies on the biosafety of *Guaiacum* saponins must be performed to determine whether they can be regarded as safe for food, feedstuff and cosmeceutical applications. Considering that a decoction made from Guaiac wood chips is used for the treatment of various ailments by tribals in the Caribbean region, *Guaiacum* saponins should be safe for human consumption. Because of the low absorption of saponins in the human body, they generally pass through the intestine without producing any adverse biological effects. However, the maximum safe dose must be determined. Additionally, studies on the cleansing properties of *Guaiacum* saponins can determine their potential for use in products such as shampoos and soaps. Overall, *Guaiacum* saponins could have promising applications in the food and drugs industries.

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