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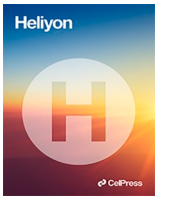

Research article

Characterization of saponins from the leaves and stem bark of *Jatropha curcas* L. for surface-active properties

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ABSTRACT

In this study, saponins extracted from leaves and stem bark of *Jatropha curcas* L. were investigated for surface-active properties. Conductivity and surface tension measurements revealed the micellar character of *J. curcas* saponin, with the average CMC, determined to be 0.50 g/L and 0.75 g/L for leaf and stem bark saponin, respectively. Stem bark saponin reduced the surface tension of water to a greater extent (γ_{CMC} = 37.65 mN/m) compared to leaf saponin (γ_{CMC} = 49.27 mN/m) indicating its efficient surface activity and potential detergency. pH measurement confirmed the weakly acidic nature of saponin with a pH value lying slightly below the range suitable for hair and skin. Stem bark saponin showed better cleaning ability, foaming ability and foam stability than leaf saponin, due to a sufficient reduction in the surface tension of water. The results obtained suggest that the saponin extracted from both the leaves and stem bark of *J. curcas* can be used as environmentally friendly alternatives to synthetic surfactants.

1. Introduction

Surfactants are chemical compounds that when added to water or any other solvents reduce their surface tension or interfacial tension [1]. These are the primary ingredients in detergents and other household cleaners [2]. Besides, they are also widely used in textile printing and dying, hygiene products, food processing, paper manufacturing, oilfield chemicals, agrochemicals, pharmaceuticals, textile products, microemulsions, and other market products [3,4]. Surfactants available in the modern marketplace are predominantly synthetic thanks to the low cost involved in manufacturing them from petroleum-based raw materials [5]. However, synthetic surfactants are associated with environmental concerns such as poor biodegradability, toxicity, dispersal of pollutants, and persistent contamination of water [6]. In response to these concerns, recently, research interest is growing toward finding their natural equivalents [7] that are biocompatible, biodegradable, less toxic, and better from the viewpoint of sustainability [8,9]. As with many other natural products, plants are the most abundant source of natural surfactants. Of many, saponins are also one of the plant-based natural surfactants [10,11].

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Saponins are secondary metabolites produced by plants belonging to more than 500 species [12–14]. Chemically, they are amphiphilic glycosides with hydrophilic glycones composed of sugar units attached to hydrophobic aglycones (steroids or terpenoids) [15,16] (Fig. 1(a)). Owing to their amphiphilic nature similar to that of a typical surfactant (Fig. 1(b)), they tend to form micelles and reduce surface tension when dissolved in a solvent. Hence, saponins are considered naturally occurring surface-active compounds [17] that are environmentally safe, biodegradable, renewable, and ecologically adaptable [18]. Their unique physicochemical and biological properties, make them useful as a substitute for soap in a variety of folk medicines and cleaning products [19]. Commercially important saponins are obtained from plants such as *Chlorogalum pomeidianum, Quillaja saponaria, Saponaria officinalis, Sapindus saponaria, Sapindus mukorossi,* and *Glinus* sp [15,18]. So far, some of the traditionally used saponins have been thoroughly investigated for their surface-active properties while few of them are already in use as natural surfactants in industrial products [20].

Although various saponin-rich plants have been studied earlier, the search for new sources continues. Its wide occurrence in the plant kingdom suggests that other sources of saponins are yet to be identified and explored. Therefore, with an aim of adding a continuum in the search for new sources of saponins, we investigated the potential of the leaves and stem bark of *Jatropha curcas* L. as a novel source of saponins. Our investigation was primarily based on two preliminary facts: (a) like soap, bubbles can be generated with the milky sap from its leaf and leaf petiole and (b) the use of chewing sticks made from its stems and twigs to clean teeth. *Jatropha curcas* is a plant belonging to the Euphorbiaceae family [21,22] and is widely cultivated in Asia and Africa as a hedge plant to protect farmlands from grazing animals [21,23]. Over the past few years, it has gained worldwide popularity for the production of biodiesel from its seeds and hence its commercial cultivation has accelerated among both private and public sectors [24–28]. However, its leaves and stems are underutilized considering biomass waste possibly also due to its toxicity [29]. In various earlier findings, the presence of various bioactive components including saponins has been reported in the leaves and stem bark of *J. curcas* [30–33]. Yet, no surfactant relevant study has been documented so far, being limited only to the phytochemical screening and analysis of biological activities. With this scenario, the present work reports the evaluation of saponins extracted from the leaves and stem bark of *J. curcas* for their surfactant-relevant properties. To the best of our knowledge, this is the first report on the surfactant properties of *J. curcas* saponins.

2. Experimental section

2.1. Extraction of saponins

Saponin extracts were obtained from the leaves and stem bark of *J. curcas* following previously reported methods [34–37]. The leaves and stem bark of *J. curcas were* collected from places within Biratnagar City, Nepal around mid-July 2021. It was verified by one of the authors, Dr. Hari Prasad Devkota. A voucher specimen (No.: JC20210614) was stored at the Research Laboratory, Department of Chemistry, Mahendra Morang Adarsh Multiple Campus, Tribhuvan University, Biratnagar, Nepal. The leaves as a whole and the stem bark of the plant were cut into small pieces and shade dried for approximately 20 days. Each dried plant material was pulverized and sieved through a mesh size 80 to obtain a fine powder of uniform size. The pulverized samples were dried in a hot air oven at 40 °C for an hour to remove the moisture content before carrying out the extraction process. For extraction, powdered plant material was first defatted with hexane in a Soxhlet extraction unit for 8 h maintaining the temperature at 50 °C. The defatted plant material was then collected and allowed to dry off in the oven at 40 °C for a day. It was then extracted with 70% v/v ethanol in water by the Soxhlet extraction method to obtain an alcoholic-aqueous fraction. The alcohol was distilled off to obtain an aqueous fraction. The aqueous fraction was cooled at room temperature and was re-extracted with n-butanol in the ratio of 1:3 using a separating funnel. The butanol fraction was concentrated using a rotary evaporator maintained at a temperature of 40 °C. The crude saponin extract obtained was then dried in a hot air oven at about 45 °C until the butanol was completely removed. The dried extract so obtained was stored at 4 °C in airtight containers.

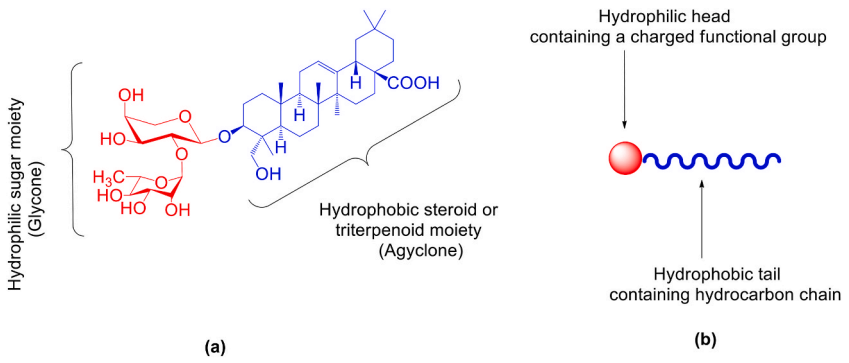

Fig. 1. (a) Molecular structure of a typical saponin molecule (sapinodoside A) with hydrophilic sugar moiety and hydrophobic aglycone representing its amphiphilic nature [14] (b) illustration of a surfactant monomer with a hydrophilic head covalently bonded to a hydrophobic tail.

2.2. Characterization of saponins

2.2.1. Preliminary analysis of saponins

(a) Solubility Test: Saponin extract was dried in a desiccator over CaO. Its solubility in water, methanol, ethanol, chloroform, petroleum ether, dil. NaOH, and dil. HCl was determined as follows: 1 mg of the dried extract was dissolved in 1 mL of the solvent till saturation at room temperature. The saturation of the solution was confirmed by the appearance of solid residues in the solution. Its solubility in each solvent was assessed qualitatively by visual observation.
(b) Foam Test: Details of this experiment are given in Section 2.3.6.

2.2.2. Fourier transform infra-red spectroscopy (FTIR)

Using an Alpha FT-IR spectrometer (Bruker, Germany), the FTIR spectra were obtained to analyze the existence of various chemical bonds in the saponin extracts. The equipment was calibrated against air before measuring the samples. All spectra were obtained for wavelengths between 4000 and 400 cm^{-1} with 32 scans per specimen at 4 cm^{-1} resolution.

2.3. Evaluation of surfactant properties

2.3.1. Preparation of stock solution

The crude saponins extract was used without further purification for the preparation of a natural surfactant (NS) solution. A stock solution of 2 g/L was prepared by dissolving crude saponins in doubly distilled water which was later diluted as per requirements. This solution was used to determine conductivity, surface tension, pH, cleaning ability, foaming ability and foam stability. All experiments were performed at 25 ± 1 °C.

2.3.2. Conductivity measurement

An auto-ranging Conductivity/TDS meter TCM-15+ with a conductivity cell having a cell constant of 1.001 cm^{-1} was used to measure the conductivity (κ). The conductivity cell was first calibrated with standard potassium chloride (0.01 M) keeping the temperature of the solution within 25 ± 1 °C.

For the conductivity measurement, the conductivity cell was first rinsed with distilled water followed by acetone and left to dry naturally. Then a clean and dry graduated glass beaker of 50 mL was filled with 30 mL of NS solution. The cell was then dipped into the solution and the corresponding conductivity value shown by the conductivity meter was noted. The solution was subsequently diluted by the serial dilution method by first withdrawing 2 mL of the test solution and adding 2 mL of distilled water. The conductivity values of the diluted solutions were determined similarly. The diluted solutions were stirred with a magnetic stirrer after each successive addition of water and a Refrigerated Bath Circulator was used to maintain the temperature at 25 ± 1 °C.

2.3.2.1. Degree of ionization. The ratio of post micellar slope (S_2) to the premicellar slope (S_1) provides an estimate of the degree of ionization (α):

$$\alpha = \frac{S_2}{S_1} \tag{1}$$

2.3.3. Surface tension measurement

The surface tension (γ) was measured by the Wilhelmy plate method using a KRUSS K20S Easy Dyne Tensiometer.

For the surface tension measurement, the platinum plate was first repeatedly rinsed with water followed by ethanol and acetone and then dried with a hair drier before each successive use. Then, 30 mL of the NS solution was taken in a 50 mL clean and dry sample vessel. The platinum plate hanging from a force-measuring balance was lowered into it and slowly pulled out. This gave the corresponding value of the surface tension which was noted. The solution was subsequently diluted by the serial dilution method by first withdrawing 2 mL of the test solution and adding 2 mL of water. The surface tension values of the diluted solutions were determined similarly. The diluted solutions were stirred with a magnetic stirrer after each successive addition of water and a Refrigerated Bath Circulator was used to maintain the temperature at 25 ± 1 °C.

2.3.3.1. Related surface properties and thermodynamic parameters. The surface pressure at CMC (π_{CMC}) is given by

$$\pi_{CMC} = \gamma_0 - \gamma_{CMC} \tag{2}$$

Here, γ_0 = surface tension of distilled water and γ_{CMC} = surface tension of NS solution at CMC.

Surface tension is found to vary linearly with the natural logarithm of the saponin concentration, so the maximum surface excess concentration at the air-water interface (Γ_{max}) is calculated by using the Gibbs adsorption isotherm [38],

$$\Gamma_{max} = -\frac{1}{RT}\left(\frac{d\gamma}{d\ln C}\right)_{T,P} \tag{3}$$

Here, γ = surface tension, R = Universal gas constant, T = absolute temperature, C = surfactant (saponins) concentration, and $\left(\frac{d\gamma}{d\ln C}\right)$ =

slope of γ vs $ln\ C$ curves in the premicellar region.

The area occupied by one saponin molecule (A_{min}) at the air-solution interface is calculated by using the equation [38,39]:

$$A_{min} = \frac{1}{N_A \Gamma_{max}} \quad (4)$$

Here, N_A = Avogadro's number.

The standard Gibbs free energy of micellization (ΔG_m°) is calculated using the equation

$$\Delta G_m^\circ = RTln\chi_{CMC} \quad (5)$$

Here, χ_{CMC} = mole fraction of saponins at CMC.

The standard Gibbs free energy of adsorption (ΔG_{ads}°) air/saturated monolayer interface is calculated using the equation

$$\Delta G_{ads}^\circ = \Delta G_m^\circ - \frac{\pi_{CMC}}{\Gamma_{max}} \quad (6)$$

2.3.4. pH measurement

A Eutech pH meter was used to measure the pH of the NS solutions. The pH electrode was initially calibrated using standard buffer solutions with pH values of 4 and 7 while keeping the solution's temperature constant at 25 ± 1 °C.

For pH measurement, the pH electrode was first rinsed with distilled water followed by acetone and left to dry naturally. Then a clean and dry graduated glass beaker of 50 mL was filled with 30 mL of NS solution. The electrode was then dipped into the solution. After that, the corresponding pH value shown by the pH meter was noted down. The pH values of the NS solutions of different concentrations were determined similarly maintaining the temperature at 25 ± 1 °C.

2.3.5. Cleaning ability

The cleansing capacity of saponins was investigated following previously described procedures [40–42] as follows: Cotton wool was dipped in water for a few hours, dried, and then weighed. It was then immersed in stimulated dirt, which was prepared by dissolving 1 g mustard oil and 1 g coconut oil in 100 mL hexane. It was then taken out, dried, and weighed. This cotton wool was soaked in NS solution in a flask and agitated for 5 min with a magnetic stirrer. It was taken out, cleaned with water, dried and weighed. The investigation was performed at room temperature (25 ± 1 °C).

2.3.6. Foaming ability and foam stability

The foaming ability and foam stability of saponins were investigated using the cylinder shake method as reported previously [40–42]. It can be summed up as follows:

50 mL of NS solution in a taken 100 mL graduated cylinder was shaken vigorously ten times. The foam height was measured immediately after shaking and 5 min afterwards. A stopwatch was used to keep track of the foam decomposition. The investigation was performed at room temperature (25 ± 1 °C).

3. Results and discussion

3.1. Extraction yield

The extraction yield of saponins from dried plant material was calculated in percentages as follows:

$$\text{Saponin extraction yield } (\%) = \frac{\text{Weight of the crude saponin}}{\text{Weight of the powdered plant material}} \times 100$$

The percentage yields of saponins extracted from *J. curcas* leaves and stem barks are shown in Table 1.

For the almost same amount of the dried plant material taken, the percentage yield of saponins from leaves was found to be greater (20.1%) than that of the stem bark (8.6%). This reports higher saponin content in the leaves than the stem bark. The leaf saponins so obtained were solid and dark brown while the stem bark saponins were pasty mass and light green in color (Fig. 2). The green color of the stem bark saponin can be attributed to the conjugation of the available >C]O group with the C]C double bonds. This probably contributes to reducing the energy difference between the ground state and the excited states leading to absorption in the lower energy region of the visible spectrum such as red. Similarly, as in leaf saponin, the >C]O concentration is low, and absorption occurs in a

Table 1
Extraction yield of saponins in percentage derived from leaves and barks of *J. curcas* and their characteristics.

Saponins Source	Weight of the powder taken (g)	Weight of the crude saponins (g)	Extraction yield (%)	Characteristics of extracts	
				Color	Consistency
Leaves	15	3.01	20.1	Dark brown	Solid
Stem bark	15	1.29	8.6	Light green	Pasty mass

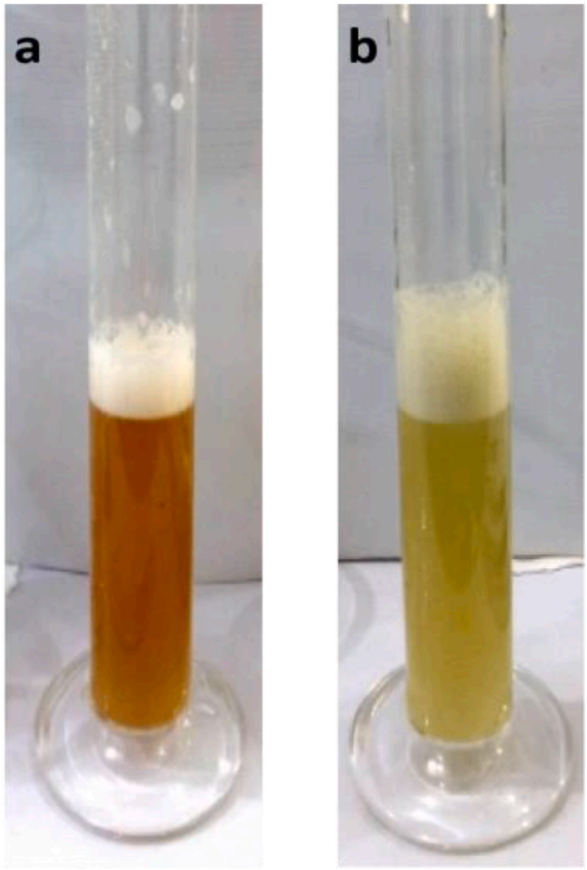

Fig. 2. Photograph of positive foam test given by the aqueous solution of a leaf (a) and stem bark (b) saponins.

higher energy region resulting in the complementary red color of the solution. These differences in the color and texture of the crude saponin extracts indicated the presence of different types of saponins in the leaf and stem bark of *J. curcas*. Earlier studies also reported variations in the composition and concentration of saponins within different parts of the same plant [38], [82].

3.2. Characterization of saponins

3.2.1. Preliminary analysis

Solubility and foam tests were performed for the preliminary analysis of saponins in the crude extract. The results are presented in Table 2.

The stem bark extract was completely soluble in water whereas the leaf extract was sparingly soluble with discrete particles. Both the leaf and stem bark saponins were sparingly soluble in methanol and ethanol but insoluble in petroleum ether and chloroform. Saponins are polar compounds that are soluble in polar solvents such as water and alcohol but insoluble in non-polar organic solvents like chloroform, ether and acetone [12]. Similar to our results, Schreiner et al., reported the maximum solubility of saponins in water (90% approx.) which is a polar protic solvent, followed by ethanol (6% approx.), ethyl acetate (2% approx.) which are polar aprotic

Table 2
Preliminary analysis of crude extract for the presence of saponins.

Test of Saponins		Leaf	Stem
1. Solubility Test	Water	+	++
	Methanol	+	+
	Ethanol	+	+
	Petroleum ether	−	−
	Chloroform	−	−
	Dilute NaOH	+	++
	Dilute HCl	−	−
2. Foam Test		+	+

(++) Soluble, (+) sparingly soluble and (−) Insoluble.

solvents and are sparingly soluble in non-polar *n*-hexane (less than 0.5%) [44]. The solubility of the crude extracts was also tested in dilute NaOH and dilute HCl. Both extracts were soluble in dilute NaOH but insoluble in dilute HCl. This indicates the acidic nature of both extracts. Positive foam test results were reported for both extracts (Fig. 2). Each of these tests qualitatively confirmed the presence of saponins in both crude extracts.

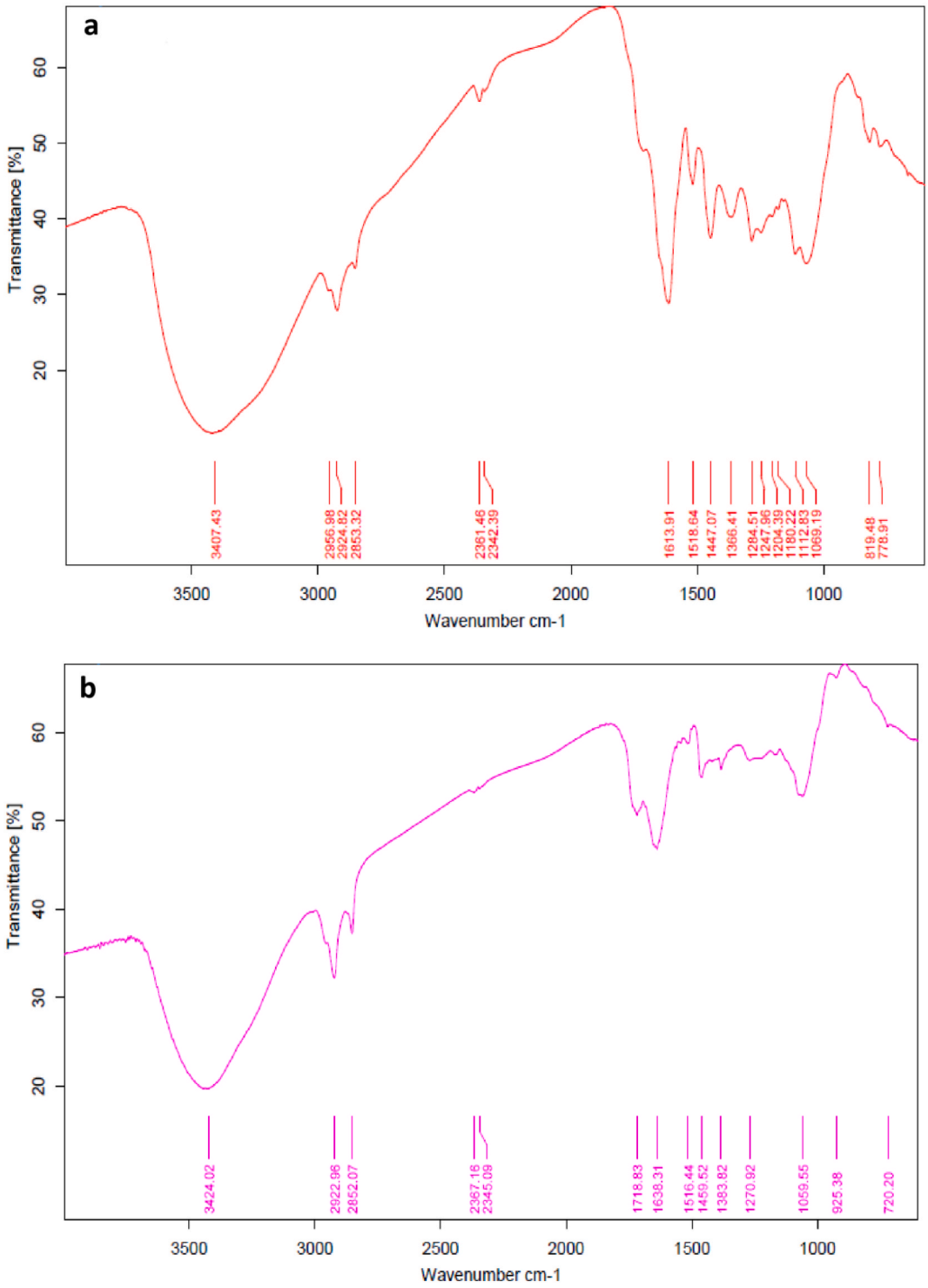

Fig. 3. FTIR spectrum of *J. curcas* leaf saponins (a) and stem bark saponins (b).

3.2.2. FTIR analysis

Fig. 3 (a) and (b) show the FTIR spectra of the leaf and stembark extracts, respectively. Both crude extracts exhibited FTIR absorption spectra characteristic of saponins thereby indicating their presence in the crude extracts. The characteristic infrared absorption of the polymeric hydroxyl group (OH) was observed at 3407.43 cm^{-1} and 3424.02 cm^{-1} for leaf and stem bark saponins, respectively. The carbon-hydrogen (C–H) absorption was observed at 2956.98 cm^{-1} for leaf extract and 2922.96 cm^{-1} for stem bark extract. The absorbance due to C]C stretching was observed at 1613.91 cm^{-1} and 1638.31 cm^{-1} for leaf and stem bark respectively. Similarly, the absorbance due to C]O stretching was observed at 1718.83 cm^{-1} for stem bark extract which was attributed to carboxylic acid groups such as glucuronic acids [44]. However, no absorption peak was obtained within the range of 1730–1700 cm^{-1} for the leaf saponins confirming the absence of carboxylic groups [17]. The absorption spectra for oligosaccharide bonding to sapogenins, that is C–O–C, were evident at 1069.19 cm^{-1} and 1059.55 cm^{-1} for leaf and stembark extract respectively. FTIR analysis also confirmed the amphiphilic nature of the saponins molecule. The polar hydrophilic portion comprises hydroxyl, carbonyl and carboxylate, while the hydrophobic portion is alkanes and alkenes [45]. The above-mentioned infrared functional group absorptions specific to saponins have been reported by Refs. [41,44,46–50].

However, further advanced spectroscopic studies are required for the structural elucidation and identification of saponins and other bioactive metabolites present in crude saponin extracts.

3.3. Evaluation of surfactant properties

3.3.1. Conductivity measurements

Conductivity measurement is the most accurate and practical approach for analyzing the characteristics of ionic surfactants due to the notable fluctuations in specific conductivity with surfactant concentration in the pre-and post-micellar areas [17]. The ions and their mobility in the solution are what cause conductivity [51]. The conductivity of the surfactant solution at the premicellar region is also reported due to the ionization of surfactant molecules. In this region, conductivity increases sharply and varies linearly with the surfactant concentration. After the critical micelle concentration (CMC) is reached, in the post-micellar region, there is only a gradual increase in conductivity due to the formation of micelles which have reduced mobility compared to that of ionized surfactant molecules.

The plots of conductivity of NS solution as a function of saponin concentration are presented in Fig. 4 for the leaf and stem bark NS solutions. Saponins in general are considered to be non-ionic surfactants. The ionic activity of saponins has been reported to be due to the ionization of the carboxylic acid groups present in them [43,52]. However, the ionization of the carboxylic acid groups associated with a saponin molecule depends on its position in the molecule. Only the carboxylic acids in the hydrophilic sugar of the molecule are ionizable which dissociates to give free carboxyl anions in the aqueous medium, thereby being responsible for the conductivity of the aqueous saponin solution. This also increases the solubility of saponin in water. In contrast, the ionization of the carboxylic acid groups attached to the hydrophobic aglycone is very limited resulting in low conductivity of the saponin solution and low solubility of saponins in water [15]. For quillaja saponins, glucuronic acids have been reported to be the only ionizable carboxylic acid groups [52] whereas other acids are linked to the main structure via ester bonds [44]. The ionization of glucuronic acid in an aqueous medium furnishes ions thereby inducing electrostatic effects [48,53,54] resulting in the conductivity of saponin solution. As the saponin content in the solution increased, the number of ions producing electrostatic effects increased and thus, the conductivity of the solution increases.

In this study, it is evident from Fig. 4 that the stem bark NS solution is more conductive than the leaf NS solution. The conductivity of both solutions increased with an increase in saponin concentration. However, this increment was more prominent for the stem bark NS solutions. The difference in conductivity values of leaf saponins and stem bark saponins may be due to the different positions of the carboxylic acid groups as reported in the previous paragraph. According to the conductivity report, stem bark saponin contains

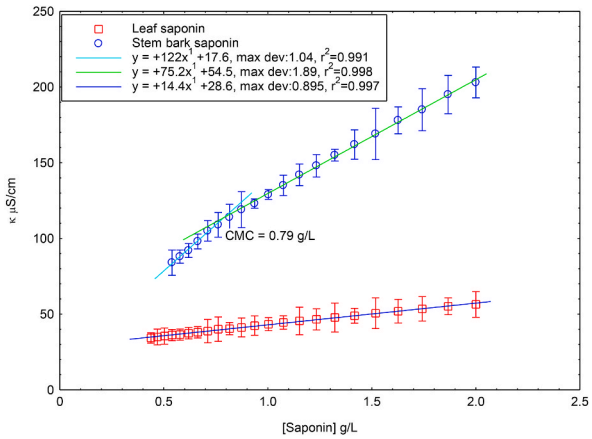

Fig. 4. Graph of conductivity as a function of saponin concentration for leaf and stem bark NS solutions.

ionizable carboxylic acid groups in the hydrophilic sugar portion whereas leaf saponin contains the least ionizable carboxylic acid groups in the hydrophobic sugar portion of the molecule. This was further evidenced by the solubility test, which reported greater water solubility of stem bark saponins than leaf saponins. This evidence supports the greater conductivity of the stem bark NS solution than that of the leaf NS solution.

By conductivity measurements, CMC is observed as a point of inflection at which a sharp change in the slope of conductivity versus concentration is reported for ionic surfactants. Non-ionic surfactants, on the other hand, exhibit a linear relationship between conductivity and surfactant concentration with very insignificant variation in the slope of the line [17]. In Fig. 4, the conductivity of the leaf NS solution showed a linear variation with saponin concentration. A sharply visible breakage was not observed in this case and behaved like a non-ionic surfactant. Therefore, CMC could not be determined for leaf NS solutions by the conductivity method. However, for the stem bark NS solution, a visible break was observed in the conductivity versus saponin concentration plot as in the case of an ionic surfactant. The CMC value of the stem bark saponin was determined to be 0.79 g/L. At this point, a slight change in the slope of the conductivity versus concentration curve is observed. The slopes of the lines corresponding to the pre-micellar region (S_1) and post-micellar region (S_2) were 122 and 75.2 respectively.

For stem bark saponins, the value of α was determined to be 0.62 (using Eq. (1)). This indicated moderate dissociation of stem bark saponins in its aqueous solutions.

3.3.2. *Surface tension measurements*

Fig. 5 shows the plots of surface tension as a logarithmic function of saponin concentration of leaf and stem bark NS solutions in water. It is observed that in both cases the surface tension decreased with increasing saponin concentration. The reduction in surface tension indicated the adsorption of saponin molecules at the air-solution interface thereby indicating the surface-active properties of both leaf and stem bark saponins. The stem bark saponins reduced the surface tension to a greater extent (37.4 mN/m at 0.9 g/L) than leaf saponins (49.9 mN/m at 0.9 g/L). The maximum decrease in surface tension by stembark saponin indicates that there is closer packing of saponin molecules at the air-solution interface. A reduction of surface tension to a value between 32 and 37 mN/m signifies efficient surface activity and detergency of the surfactant [55]. Herein, we report the efficient surface activity of stem bark saponins and the moderate surface activity of the leaf saponins. The reduction in surface tension caused by *J. curcas* saponins is slightly less than that of saponins extracted from other sources (Table 4).

By surface tension measurements, the CMC is obtained as a breakpoint in a graph of surface tension versus the logarithm of the saponin concentration curve. For both the leaf and stem bark NS solutions, a clear breakpoint was observed (Fig. 5) and the corresponding surface tension value at the CMC is defined as γ_{CMC}. The CMC and γ_{CMC} values obtained by the surface tension measurement method are listed in Table 3. For stem bark NS solution, a higher CMC (0.71 g/L) and lower corresponding surface tension (γ_{CMC} = 37.65 mN/m) are observed compared to leaf NS solution (CMC = 0.5 g/L and γ_{CMC} = 49.27 mN/m). A higher CMC indicates that a higher amount of stem bark saponin molecules are incorporated into the interfacial film at the air/solution interface [56]. The higher potential of stem bark saponins to reduce surface tension than leaf saponins is most probably due to delayed micellization in water [41]. Different CMC values for the leaf and stem bark NS solutions have been reported which may be because saponins derived from different parts of the same plant have varied chemical compositions and molecular structures [34]. Differences in the structure of aglycone, type and number of sugar units, alter the chemical composition of saponins and affects the surface properties of the molecules and hence the micellization phenomena [48]. Meanwhile, the presence of other bioactive metabolites in the crude saponin extracts also affects CMC [57].

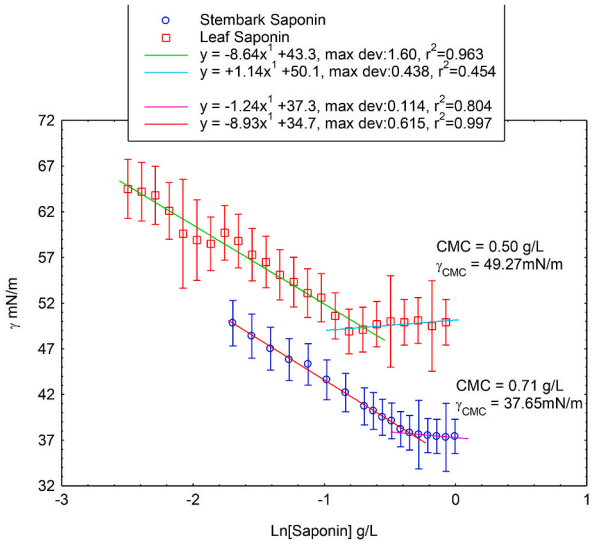

Fig. 5. Graph of surface tension as a logarithmic function of saponin concentration for leaf and stem bark NS solutions.

Table 3
Comparative table for CMC obtained by conductivity and surface tension method.

Saponin Source	CMC by conductivity method (g/L)	CMC by surface tension method (g/L)	Average	Standard deviation
Leaf saponin	–	0.50	0.50	–
Stem bark saponin	0.79	0.71	0.75	0.057

The CMC values for the stem bark saponins obtained by the conductivity method were compared with the results obtained from the surface tension measurements and were within the experimental error range. Hence, in further discussion, the average value of CMC will be considered i.e., 0.75 g/L for stem bark saponins. This agreement between the CMC values obtained by the two different methods provides strong evidence that saponins from the stem bark of *J. curcas* form micelles in an aqueous solution above a well-defined critical concentration. The CMC value of *J. curcas* saponin extract is in the range of the literature values reported for saponins extracted from other sources (Table 4).

Based on these results, we concluded that the CMC value of saponins varies depending on the source [13]. Because saponins from different plant sources have different molecular structures [17], ionic character and surface activity [60–62].

The π_{CMC}, Γ_{max}, A_{min}, ΔG°_{m} and ΔG°_{ads} calculated by using Eqs. (2)–(6) are presented in Table 5.

π_{CMC} is the measure of the effectiveness of a surfactant to decrease the surface tension of the water. A surfactant that reduces the surface tension of water the most is effective. This study reports stem bark saponins exhibit maximum effectiveness over leaf saponins.

Γ_{max} is an effective measure of adsorption at the air/solution interface. It estimates the number of saponins adsorbed per unit surface area at the air/solution interface. A_{min} provides information on the saponin molecules at the air/solution interface; the larger the minimum area, the more area is occupied by one saponin molecule at the interface [10].

The computed values of Γ_{max} for leaf and stem bark were 3.49 μmol/m^2 and 3.60 μl/m^2 with A_{min} values of 0.48 nm^2/molecule and 0.46 nm^2/molecule respectively. As per the value determined, a leaf saponin molecule occupies a large surface area than a stem bark saponin molecule at the air/solution interface. Hence, more amount of stem bark saponin molecules are adsorbed at the interface which is also confirmed by the large Γ_{max} value for stem bark saponins. There was no literature data for Jatropha saponins to compare. However, the values of A_{min} obtained for Jatropha saponins were in the range reported to that for Quillaja saponins (0.37–1.19 nm^2) [39]. Santini et al., 2019 reported slightly lower $\Gamma_{max} = 2 \pm 0.1$ μmol/m^2 for Quillaja saponins. Other literature data are also available for saponins from different sources: *Gleditsia sinensis* fruit saponins $\Gamma_{max} = 2.33$ μmol/m^2 and $A_{min} = 0.712$ nm^2 [63]. The reported A_{min} value for Chubak saponins (0.74 nm^2), purified saponins (0.58 nm^2) [38], Yucca saponins (0.87 nm^2) [10]. This difference in values might be due to the difference in the saponin structure from different sources and the different extraction protocols employed.

ΔG°_{m} denotes the spontaneity with which the micellization process occurs. The more negative ΔG°_{m} denotes greater spontaneity of micellization. Table 5 reports negative ΔG°_{m} values for both leaf and stem bark saponins in water. The ΔG°_{m} is found to be more negative for leaf saponins than stem bark saponins indicating more favorable micellization. The higher negative ΔG°_{ads} the value indicated the spontaneous adsorption of saponin molecules on the air-solution interface and was more favorable than micellization. Further adsorption is more favorable for stem bark saponins than leaf saponins at the air-solution interface. The greater the adsorption, the

Table 4
Comparison of crude saponin content, CMC and surface tension values of aqueous saponin solution extracted from different plant sources.

Scientific Name of plant source	Part used	Crude Saponin content (%)	CMC (g/L)	Surface Tension (mN/m)	References
Bellis perennis	Flower	24.5	0.076	36.8	[53]
Betula pendula	Leaf	26	0.240	45.7	[53]
Camellia oleifera	Seed		0.4	31.4	[55]
			0.5	30.01	[50]
		8.34			[58]
Chenopodium quinoa	Seed		3.3	31.06 ± 0.29	[52]
Equisetum arvense	Haulm	9.5	0.033	37.9	[53]
Glycine max	Seed		5	32.41 ± 0.08	[52]
Hedera sp.	Leaf		0.5	40	[13]
Malpighia emarginata	Fruit		5	33.09 ± 0.18	[52]
Panax ginseng	Root		0.830	38.921	[49]
Panax ginseng	Root		0.09		[59]
Quillaja saponaria	Bark		0.65		[8]
			0.51		[48]
			0.5–0.8		[20]
			0.5–0.7		[17]
Ruscus aculeatus	Root		0.756		[44]
Sapindus mukorossi	Fruit Pericarp	43.5	0.243	41.8	[53]
			0.45		[48]
	Pod		7.5	35.30	[41]
Tribulus terrestris	Fruit		0.589		[44]
Trigonella foenumgraecum	Fruit		0.904		[44]
Verbascum densiflorum	Flower	34.5	0.355	41.5	[53]
Zephyranthes carinata	Bulb		0.64	40.46	[41]

Table 5
CMC, γ_{CMC}, surface properties and thermodynamic parameters of leaf and stem bark saponins in water.

Surfactant	CMC (g/L)	γ_{CMC} (mN/m)	π_{CMC} (mN/m)	Γ_{max} (μmol/m^2)	A_{min} (nm^2/molecule)	ΔG°_{m} (kJ/mol)	ΔG°_{ads} (kJ/mol)
Leaf saponin	0.50	49.27	22.23	3.49	0.48	−18.84	−25.22
Stem bark saponin	0.75	37.65	33.85	3.60	0.46	−17.84	−27.23

maximum reduction in surface tension.

3.3.3. pH measurements

In surfactant evaluation studies, pH is an important parameter to consider. A surfactant solution with a pH of 5.5 does less harm to the skin and hair [41,55]. The nature of stem bark and leaf saponins was determined by measuring the pH of their NS solution. Table 6 shows the pH values of the NS solutions at various concentrations.

The pH of both of the NS solutions was found to be in the acidic range. They are reported to be weakly acidic owing to the hydrolysis of sugar units [55,64,65]. The pH of the stem bark NS solutions decreased with an increase in saponin concentration, thereby confirming the hydrolysis of sugars [48]. While the pH of leaf saponins decreased less prominently as compared to that of the stem bark saponins. This might be due to the limited hydrolysis of the glycosides as discussed in the earlier section. As the pH of both the NS solutions is found to be slightly below the range recommended for skin and hair, it may cause irritation on use.

3.3.4. Cleaning ability

The cleaning ability of NS solutions was investigated for concentrations ranging from 0.1 to 1 g/L at 25 ± 1 °C. The cleaning ability was determined in percentage and calculated by using the following formula:

$$\text{Cleaning ability} = \frac{W_2 - W_3}{W_2 - W_1} \times 100\% \tag{7}$$

where, W_1 = Initial weight of the cotton wool. W_2 = Weight of the cotton wool with the stimulated dirt. W_3 = Weight of the cotton wool after being cleaned with the NS solution.

The cleaning ability of both leaf and stem bark NS solutions is reported in Fig. 6. From the figure, it is clear that cleaning performance increases with an increase in saponin concentration, as expected and accelerates near the CMC. Because the micellar aggregates are formed in water once the CMC is crossed. The hydrophilic heads form the outer shell and the hydrophobic tails form the micelles' core. The hydrophobic filth and oil are retained and solubilized within the core of the micellar aggregates, making them water-soluble [66]. Stem bark saponins showed better cleaning ability (60.34% at 1 g/L) than leaf saponins (22.46% at 1 g/L), which may be because stem bark saponins reduced the surface tension of water sufficiently.

3.3.5. Foaming ability and foam stability

In this work, the foaming properties of NS solutions were investigated within concentrations ranging from 0.1 to 1 g/L at a temperature of 25 ± 1 °C. The maximum foam height obtained immediately after shaking gave foaming ability which is represented by the column graph in Fig. 7(a) and (b). The stem bark saponins were ranked the highest for foam height at 1 g/L concentration of the solutions. The foam height of stem bark and leaf NS solutions were 3.5 cm and 1.8 cm respectively. The foam height of leaf saponins was 51.4% of stem bark saponins. Further stem bark saponins produced more dense foam than the leaf saponins. This might be due to the greater solubility of stem bark saponins in water [41]. Foam height increased gradually with concentration. So, stem bark saponins were found to exhibit better foaming ability than leaf saponins as depicted in Fig. 8.

Foam stability was expressed in terms of the R_5 parameter and calculated using the following equation [67]:

$$R_5 = \frac{h_5}{h_0} \times 100\% \tag{8}$$

where, h_0 = Maximum foam height attained. h_5 = Foam height after 5 min.

Fig. 9 represents the foam stability of leaf and stem bark saponins in terms of the R_5 parameter. At 0.1 g/L concentration of the solution, the foam stability of stem bark saponins and leaf saponins was 25% and 12.5%, respectively. Foam stability increased initially

Table 6
pH of NS solutions at different concentrations.

S·N	Concentration (g/L)	Leaf NS solution	Stem bark NS solution
1.	0.1	5.17 ± 0.01	5.15 ± 0.01
2.	0.2	5.16 ± 0.01	5.13 ± 0.01
3.	0.3	5.15 ± 0.02	5.04 ± 0.01
4.	0.4	5.15 ± 0.01	4.97 ± 0.01
5.	0.5	5.13 ± 0.01	4.91 ± 0.01
6.	0.7	5.12 ± 0.01	4.87 ± 0.03
7.	0.9	5.10 ± 0.02	4.83 ± 0.02

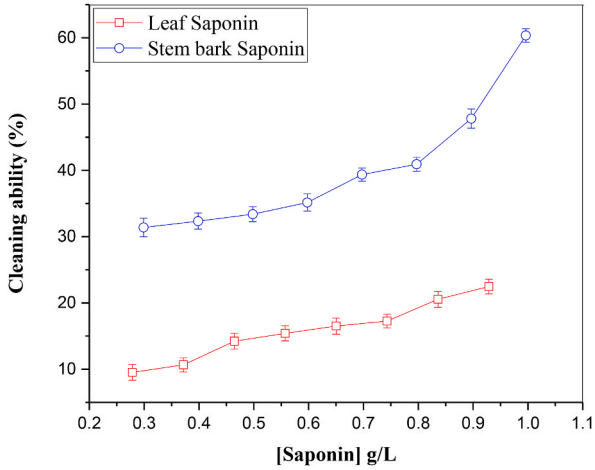

Fig. 6. Graph of cleaning ability as a function of saponins concentration.

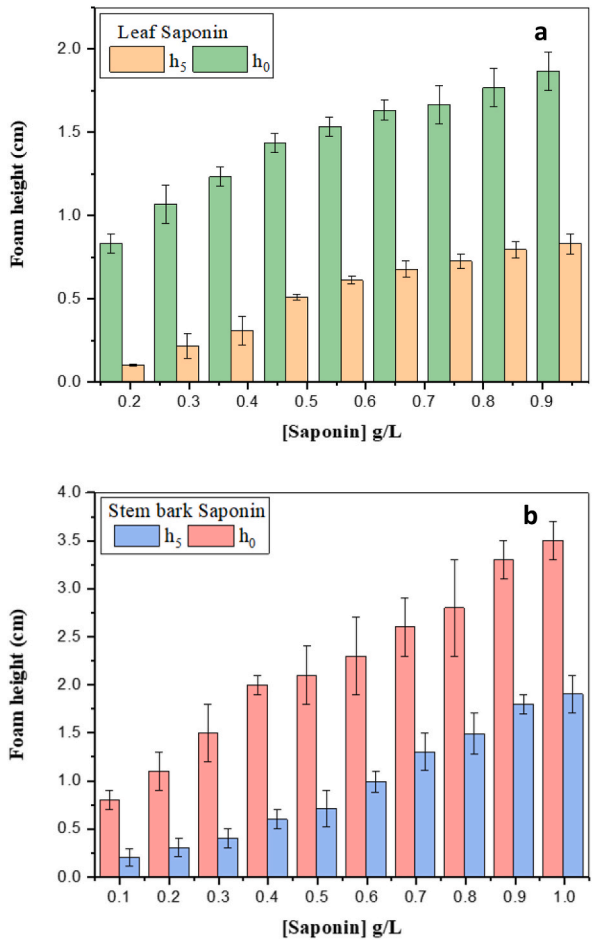

Fig. 7. Foaming height of leaf saponins (a) and stem bark saponins (b) at different concentrations (h_0 = maximum foam height and h_5 = foam height after 5 min).

with concentration for both saponins due to a decrease in surface tension. However, after CMC was achieved, it remained almost constant at 53.6% and 40.6%, respectively, for stem bark and leaf saponins. It is because the surface tension of the solution does not change further and the surface of the system remained relatively unchanged [68]. Foam is a thermodynamically unstable system. So,

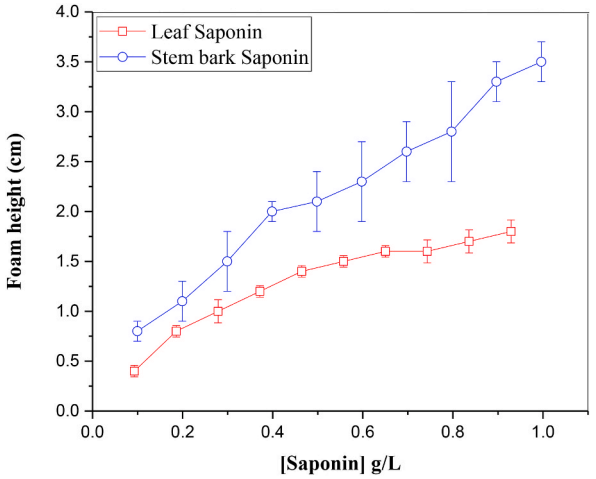

Fig. 8. A comparative plot of the foaming ability of leaf and stem bark saponins at different concentrations.

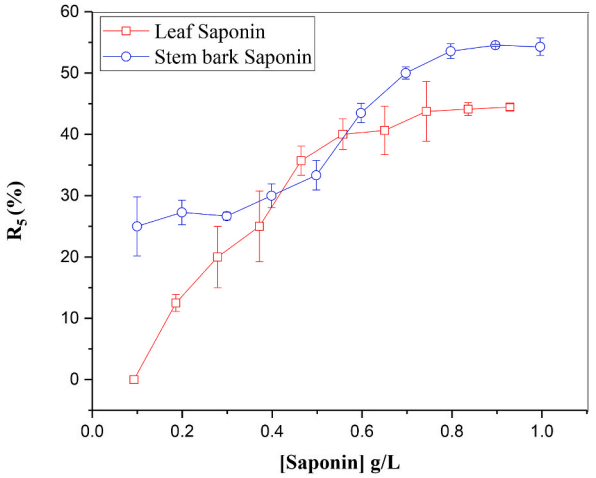

Fig. 9. R_5 value as a function of saponin concentration.

the foam breakdown occurs continuously throughout the whole foaming process. Beyond CMC, an equilibrium is maintained between the foaming and foam breakdown phenomenon. Hence the foamability of the solution doesn't increase anymore [50] Foams with R_5 value of 50% are considered metastable, whereas R_5 values lower than 50% indicate low stability [67]. Stem bark saponins produced metastable foam while leaf saponins produced relatively less stable foam.

4. Conclusions

In this study, *Jatropha curcas* leaves and stem bark were investigated as a new source of natural surfactants (saponins). Various physicochemical properties were investigated for characterizing the micellization behavior of saponin in aqueous solution, cleaning and foaming abilities. Our results showed that the stem bark extract provides good foaming power and distinct cleaning ability. It cleans well by reducing surface tension sufficiently. The results also showed that saponins are weakly acidic with a pH slightly lying below the range recommended for use in skin and hair. So, its direct use must be avoided. Stem bark saponins showed greater cleaning and foaming ability than leaf saponins. It also produced stable foam as compared to leaf saponins. Hereby we conclude that stem bark saponins from *J. curcas* have good surfactant properties while leaf saponins have moderate surface activity. Jatropha saponins are biodegradable and renewable compared to synthetic surfactants. Hence, plant-based surfactants extracted from *J. curcas* be used as an alternative to synthetic surfactants. This study contributes to the identification of a new saponin source for the development of formulations for biomedical and industrial applications to reduce the use of synthetic surfactants in commercial products. We propose using this plant-based surfactant to create these novel biodegradable and renewable alternatives. In this work, crude saponins extract was used directly instead of chemically isolated pure saponins. This research is preliminary, and more detailed studies with chemically pure saponins extracts are recommended for better insights and understanding. More research into the extraction and characterization

of saponins from *J. curcas* along with detailed studies regarding the processing to remove toxic compounds will help to improve its commercial application.

Author contribution statement

Summi Rai: Conceived and designed the experiments; Performed the experiments, Analyzed and interpreted the data; Wrote the paper.

Ananda Kafle, Hari Prasad Devkota: Analyzed and interpreted the data; Wrote the paper.

Ajaya Bhattarai: Conceived and designed the experiments; Contributed reagents, materials, analysis of tools or data; Wrote the paper.

Data availability statement

Data are included in the article. The datasets produced and/or analyzed during the study can be obtained from the corresponding author upon request.

Disclaimer

The findings and views presented in this paper are solely those of the authors and do not necessarily represent the views of the Water Resource Research and Development Centre or any affiliated organizations.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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