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Antibacterial and Antioxidant Activities of Aqueous Extract of Soapnuts (*Sapindus mukorossi*)

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Abstract

As an alternative to chemical surfactants, the biosurfactants obtained from plants are renewable, biocompatible, biodegradable, less toxic and less expensive. In the present report, the aqueous extract prepared from the pericarps of soapnuts fruits is employed, which are known to exhibit a myriad of biological properties. The extract was characterized using Fourier transform infrared spectroscopy (FTIR) and zeta analyzer for identifying the functional groups and surface charge, respectively. The extract showed abundance of saponins, triterpenoids, flavonoids and negative charge of –8.9 mV. The free radical scavenging and antibacterial activities of the extract were evaluated with DPPH scavenging and well diffusion assays. The DPPH scavenging (%) increased with an increase in extract concentration and showed a significant radical scavenging potential of 85.3% at a concentration of 250 µg/mL. The extract didn't show antibacterial action on Gram–negative bacteria at the selected concentrations. But, it demonstrated significant inhibitory action on Gram–positive bacteria; *Bacillus subtilis* and *Micrococcus luteus* with inhibition zones of 4.0 mm and 12.5 mm at 43.75 mg of crude saponins, respectively. Thus, the green extract used in the present study finds its application as a natural, antibacterial and antioxidant biosurfactant in cosmetic and food industries, as a substitute to chemical surfactants.

Key words: Antibacterial: Antioxidant: Biosurfactant: Saponin: Soapnut

Introduction

The tree *Sapindus mukorossi* (Sapindaceae family) is generally known soapnut, soapberry, washnut, *kunkudu, reetha* etc. The fruits of the tree are extensively used in Asian countries for bathing, washing hair, silk and woolen clothes; kitchen utensils, and polishing tarnished gold and silver ornaments due to its excellent cleansing activity. The pericarps of the fruit are traditionally used in Ayurvedic and folk medicine for curing epilepsy, eczema, psoriasis, migraine etc. The main phytoconstituents of the fruit are saponins (10–11.5%), sugars (10%) and mucilage. The saponins present in the pericarps, main constituent of the aqueous extract are non–ionic glycosides containing sugars such as D–glucose, D–xylose, L–arabinose, L–rhamnose, and glucuronic acid. The saponins are classified under triterpenoidal saponins and mainly of three types i.e. oleanane, dammarane and tirucullane (Fig. 1). The constituent saponin acts as natural surfactant and classified under biosurfactant category. Also, the vitamins present in extract such as A, D, E, and K acts as natural conditioner and impart shine and smoothness to hair, after application. (Suhagia et al., 2011; Upadhyay and Singh, 2012; Yang et al., 2010).

It is significant to note that the extracts of soapnut fruit is known to possess a myriad of

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biological properties including hepatoprotective, antiinflammatory, anxiolytic, antiplatelet aggregation, anticancer, antiprotozoal, anti trichomonial, antifungal, antibacterial, free radical scavenging, spermicidal, piscicidal and molluscicidal activities (Ibrahim et al., 2006; Köse and Bayraktar, 2016; Suhagia et al., 2011; Upadhyay and Singh, 2012). As the extract is insecticidal, it is traditionally used for the killing and removal of *Pediculus humanus*, a human lice that infects the scalp (Suhagia et al., 2011; Upadhyay and Singh, 2012). Also, it is known to cure bacterial and fungal based scalp infections, including dandruff by its antidermatophytic activity (Tamura et al., 2001). The saponin containing aqueous extract exhibits functional properties such as superior emulsification activity in comparison with synthetic surfactant sodium dodecyl sulfate. Thus, implying its utilization as a commercial, economical biosurfactant (Ghagi et al., 2011).

Biosurfactants are biological substitutes, derived from plants, bacteria and fungi and typically used as emulsifiers, deemulsifiers, wetting and foaming agents, functional food ingredients and detergents. There are many advantages of plant derived biosurfactants in comparison with the chemical surfactants. They are renewable in nature, sustainable, easily available, less expensive, biocompatible and biodegradable under both aerobic and anaerobic conditions. They also exhibit lower human toxicity and allergenicity; less toxic to environment and helps in remediation of various hydrophobic contaminants in water and soil. Also, their production does not deplete the existing limited petroleum resources (Ghagi et al., 2011; Rao and Paria, 2009; Vijayakumar and Saravanan, 2015).

While, most of the personal care and household products such as soaps, shampoos, body lotions, dishwashing soaps, cosmetics, varnishes, paints, inks etc contain 1, 4–dioxane. It is a byproduct formed during the manufacturing of commercial chemical surfactant/detergent, sodium lauryl sulfate by ethoxylation process. It is volatile, irritant and listed under probable human carcinogen by Environmental Protection Agency (EPA). Notably, the soapnuts are natural and its utilization as a biosurfactant is known for centuries all over the world, widening its application in cosmetic, pharmaceutical, petrochemical, mining, metallurgical, agrochemical and food industries. In this perspective, an attempt has been made to study the antibacterial and free radical scavenging potential of the aqueous soapnut fruit extract. Thus, the study paves a way for finding its application as a natural, antibacterial, antioxidant biosurfactant in our day to day life, an alternate to chemical surfactants.

Materials and Methods

Soapnut fruits were obtained from the local market. Absolute ethanol (Shymlakhs International, London, UK), 1, 1 diphenyl picryl hydrazyle (DPPH) (Thomas Baker Chemicals Pvt. Ltd, Mumbai, India), streptomycin sulphate (Sigma–Aldrich, Bengaluru, India), nutrient broth and Mueller Hinton agar (HiMedia Chemicals Pvt. Ltd, Mumbai, India) were used during this study. The medium nutrient broth made up of sodium chloride (5 g/L), yeast extract (1.5 g/L), peptone (5 g/L) and beef extract (1.5 g/L). The Mueller Hinton agar (pH 7.4 ± 0.2) composed of starch (1.5 g/L), beef extract (2 g/L), casein acid hydrolysate (17.5 g/L) and agar (20 g/L). All the solutions were prepared in ultra pure water of 18.2 MΩ–Cm resistivity; produced from Elga Purelab Flex 3 water polishing unit (High Wycombe, England). At 121ºC for 20 min, glassware, plasticware and media used in the present study were sterilized in Obromax vertical autoclave (Delhi, India).

Preparation of aqueous extract of soapnut pericarps: The soapnut fruits were dried at 50ºC in Osworld JRIC–7/A laboratory hot air oven (Mumbai, India) for 24 h. The acquired pericarps after removal of the seeds were pulverized into fine powder using Prestige Deluxe–Vs mixer grinder (Bengaluru, India) and sieved (Jayant Scientific Industries, Mumbai, India) to obtain a

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particle size of 300 µm. The collected powder was stored in an air tight container at room temperature. A 15% (w/v) aqueous solution of the pericarps in ultrapure water was prepared by continuous stirring with Tarson Spinit magnetic stirrer (Kolkata, India) at room temperature for 2.5 h. The extract was centrifuged using Remi R–24 Research centrifuge (Mumbai, India) at 8, 000 rpm for 10 min and the obtained supernatant was filter sterilized with sterile Millipore 0.22 µm syringe filter (Bengaluru, India). Thus, the obtained aqueous extract was used for all the studies (Fig. 2).

Characterization of the aqueous soapnut extract: The zeta potential of the extract was determined with Malvern Zetasizer Nano ZS90 (Malvern, UK). Using Labconco Freezone 4.5L Plus benchtop cascade freezedry system (Kansas City, USA), the aqueous extract solution was made into powder. The IR spectrum of the lyophilized powder was recorded at a wave number range of 1000–4000 cm^{-1} with Bruker Optics TENSOR 27 FTIR spectrometer (Ettlingen, Germany),

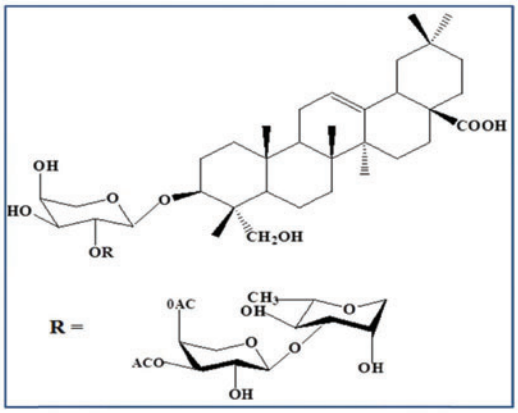

Fig. 1. The typical structure of a saponin from *Sapindus mukorossi* (Rao and Paria, 2009)

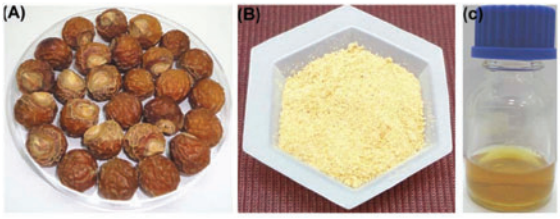

Fig. 2. Digital photographs showing the soapnut (a) dried fruits, (b) powder and (c) aqueous extract (15%)

DPPH scavenging activity: The antioxidant activity of the extract was investigated by scavenging (1'–1' diphenyl picryl–hydrazyle) (DPPH). The DPPH is a purple coloured, stable free radical. The solutions of 100 µM DPPH prepared in absolute ethanol were mixed with 62.5–100 µg/mL concentration of the extract and incubated in dark at room temperature for 60 min. The ascorbic acid solution (50 µg/mL) and water were used as positive and negative controls, respectively. The absorbance was noted at 520 nm with Analytic Jena AG Specord 200 Plus UV–visible spectrophotometer (Jena, Germany). The DPPH scavenging (%) by applying the equation: DPPH scavenging (%) = (DPPH absorbance–sample absorbance/DPPH absorbance) × 100 (Kora and Rastogi, 2018). The experiment was carried out in triplicate and the values were expressed as mean ± SD.

Antibacterial activity: The antibacterial activity of the extract was checked with well diffusion method. American Type Culture Collection (ATCC) strains, *Escherichia coli* 25922 and *Pseudomonas aeruginosa* 27853; and *Bacillus subtilis* 6633 and *Micrococcus luteus* 10240 were used as representive test strains for Gram–negative and Gram–positive bacteria, respectively. The Mueller Hinton agar plates were inoculated with bacterial suspension by spread plate method. The suspension was prepared from the nutrient broth grown overnight culture by turbidity adjustment to 0.5 McFarland standard. The aqueous extract containing 12.5, 25, 37.5 and 43.75 mg of crude biosurfactant were added to the 8 mm diameter wells made in the solid agar medium. The negative and positive control wells were maintained with water and the antibiotic streptomycin (10 µg), respectively. The plates were incubated at 37ºC

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for 24 h in Remi CIS–24 Plus bacteriological incubator (Mumbai, India). The inhibition zone was calculated by deducing the well diameter from the total inhibition zone diameter. The average value was obtained from the three independent experiments carried out with each bacterial strain (Kora and Rastogi, 2018).

Results and discussion

Fourier transform infrared spectroscopy (FTIR): The IR spectrum of the lyophilized aqueous extract was noted for identifying the functional groups of the biomolecules present in the soapnut pericarps (Fig. 3). The major absorbance bands in the spectrum are at 3343, 2924, 2855, 2130,

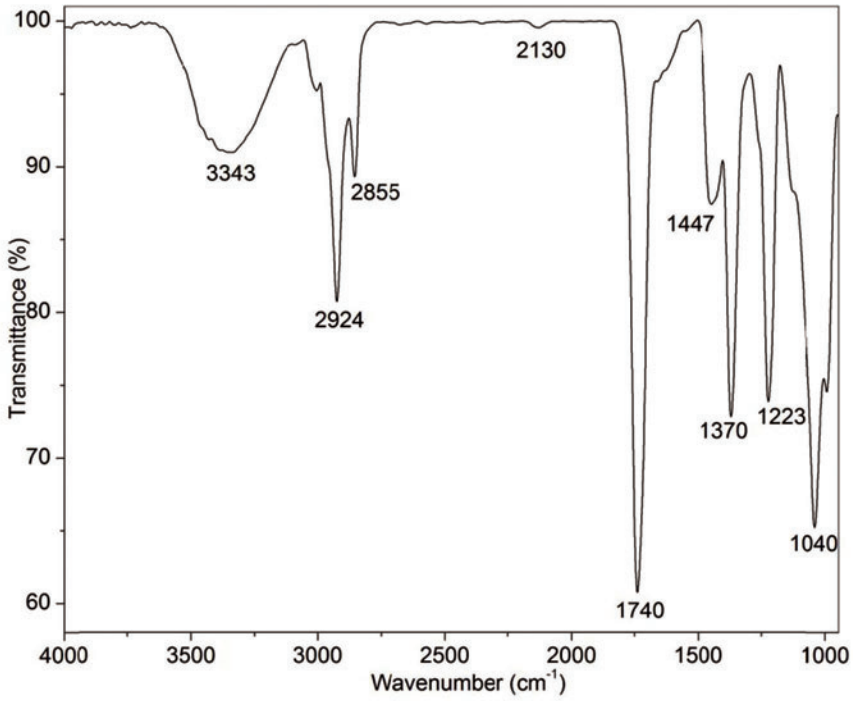

Fig. 3 The FTIR spectrum of freeze dried powder of the soapnut aqueous extract

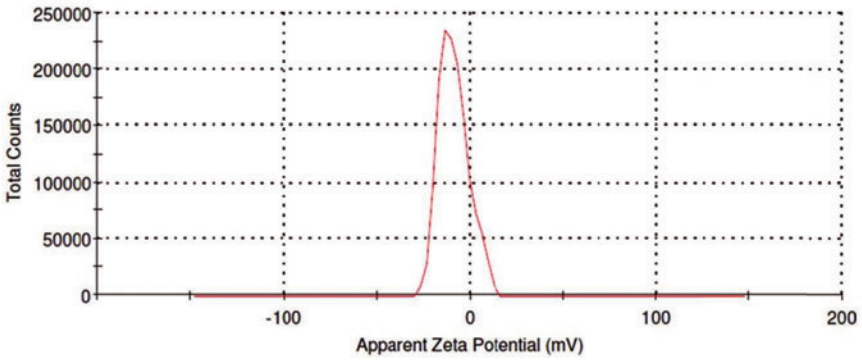

Fig. 4 The zeta potential distribution of the soapnut aqueous extract

1740, 1447, 1370, 1223 and 1040 cm^{-1}, respectively. The broad band observed at 3343 cm^{-1} could be assigned to stretching vibrations of hydroxyl groups in alcohols and phenolics. The bands at 2924 and 2855 cm^{-1} correspond to respective asymmetric and symmetric stretching vibrations of methylene groups. The broad band at 2130 cm^{-1} arises from various carbonyl species. The peak at 1740 cm^{-1} could be assigned to carbonyl stretching vibrations in aldehydes, ketones and carboxylic acids. The symmetrical stretch of carboxylate group can be attributed to the bands present at 1447 and 1370 cm^{-1}. The peaks at 1223 and 1040 cm^{-1} correspond to C–O stretch of phenolic and alcoholic groups, respectively. Hence, the distinctive peaks observed in the spectrum denote the abundance of various bioactive molecules such as saponins, triterpenoids, flavonoids, fatty acids etc in the fruit extract (Du et al., 2014; Sharma et al., 2013; Suhagia et al., 2011). The aqueous extract exhibited a zeta potential value of –8.9 mV (Fig. 4). The negative charge of the extract is due to the presence of various saponins in the extract (Rao and Paria, 2009).

Antioxidant activity*:* The antioxidant capacity of the extract was quantified with DPPH scavenging assay (Fig. 5). With time, the colour of the DPPH solution containing the soapnut extract slowly changed from purple to pale yellow (Inset of Fig. 5). The DPPH scavenging (%) increased from

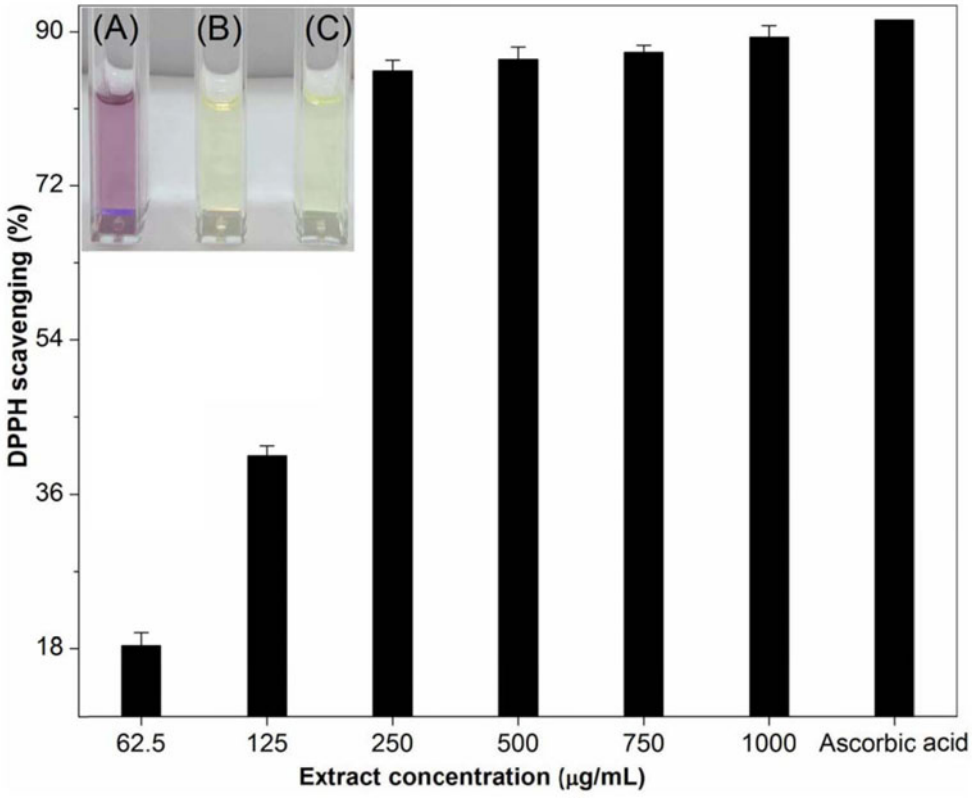

Fig. 5 The DPPH radical scavenging activity of soapnut aqueous extract at different concentrations (62.5–1000 µg/mL). Inset: DPPH solution colour (a) before and after treatment with (b) soapnut extract (250 µg/mL) and (c) ascorbic acid (50 µg/mL)

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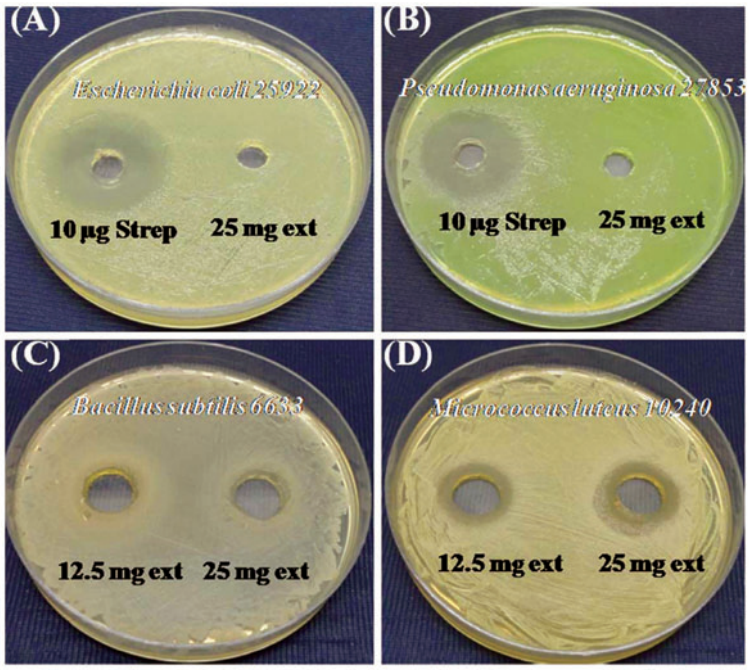

Fig. 6. Bacterial culture plates showing the inhibition zones around the wells loaded with different amounts of soapnut extract and streptomycin, (a) *Escherichia coli* 25922, (b) *Pseudomonas aeruginosa* 27853, (c) *Bacillus subtilis* 6633 and (d) *Micrococcus luteus* 10240

18.1–85.3% with an increase in extract concentration from 62.5-250 µg/mL. Further, the value was marginally increased and reached 89.3% at 1000 µg/mL. Whereas, the positive control ascorbic acid at 50 µg/mL concentration showed a scavenging activity 91.3%. The negative control water did not show any colour change from purple. The extract demonstrated significant radical scavenging potential at a concentration of 250 µg/mL. Thus, the extract is meeting the requirements of an effective antioxidant. The exhibited antioxidant activity of the extract could be attributed to the reducing and chelating properties of the different complex biomolecules of the extract, including saponins, phenolics and flavonoids (Bahri-Sahloul et al., 2014; Kora and Jayaraman, 2012).

Antibacterial activity*:* The well diffusion assay was used for evaluating the antibacterial activity of the extract. The results showed that there was no antibacterial action on Gram–negative bacteria at the selected concentrations of the extract. While, the extract demonstrated significant inhibitory action on Gram–positive bacteria (Table 1). For *B. subtilis*, the inhibition zones ranged from 2.5-4.0 mm in the range of 12.5–43.75 mg of crude saponins. In the case of *M. luteus*, the inhibition zones were higher and ranged from 8.0–12.5 mm. Interestingly, the highly inhibited *M. luteus* strain is a part of the normal flora of the mammalian skin and commonly colonizes on mucosal tracts of humans. As expected, the positive control streptomycin showed higher inhibition action on all the test strains at 10 µg, ranged from 24.6–30.0 mm.

These results are in accordance with earlier study carried out with polyphenolic rich callus culture extracts of *Crataegus azarolus* L. var.

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Table 1. The inhibition zones observed with different bacterial culture plates loaded with soapnut extract and streptomycin

Test compound	Zone of inhibition (mm)*			
	***E. coli* 25922**	***P. aeruginosa* 27853**	***B. subtilis* 6633**	***M. luteus* 10240**
Extract (12.5 mg)	0	0	2.5 ± 0.7	8.0 ± 0
Extract (25 mg)	0	0	3.5 ± 0.7	9.0 ± 0
Extract (37.5 mg)	0	0	3.8 ± 0.5	10.0 ± 0
Extract (43.75 mg)	0	0	4.0 ± 0	12.5 ± 0.7
Streptomycin (10 µg)	29 ± 0	24.6 ± 1.5	28.3 ± 0.5	30.0 ± 0

*Values are mean ± SD (n = 3).

aronia. The differential activity of the extract towards Gram–positive and Gram–negative bacteria is based on the difference in the cell wall structure. The hydrophilic outer membrane of the Gram–negative bacteria acts as a permeability barrier, thus exhibits resistance towards antibacterial compounds. Whereas, the lipophilic cell wall of Gram–positive bacteria facilitates the penetration of hydrophobic compounds. The inhibition of Gram–positive bacteria by aromatic compounds could be due to the inhibition of enzyme production, cell wall deterioration and cell lysis (Bahri-Sahloul et al., 2014). Previous study on antimicrobial activities of pericarp saponins showed moderate growth inhibitory action only on Gram–positive bacteria, but not against Gram–negative bacteria (Tamura et al., 2001). The data is further supported from the earlier reported study on preservative efficacy of crude saponin extract. It is reported that the extract is an effective preservative against Gram–positive *Staphylococcus aureus*, but ineffective against Gram negative *E. coli* (Yang et al., 2010).

The current study employs the aqueous extract obtained from the pericarps of soapnuts as an antioxidant and antibacterial agent. The saponins present in the extract act as renewable, biocompatible, biodegradable and non–toxic biosurfactant. The IR analysis reveals the abundance of various bioactive molecules such as saponins, triterpenoids, flavonoids, fatty acids etc in the extract. The extract exhibited significant radical scavenging potential at 250 µg/mL concentration, thus qualifies as an effective antioxidant. Also, the extract showed inhibitory action on Gram–positive bacteria. The results of the present report highlights the applications of the soapnut extract in various fields including cosmetics and food. It has potential as a functional food additive/supplement during chemotherapy and antibiotic treatment. It could be utilized as a substitute for the chemical surfactants in conditioners and hand wash for effective control of skin bacterial flora, with no side effects.

Ethics Statements

Not applicable.

Conflict of interest

The author declares no conflict of interest.

Acknowledgement

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