

Study of functional properties of *Sapindus mukorossi* as a potential bio-surfactant

Rupeshkumar Ghagi¹, Surekha K. Satpute², Balu A. Chopade² and Arun G. Banpurkar¹

¹Center for Advanced Studies in Materials Science and Condensed Matter Physics, Dept. of Physics;

²Dept. of Microbiology, Institute of Bioinformatics and Biotechnology, University of Pune, Pune -411 007, India
agb@physics.unipune.in

Abstract

Functional properties such as critical micellar concentration, emulsification and hemolytic activity of raw ritha (*Sapindus mukorossi*) aqueous solution have been investigated. Commercially available ritha fruits were soaked overnight to prepare concentrated stock solution. Critical micellar concentration was found to be 0.017 gm/cc (1.7 wt %) at which the surface tension of aqueous solution remained constant to minimum value of 38 mN/m. Emulsification activity for water-kerosene and various plant oils was found to be excellent in comparison with synthetic surfactant like sodium dodecyl sulfate (SDS). These functional properties are comparable to the saponin composite, which is chemically extracted from ritha hence crude ritha could be used as an economical bio-surfactant.

Keywords: Biosurfactant, *Sapindus mukorossi*, ritha, saponin, sopanut, plant.

Introduction

There is a growing interest in the natural and green surfactants due to its excellent functional properties and being biologically and environmentally safe as well as ecologically adaptable (Fiechter, 1992; Desai & Banat, 1997; Dembitsky, 2004). These surfactants can be mainly obtained from microorganisms like bacteria, yeast, fungi (Kitamoto *et al.*, 1990; Desai & Banat, 1997; Kokare *et al.*, 2007) and plants (Ishigami & Suzuki, 1997). Saponin is one of the most commonly known plant based surfactants. Saponin is largely found in plants like *Sapindus mukorossi*, soyabean (Berhow *et al.*, 2000), Quillaja bark (Mitra & Dungun, 1997) and *Fagonia indica* (Shaker *et al.*, 1999). The nut obtained from trees of *Sapindus mukorossi* and *Sapindus emarginatus* is commonly known as 'sopanut' or 'ritha'. These trees are found in the different regions of India, Pakistan and other tropical and sub-tropical regions of the world. However, *Sapindus mukorossi* is abundant in the most part of northern India. This belongs to the main plant order Sapindaceae and family Sapindeae. The fruits pericarp contains saponin which is known for the surfactant action. The saponin content in the ritha varies from 6 to 10 wt % (Kommallpatti *et al.*, 1998). Saponin is also widely used in the pharmaceutical industries (Robber & Tyler, 1996; Edeoga *et al.*, 2006), detergents (Cheeke, 1999) and environmental remediation (Urum & Pekdemir, 2004). There are several reports on the study of the saponin, extracted from ritha. Rao *et al.* (1992) has presented basic chemical method for the extraction of saponin. Row and Rukmini (1996) have studied the chemical properties of saponin (Row & Rukmini, 1996). Urum and Pekdemir (2004) have studied the ability of aqueous saponin solution as a biosurfactant for applications in washing and crude oil contaminated soil. Zhang *et al.* (1998) have observed the excellent biodegradability and contaminated soil washing property of ritha over the synthetic

surfactants. Very recently Balakrishnan *et al.* (2006) have studied functional properties of aqueous *Sapindus* saponin solution, like critical micellar concentration (CMC), aggregation number, solubilisation of crude oils etc.

Most of these reports are mainly on the properties of pure saponin chemically extracted from either ritha or other sources (Mitra & Dungun, 1997; Ishigami & Suzuki, 1997; Shaker *et al.*, 1999; Berhow *et al.*, 2000). It is well known that ritha in the form of powder or organic extracts are being used as one of the major ingredients in the herbal products like bath soaps, fabric washing, hair dyes, shampoo, body-care lotions etc. In a recent report, Ibrahim *et al.* (2006) have studied anti-microbial activity against *H. pylori* using extracts from ritha. In view of these applications, it is interesting to study the functional properties of crude ritha as an economically viable bio-surfactant. We considered basic aqueous solution of 'ritha', as crude bio-surfactant for analyzing the various functional properties viz. surface tension, CMC, emulsification and hemolytic activity. The present study showed that crude bio-surfactant from ritha maintains similar functional properties as a chemically extracted and purified saponin.

Material and methods

Sample collection & processing

We used commercially available, well dried ritha fruits of *Sapindus mukorossi* for preparation of aqueous solution. In the beginning ritha pericarp was separated from hard seed then 20 gm of pericarp was soaked overnight in 200 ml double distilled water at 23°C. This mixture was further vortexed using magnetic stirrer for 2 h at room temperature and then filtered using stainless steel sieve (pore size = 58 µm). The ritha concentration in this solution was determined on the basis of the ratio of weight of ritha pericarp to that of water volume. Thus the

concentration of ritha in the above mentioned solution becomes 0.1 gm/cc or 10 wt %. This solution was used for further studies and henceforth referred as ritha-a. This solution was further subjected to centrifugation at 5,000 rpm for 20 min and at room temperature to get suspended free solution and henceforth referred as ritha-b solution. These aqueous solutions were found to be acidic, pH= 4.6.

Critical micelle concentration (CMC) measurement

The CMC for ritha solution was estimated by employing Wilhelmy plate tensiometer (DCAT 11), from Dataphysics, Germany. Surface tension of double distilled water was measured using Wilhelmy plate (platinum-iridium plate-PT 11). Then a controlled volume of ritha-a solution was added using calibrated micropipettes (20-200 μ l, 200-1000 μ l) into the double distilled water. This solution was stirred using Teflon needle for 60 sec duration and then allowed to equilibrate for 5 min before the surface tension measurement. The concentration of aqueous ritha solution was increased gradually by adding stock solution and surface tension was measured. The CMC was determined by noting the concentration above which the surface tension remained constant to the minimum value. The CMC using ritha-b solution as a mother solution was also determined.

Determination of emulsification activity with kerosene

The aqueous solutions viz. *ritha-a*, *ritha-b* and *ritha-c* solution at CMC (referred as *ritha-c*), were tested separately for the emulsification activity. This activity was checked by adding 1 ml of *ritha-a* solution in 4 ml of water and insoluble 6 ml of kerosene. Further, this mixture was vortexed vigorously for 2 min to obtain maximum emulsification. Same procedure was followed to determine emulsification activity for *ritha-b* and *ritha-c* solutions. We also studied emulsification activity of aqueous SDS at CMC concentration (8.1 mM), henceforth we referred as SDS-c. After 48 h of settling down time, emulsification index was calculated by measuring percentage emulsion layer height i.e. ratio of height of emulsion layer to total height of liquid column (Ellaiah *et al.*, 2002). The results were obtained by averaging more than 3 realizations.

Emulsification assay with plant oils

Emulsification activity to the various plant oils in water medium is also tested. In this case 3 ml of *ritha-a* solution was mixed with 0.5 ml of plant oils (coconut, mustard, soyabean, almond, castor, sunflower & olive) separately. It was vortexed vigorously for 2 min and incubated at room temperature for 1 h without disturbance for separation of aqueous and oil phase. Aqueous phase was removed carefully with the help of 1 ml micropipette and absorption was measured. Same procedure was followed for *ritha-b*, *ritha-c* and SDS-c solutions. Ritha solution without any oil was taken as a blank. Absorbance of aqueous phase was measured by using spectrophotometer (UV1601 Shimadzu

Corporation, Japan) at wavelength of 400 nm. Emulsification activity per ml (EU/ml) was calculated by using the formula:

Emulsification unit = $0.01 \times$ dilution factor (Patil & Chopade, 2001). Such experiments were repeated more than thrice and mean value of EU was considered for activity.

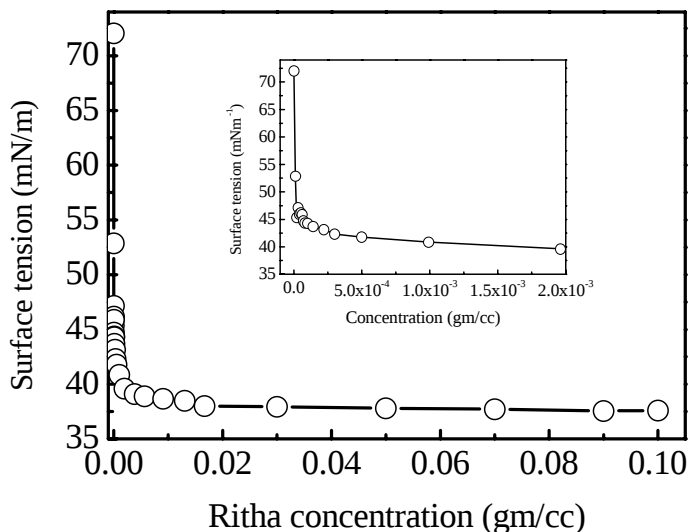
Hemolytic activity

Nutrient agar, containing (g/l) beef extract: 1.0, yeast extract: 2.0, peptone: 5.0, NaCl: 5.0, agar: 15, supplemented with 5% fresh whole human blood (Mulligan *et al.*, 1984; Carrillo *et al.*, 1996) was used to determine the hemolytic activity of all three ritha solutions (*ritha-a*, *ritha-b* & *ritha-c*). 10 μ l of each sample was loaded on blood agar plate and incubated at room temperature for 48-72 h. Observations were done using digital photographs for hemolytic activity.

Results and discussion

The decrease in the surface tension of the aqueous solution as a function of ritha concentration is plotted in Fig. 1. It can be seen that the surface tension of aqueous surfactant solution decreases rapidly with increase in ritha concentration. For pure water it is 72 mN/m and saturates to minimum value of 38 mN/m when ritha concentration becomes 0.017 gm/cc (1.7 wt %). Both *ritha* solution (*ritha-a* & *ritha-b*) shows a same surface tension activity. Thus CMC for the crude *ritha* was found to be 0.017 g/cc, or 1.7 wt %. Considering the fact that pericarp contains 6-10 wt % saponin (Kommelpatti *et al.*, 1998). The CMC value for crude *ritha* is rather close to the reported value for chemically purified *Sapindus* saponin (Balakrishnan *et al.*, 2006). Bio-surfactant from *ritha* (soap nuts) contains mainly saponin which is responsible for its various functional properties (Row & Ruckmini, 1966; 1996a). It is clear that the crude *ritha*

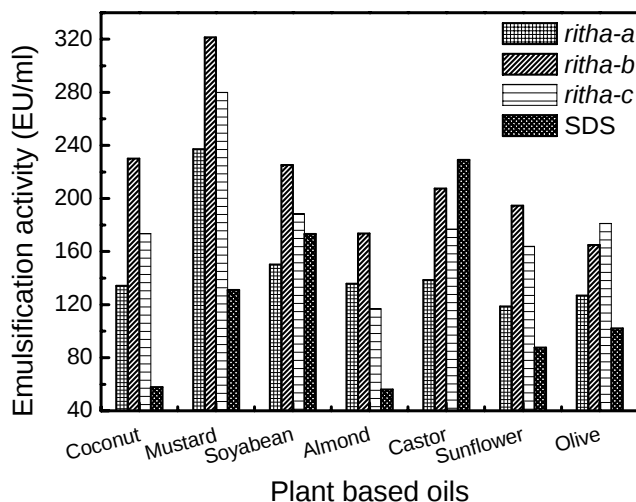
Fig. 1. Variation of the surface tension of aqueous solution with ritha concentration (Inset shows enlarged plot near CMC).



maintains CMC value, which is an essential functional property of a surfactant and could be used as an economical bio-surfactant. Nevertheless, extensive research work has been done on the properties of saponin complex derived from ritha and other plants (Balakrishnan *et al.*, 2006).

Emulsification activity is one of the important properties of a potent bio-surfactant. Due to amphiphilic nature of biosurfactant it can solubilize water insoluble substance/hydrocarbons (Balakrishnan *et al.*, 2006). Therefore, we observed emulsification index for ritha solutions. Emulsification index was tested for all 3 ritha and aqueous SDS-c solutions with kerosene. Emulsification activity for all 3 ritha solutions was approximately 67%. It is also seen that emulsification activity of ritha with kerosene is comparable to that of SDS-c solution, which is also 67%. Hence, ritha proves to be a good substitute for emulsification in comparison with synthetic surfactants. Fig. 2 shows a variation in the emulsification activity using 3 ritha and SDS-c solutions for different plant oils. Excellent emulsification activity was shown by all 3 ritha solutions with the tested oils. These solutions show a highest activity for mustard oil followed by castor, soyabean and coconut oil. Among 3 ritha solutions, ritha-b demonstrates superior emulsification activity. It is important to note that SDS-c solution exhibits least activity for coconut, almond, sunflower and olive oils. Relatively good activity was observed for castor and soyabean oils. This is the first report dealing with the emulsification activity of ritha solutions with respect to the various plant oils. Being a plant surfactant ritha emulsified various plant oils. In addition to the above mentioned properties, we also studied hemolytic activity of all ritha and SDS-c solutions. All 3 ritha solutions have lysed red blood corpuscle (RBC), leading the medium around the suspension colourless and thus demonstrated β -hemolytic activity, whereas SDS-c did not show any hemolytic activity.

Fig. 2. Graph shows the emulsification activity using 3 different aqueous ritha SDS-c & solutions with various plant oils.



Therefore raw ritha could not be directly used in hemolytic areas.

Conclusion

The basic functional properties like critical micellar concentration (CMC), emulsification and hemolytic activity of crude ritha in aqueous medium were examined. CMC in aqueous medium was found to be 0.017 gm/cc (1.7 wt %) and value of surface tension of aqueous medium remained constant at 38 mN/m. Emulsification activity using kerosene and various plant oils was found to be excellent in comparison with commercially available SDS surfactant. The study is highlighting the economical use of crude ritha as a green surfactant similar to the purified *Sapindus* saponin.

Acknowledgements

One of the authors (AGB) would like to thank DST (Govt. of India) for the financial support. Surekha K. Satpute thanks to the UGC (UGC: F.17-37/98(SA-I)), Govt. of India for financial support.

References

- Balakrishnan S, Varughese S and Deshpande AP (2006) Micellar characterization of saponin from *Sapindus Mukorossi*. *Tenside Surf. Det.* 43(5), 262-268.
- Berhow MA, Wagner ED, Vaughn SF and Plewa MJ (2000) Characterization and antimutagenic activity of soybean saponins. *Mut. Res.* 448(1), 11-22.
- Carrillo PG, Mardaraz C, Pitta-Alvarez SI and Giuliett AM (1996) Isolation and selection of bio-surfactant producing bacteria. *World J. Microbiol. Biotechnol.* 12(1), 82-84.
- Cheeke PR (1999) Actual and potential applications of *Yucca schidigera* and *Quillaja saponaria* saponins in human and animal nutrition. In proc. of the Amer. Soc. of Animal Sci. pp1-10.
- Dembitsky MV (2004) Astonishing diversity of natural surfactants: 1. Glycosides of fatty acids and alcohols. *Lipids* 39(10), 933-953.
- Desai JD and Banat IM (1997) Microbial production of surfactants and their commercial potential. *Microbiol. Mol. Biol. Rev.* 61(1), 47-64.
- Edeoga HO, Omosun G and Uche LC (2006) Chemical composition of *Hyptis suaveolens* and *Ocimum gratissimum* hybrids from Nigeria. *Afri. J. Biotechnol.* 5(10), 892-895.
- Ellaiah P, Prabhakar T, Sreekanth M, Taleb AT, Bhima P and Saisha V (2002) Production of glycolipids containing bio-surfactant by *Pseudomonas* species. *Ind. J. Exp. Biol.* 40(9), 1083-1086.
- Fiechter A (1992) Biosurfactants: Moving towards industrial application. *Tibtech.* 10(6), 208-217.
- Ibrahim M, Khan AA, Tiwari SK, Habeeb MA, Khaja MN and Habibullah CM (2006) Antimicrobial activity of *Sapindus Mukorossi* and *Rheum emodi* extracts against *H. pylori*. In vitro and in vivo studies. *World J. Gastroenterol.* 12(44), 7136-7142.



11. Ishigami Y and Suzuki S (1997) Development of biochemicals functionalization of bio-surfactants and natural dyes. *Prog. Org. Coat.* 31(1-2), 51-61.
12. Kitamoto D, Akiba S, Hioki C and Tabuchi T (1990) Extracellular accumulation of mannosylerythritol lipids by a strain of *Candida antarctica*. *Agric. Biol. Chem.* 54(1), 31-36.
13. Kokare CR, Kadam SS, Mahadik KR and Chopade BA (2007) Studies on bioemulsifier production from marine *Streptomyces* sp. S1. *Ind. J. Biotechnol.* 6, 78-84.
14. Kommalpatti RR, Valsaraj KT, Constant WD and Roy D (1998) Soil flushing using CGA suspensions generated from a plant-based surfactant. *J. Haz. Mat.* 60(1), 73-87.
15. Mitra S and Dungun S (1997) Micellar properties of *Quillaja* saponin. Effects of temperature, salt and pH solution properties. *J. Agric. Food Chem.* 45(5), 1587-1595.
16. Mulligan CN, Cooper DG and Neufeld RJ (1984) Selection of microbes producing bio-surfactants on media without hydrocarbons. *J. Ferment. Technol.* 62(4), 311-314.
17. Patil JR and Chopade BA (2001) Studies on bioemulsifier production by *Acinetobacter* strains isolated from human skin. *J. Appl. Microbiol.* 91(2), 290-298.
18. Rao ASVS, Basa SC and Srinivasulu C (1992) Improved process for the production of saponin from soapnuts. *Res. Industry.* 37, 209.
19. Robber JM and Tyler VS (1996) Pharmacognosy, pharmacobiotechnology. Williams and Wilkins, Baltimore. pp:1-14.
20. Row LR and Ruckmini C (1966) Saponin of *Sapindus emarginatus* Vahl. *Ind. J. Chem.* 4(1), 149-150.
21. Row LR and Ruckmini C (1996a) Chemistry of saponins: Part I-saponin of *Sapindus mukorossi* Gaertn. *Ind. J. Chem.* 4, 36-38.
22. Shaker KH, Bernhardt M, Elgamal MHA and Seifert K (1999) Triterpenoid saponins from *Fagonia indica*. *Phytochem.* 51(8), 1049-1053.
23. Urum K and Pekdemir T (2004) Evaluation of bio-surfactant for crude oil contaminated soil washing. *Chemosphere.* 57(9), 1139-1150.
24. Zhang C, Valsaraj KT, Constant WD and Roy D (1998) Surfactant screening for soil washing: comparison of foamability and biodegradability of a plant-based surfactant with commercial surfactants. *J. Environ. Sci. Hea. A.* 33(7), 1249-1273.