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Isolation and Characterization of Herbal Surfactant from Selected Medicinal Plant

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ABSTRACT

The shade dried fruits of Sapindus mukorossiwas extracted with pet ether, chloroform and ethyl acetatesuccessively by soxhlation method, water by maceration method at room temperature and Preliminary phytochemicals screening of extracts of fruits of Sapindusmukorossiwith various solvents.Then we isolate the compound with high purity by flash chromatography technique. Mobile phase Benzene: ethyl acetate: water (3:4:3) and we got 11 phytoconstituents separated in different test tube and characterized fraction with parameter UV, IR, and Surfactant properties. The above findings lead to the conclusion that the saponin was sussefully isolated in fraction as surfactant of *S. mukorossi* fruits.

Keywords : Sapindus Mukorossiwas, S. Mukorossi Fruits, UV, IR, NMR, LC-MS

I. INTRODUCTION

Sapindus mukorossi (Fam: Sapindaceae), well known as soapnuts, are used medicinally as an expectorant, emetic, contraceptive, and for treatment of excessive salivation, epilepsy, chlorosis, and migranes. Sapindus mukorossi is a popular ingredient in Ayurvedic shampoos and cleansers. Surfactants are term as surface-active agents also wetting agents,emulsifying agents or suspending agents depending on its properties and use. Surface-active agents are substances which, at low concentrations, adsorb onto the surfaces or interfaces of a system and alter the surface or interfacial free energy and the surface or interfacial tension.Surfactants are monomers, it has a characteristic structure possessing both hydrophobic groups / non-polar regions (their "tails") usually contain a C12–C18 hydrocarbon chain and hydrophilic groups / Polar Regions(their "heads").Therefore, they are soluble in both organic solvents and water, so they called amphiphilic present study carried out develop the method for extraction by various solvents, Identification of plant constituents by preliminary method, isolate plant constituents by using Flash Chromatography method, characterization of isolated plant constituents by UV, IR, NMR and LC-MS

II. MATERIALS AND METHODS

Collection and identification of plant material:
The plant material used in this study was collected during month of august in Nashik Dist, India and authentication was done from botanical survey of India pune A voucher specimen has been deposited.

Drying and grinding of plant materials:
Collected fruits of Sapindus mukorossi were dried under shade and pulverized to make coarse powder.

Preparation of the Extract:
The shade dried fruits of Sapindus mukorossi was extracted with pet ether, chloroform and ethyl acetate successively by soxhlation method, water by maceration method at room temperature, concentrated over water bath and evaporated under reduced pressure. The yields of extract were calculated

Preliminary phytochemicals screening:
The plants may be considered as a biosynthetic laboratory for a multitude of compounds like alkaloids, glycosides, tannins, saponins, flavonoids and sugars, etc. that exert physiological effects. These compounds are responsible for therapeutic effects, usually the secondary metabolites. All the extracts of the plant material were

subjected to preliminary phytochemicals screening for the detection of various plant constituents

Table: 1. Preliminary Phytochemicals Screening

Sr no	Plant constituents	Test /reagent	PTE	CHL	ETA	AQE
1	**Steroids**	**Salkovaski**	--	--	--	--
2	**Alkaloids**	**Dragendroff's test**	--	--	--	--
		Hager's test	++	++	++	++
		Mayer's test	++	++	++	++
		Wagner's test	++	++	++	++
3	**Saponins**	**Foam test**	--	--	++	++
		Haemolysis test	--	--	++	++
4	**Fats and oils**	**Filter paper test**	++	++	++	++
5	**Tannins and Phenolic**	**Ferric chloride test**	--	--	++	++
		Lead acetate test	--	++	++	++
		Pot. Dichromate	--	++	++	++
		Bromine water	--	--	++	++
6	**Flavonoids**	**Shinoda test**	--	++	++	++
		Lead acetate test	--	++	++	--
7	**Carbohydrates**	**Molisch test**	--	--	--	--
		Fehling's test	--	--	--	--
		Barfoed's test	--	--	--	--
8	**Proteins**	**Millon's test**	--	++	++	++
		Biuret test	--	++	++	++
9	**Amino acid test**	**Ninhydrine test**	--	++	++	++

+ ve -- present; -- ve absent

Isolation and Characterization

Isolation of constituents from ethyl acetate extract by flash chromatography

Flash chromatography is a fast and inexpensive separation technique for the purification of organic syntheses products e.g. in drug discovery or from natural

extracts. It is a popular alternative when other separation techniques cannot be used or are too difficult. Flash chromatography provides a rapid and inexpensive general method for the preparative separation of mixtures requiring only moderate resolution. It can be applied to normal-phase and reversed-phase separation. Flash chromatography can endure relatively high flow rate with low pressure, offering good separation in a short time under a proper chromatographic condition. In flash chromatography Columns are disposable plastic cartridges, advantage of cartridges are time save and reproducibility. Based on sample volume we may select different size of cartridges. Now a day's readily prepared cartridges are available based on particle size and stationary phase volume. Flash chromatography is cost effective and low maintenance. In the case of the target molecule or compound is in high concentration, flash Chromatography is preferable. Then we may isolate the compound with high purity. In the case of sample have more chemical constituents, without information of concentrations of that chemical constituents, preparative chromatography is preferable

Resulted Peak (ethyl acetate extract):

- ✓ Prepared a conc. of 2000µg/ml from above working standard solution (10,000 ppm).In 10 ml of volumetric flask pipette out 0.2 ml of working standard solution was mixed with 5 ml of 0.5N HCL and kept for 30 min for heating on water bath. After 30 min solution was diluted up to 10 ml with methanol.
- ✓ The prepared (2000 ppm) fractionsolution was adsorbed over silica gel (# 60 – 120) in the ratio 1:4 (drug to silica gel) and finally dried under vacuum below 60^0 C. A column of 5 litres capacity was first loaded with 1 to 2 g of silica gel (# 60-120) with chloroform as solvent (dry packing).

The adsorbed material (200 mg) was charged and eluted with chloroform: methanol gradient (100:0---90:10---80:20---70:30---60:40---50:50---40:60---30:70---20:80---0:100). Fractions of 100 ml were collected. The fractions collected were concentrated by distillation under vacuum using rota vapour and weighed

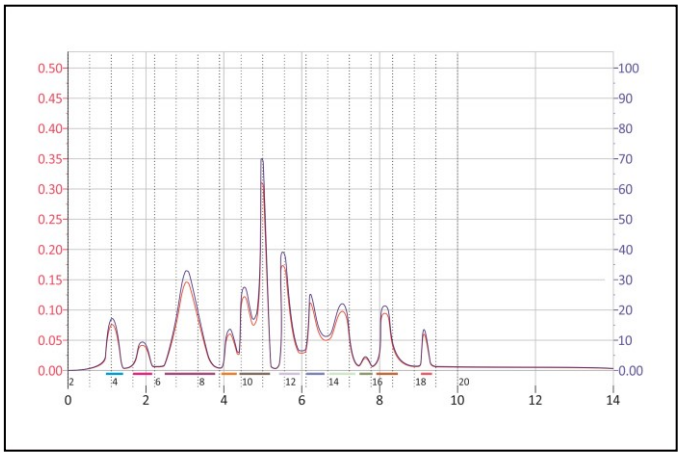

Figure 1. Flash Chromatogram of ethyl acetate extract at mobile phase benzene: ethyl acetate: water (3:4:3)

Characterization of fraction:

The structures of isolated fraction of degradation products were characterized by UV and functional groups were identified by IR spectra.

1) UV Spectra:

To analyze the collected fraction of ethyl acetate from flash chromatography samples was scanned under UV in the range of wavelength 200-400 nm. In followed UV spectra it shows change in wavelength that was at 242nm. From this result it was conclude that the fraction was isolated successfully.

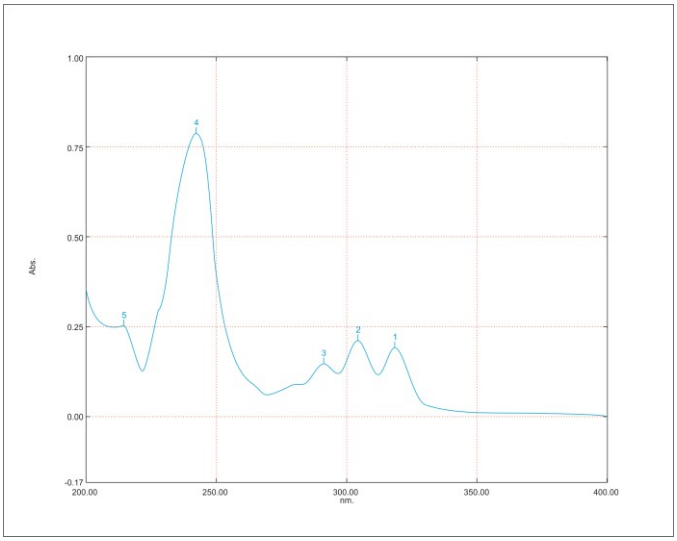

Figure 2. U V spectra of fraction

3) FT-IR:

Specification of FT-IR

Model - JASCO- M 4100 FT-IR

Preparation of sample for IR

The collected fraction adsorbed on sufficient Qty. of silica gel. This residue was then mixed with KBr in the ratio 1:300 and this sample was analyzed.

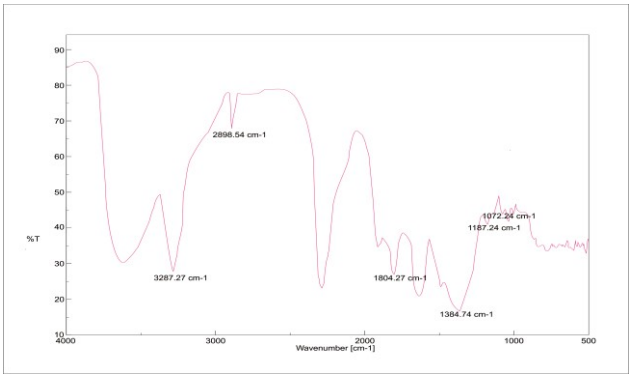

Figure 3. FT-IR Spectrum of fraction of extract

Surfactant Evaluation

Critical micelle concentration (CMC) measurement:

The CMC for fraction solution was estimated by employing Wilhelmy plate tensiometer (DCAT 11), from Data physics, Germany. Surface tension of double distilled water was measured using Wilhel my plate (platinum-iridium plate-PT 11). Then a controlled volume of fraction was added using calibrated micropipettes (20-200 µl, 200-1000 µl) 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 fraction 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 fraction solution as a mother solution was also determined.

Determination of emulsification activity with kerosene:

The fraction solution at CMC, were tested separately for the emulsification activity. This activity was checked by adding 1 ml of fraction 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. 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. 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 fraction 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. Fraction solution without any oil was taken as a blank. Absorbance of aqueous phase was measured by using spectrophotometer. Emulsification activity per ml (EU/ml) was calculated by using the formula:

Emulsification unit = 0.01 × dilution factor. Such experiments were repeated more than thrice and mean value of EU was considered for activity.

The decrease in the surface tension of the aqueous solution as a function concentration is plotted It can be seen that the surface tension of aqueous surfactant solution decreases rapidly with increase in fraction concentration. For pure water it is 72.14 mN/m and saturates to minimum value of 41.21 mN/m when fraction concentration becomes 0.04 gm/cc. The CMC value for fraction is rather close to the reported value for chemically purified Sapindus saponin. Bio-surfactant fraction contains saponin which is responsible for its various functional It is clear that the crude ritha maintains CMC value, which is an essential functional property of a surfactant and could be used as an economical bio-surfactant.

Table 2. Variation of the surface tension of aqueous solution with fraction concentration

Fraction (gm/cc)	Surface tension (mN/m)
0	72.14
0.04	41.21
0.06	12.47
0.08	2.4

0.1	1.6
0.12	1.2
0.14	1.1
0.16	0.57
0.18	0.22

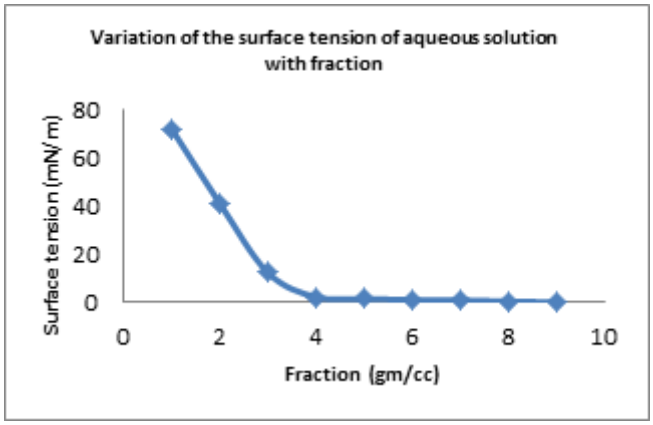

Figure 4. Variation of the surface tension of aqueous solution with fraction concentration

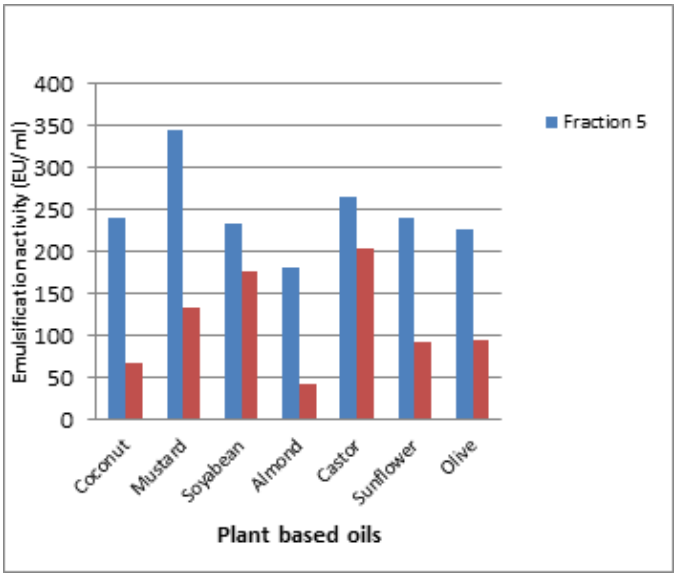

Figure 5. Emulsification activity of fraction and SDS with various plant oils.

III. DISCUSSION AND CONCLUSION

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.Therefore, we observed emulsification index for fraction. Emulsification index was tested for fraction and aqueous SDS-c solutions with kerosene. Emulsification activity for fraction solutions was approximately 72%. It is also seen that emulsification activity of fraction with kerosene is comparable to that of SDS-c solution, which is also 72%. Hence, fraction proves to be a good substitute for emulsification in comparison with synthetic surfactants. Fig. 2 shows a variation in the emulsification activity using fraction and SDS-c solutions for different plant oils. Excellent emulsification activity was shown by fraction solutions with the tested oils. These solutions show a highest activity for mustard oil followed by castor, soyabean and coconut oil. Fraction 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 fraction with respect to the various plant oils.

The shade dried fruits of *Sapindus mukorossi* was extracted with pet ether, chloroform and ethyl acetate successively by soxhlation method, water by maceration method at room temperature Preliminary phytochemicals screening of extracts of fruits of *Sapindus mukorossi.* The plants may be considered as a biosynthetic laboratory for a multitude of compounds like alkaloids, glycosides, tannins, saponins, flavonoids and sugars, etc. that exert physiological effects. These compounds are responsible for therapeutic effects, usually the secondary metabolites. All the extracts of the plant material were subjected to preliminary phytochemicals screening for the detection of various plant constituents we got positive results with Steroids, Alkaloids, Saponins, Fats and oils, Tannins and Phenolic, Flavonoids, Carbohydrates, Proteins, Amino acid test, in different solvents we have concentrate on Saponins test was performed like Foam test, Haemolysis test and saponin was presents in ethyl acetate and water extract.

Flash chromatography is cost effective and low maintenance. In the case of the target molecule or compound is in high concentration, flash Chromatography is preferable. Then we isolate the compound with high purity. In the case of sample have more chemical constituents, without information of

concentrations of that chemical constituents, preparativev chromatography is preferable Mobile phase: Benzene: ethyl acetate: water (3:4:3) and we got 11 phytoconstituents separated in different test tube. To analyze the collected fraction of ethyl acetate from flash chromatography samples was scanned under UV in the range of wavelength 200-400 nm. In followed UV spectra it shows change in wavelength that was at 242nm. From this result it was conclude that the fraction was isolated successfully. The collected fraction of fraction adsorbed on sufficient Qty. of silica gel. This residue was then mixed with KBr in the ratio 1:300 and this sample was analyzed. The observed frequencies are Observed frequency in (cm-1), 1072.24, 1187.24, 1384.74, 1804.27, 2898.54.

The decrease in the surface tension of the aqueous solution as a function concentration is plotted in Fig. 3. It can be seen that the surface tension of aqueous surfactant solution decreases rapidly with increase in fraction concentration. For pure water it is 72.14 mN/m and saturates to minimum value of 41.21 mN/m when fraction concentration becomes 0.04 gm/cc. The CMC value for fraction is rather close to the reported value for chemically purified *Sapindus saponin*. Bio-surfactant fraction contains saponin which is responsible for its various functions. It is clear that the crude ritha contain CMC value, which is an essential functional property of a surfactant and could be used as an economical bio-surfactant. Above findings lead to the conclusion that the saponin was sussefully isolated in fraction as surfactant of *Sapindus mukorossi*

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