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Rashmi Patel
Research Scholar, Botany Department, Govt. P.G. College, Satna (M.P.) India

Abha Khare
Associate Professor, Department of Botany, Govt. Girls P.G. College, Satna (M.P.) India

Corresponding Author:
Rashmi Patel
Research Scholar, Botany Department, Govt. P.G. College, Satna (M.P.) India

Extraction of Saponins from *Sapindus laurifolia* (L.)

Rashmi Patel and Abha Khare

Abstract
Functional properties such as critical micellar concentration, emulsification and hemolytic activity of raw ritha (*Sapindus laurifolia*) 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: Saponins, *Sapindus laurifolia*

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 (Dembitsky, 2004) [1]. These surfactants can be mainly obtained from microorganisms like bacteria, yeast, fungi (Desai & Banat, 1997; Kokare *et al.*, 2007) [2-3] and plants (Ishigami & Suzuki, 1997) [4]. Saponin is one of the most commonly known plant based surfactants. Saponin is largely found in plants like *Sapindus laurifolia*, soyabean (Berhow *et al.*, 2000) [5], Quillaja bark (Mitra & Dungun, 1997) [6] and *Fagonia indica* (Shaker *et al.*, 1999) [7]. The nut obtained from trees of *Sapindus laurifolia* 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 laurifolia* 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 % (Kommalpatti *et al.*, 1998) [8]. Saponin is also widely used in the pharmaceutical industries (Robber & Tyler, 1996; Edeoga *et al.*, 2006) [9-10], detergents (Cheeke, 1999) [11] and environmental remediation (Urum & Pekdemir, 2004) [12]. There are several reports on the study of the saponin, extracted from ritha. Rao *et al.* (1992) [13] has presented basic chemical method for the extraction of saponin. Row and Ruckmini (1996) [14] have studied the chemical properties of saponin (Row & Ruckmini, 1996a) [15]. Urum and Pekdemir (2004) [16] have studied the ability of aqueous saponin solution as a biosurfactant for applications in washing and crude oil contaminated soil. Zhang *et al.* (1998) [17] have observed the excellent biodegradability and contaminated soil washing property of ritha over the synthetic 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) [18-20]. These surfactants can be mainly obtained from microorganisms like bacteria, yeast, fungi (Kokare *et al.*, 2007) [21] and plants (Ishigami & Suzuki, 1997) [22]. Saponin is one of the most commonly known plant based surfactants. Saponin is largely found in plants like *Sapindus laurifolia*, soyabean (Berhow *et al.*, 2000) [23]. The nut obtained from trees of *Sapindus laurifolia* 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 laurifolia* 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 % (Kommalpatti *et al.*, 1998) [24].

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Usually, saponin is extracted by using solvent like water, ethanol and methanol (Huang *et al.*, 2008; Rao & Sang, 2006) [27-28]. Accordingly, this experiment will use ground pulp of *Sapindus laurifolia* as raw material. Meanwhile the impact brought about by different solvent, extraction times, extraction durations and different solid-liquid ratios has been investigated.

2. Material and Methods

2.1 Plant and Chemical Material

Panna is located at 24.72°N 80.2°E. It has an average elevation of 410 metres (1420 ft). Panna was a Gond settlement until the 13th or 17th century (cite reference), when the Gondi were defeated by the Chandelas they migrated to other parts of Madhya Pradesh. Until that date, there were many rulers of the area.

Fruits pulps of *S. laurifolia* were collected from forest of Satna district in November 2020. Prior to all ex-tractions, fruits pulp was dried at 60˚C for 48 h and was ground in a Wiley mill to pass a 0.5 mm poresize screen. Chromatograph solvents used during the study were of HPLC grade and the other solvents and reagents used during the study were of AR grade.

2.2 Extraction Process

The main factors that affect the extraction of saponins like extraction solvents, temperature, time, times and materials ratio (weight of the fruit pulp: volume of the extracting solvent), were studied individually. The optimum extraction conditions were determined by $L_9(3^4)$ orthogonal design of experiments i.e. three levels and three different parameters.

2.3 Estimation of Total Saponins

Saponins sample was collected from extraction solution with labware. Saponins concentration was measured in sample by HPLC (column: Symmetry TM C_{18} (3.9 mm i.d × 150 mm), 40˚C, Flowing phase: CH_3CN:H_2O (H_2O:90% → 20%, 30 min), 1ml·min^{-1}; detection wavelength: 210nm).

2.4 Stability Study

This study was carried out at vary water temperature (25, 35, 40˚C), pH (6.3, 7.0, 7.7), water hardness (50, 100, 250 mg·L^{-1}) with aqueous solutions having a saponin content of 4%. The Cmc and *γ*Cmc were determined by dynamic tension meter.

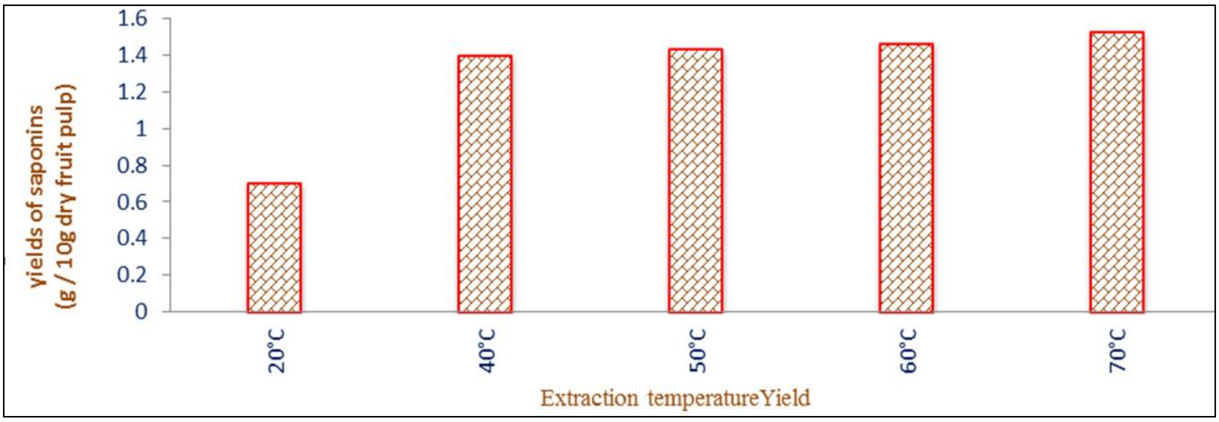
2.5 Statistical Analysis

The results are expressed as means ± SD unless otherwise stated. The evaluation of statistical significance was determined by the one-way ANOVA test, these analyses were done with SPSS for WINDOWS, version 19.0, and with small letter < 0.05, capital letter < 0.01 considered to be statistically significant.

3. Results and Discussion

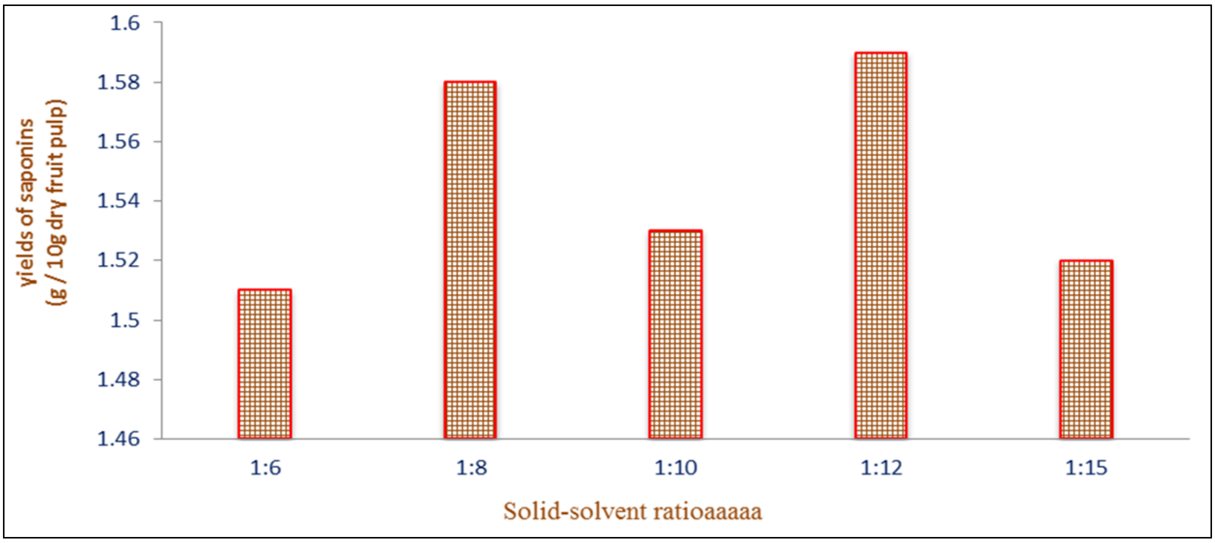
Saponins in *S. laurifolia* (L.) were extracted by nine sol-vents, and its content was measured by HPLC. The best ex-tracting solvents was ethanol which can result in the high productivity of saponin, good quality of saponin colour and volatiles (Table 1).

Graph 1 showed the yields of saponins tended to increase with a rise in the temperature range from 20˚C to 50˚C. It may be probable that the greater speed of the molecule movements in higher temperature so that saponins diffused more quickly from cell to extracting agent. But the yields of saponins could be slight changed temperature of surpassing 60˚C. Temperature’s effect on extraction is dual. On one hand, higher temperature can accelerate the solvent flow and thus increase the saponins content and on the other hand, higher temperature can decrease the fluid density that may reduce the extraction efficiency. Hence, it was found that 60˚C was the optimum temperature for extracting the saponins.



Graph 1: Effected of temperature on the yield of s. laurifolia saponins. Ethanol, solid-liquid ratio 1:20, time 6h, 2 extraction time

Graph 2 showed the yields of saponins extracted was mini-mum at 1:6 materials ratio. Further increase in the material ratio leads to slight changed in the yields of saponins. This phenomenon might be due to the fact that when the material ratio reached a certain level, the extract has well dissolved in the solution that may lead the contents of the extract become saturated and prevent further increase.



Graph 2: Effected of the solid-solvent ratio on the yield of S. *laurifolia* saponins, ethanol temperature 60° C, time 3h, 2 extraction times

The parameters and the orthogonal design of experiment for the extraction of saponins were given in the Tables 2 and 3.

Table 1: Effects of solvents on the characteristics of *S. laurifolia* (L.) Saponins (means ± SD).

	MeOH	EtOH	Acetone	Butanol	H_2O	95% MeOH	95% EtOH	95% Acetone	95% Butanol
Mass of Saponins(g)	0.68±0.13	1.54±0.08	1.00±0.11	0.98±0.01	0.63±0.10	1.19±0.08	1.51±0.05	1.37±0.11	1.45±0.08
Purity (%)	63.62	72.63	73.48	69.90	35.03	59.15	70.48	72.42	67.80
Desiccation situation	Easy	Easy	Easy	Easy	Difficult	Little viscosity	Easy	Easy	Easy
Characters of the dry substance	Yellow powder	Off-white powder	Light yellow powder	Light yellow powder	Brown glue	Yellow powder	Off-white powder	Off-white powder	Light yellow powder
Volatiles	Slightly sweet	Special fragrance	Special fragrance	Special fragrance	Heavily sweet	Slightly sweet	Special fragrance	Special fragrance	Special fragrance

Note: Temperature 60 ^{0}C, solid-liquid ratio 1:20, time 6 h, 2 extraction times.

Table 2: Factors and levels of orthogonal test.

Levels	Extraction time (A)	Solid-liquid ration (B)	Extraction time (C)
1	1	1:8	2
2	2	1:9	3
3	3	1:10	4

The factors that influence the extraction of saponins are put in the order of extraction times, extraction time, solid-liquid ratio, and the best combination is when the powder of the pulp is extracted with EtOH (solid-to-solvent ratio = 1:8, w/v) for three times at 60 ^{0}C for 3 hours. Under these conditions, about 1.63 g saponins will be extracted from 10 g raw material.

The results show that the *Sapindus laurifolia* saponins can maintain surface activity at water temperature (25 ^{0}C – 40 ^{0}C), pH (6.3 - 7.7) and water hardness (50 - 250 mg.L^{-1}) (Table 4).

Table 3: Design and result of orthogonal test.

Test No.	A	B	C	Yield of *Sapindus laurifolia* (L.) Saponins (g)
1	1	1	1	1.05
2	1	2	2	1.34
3	1	3	3	0.92
4	2	1	2	1.48
5	2	2	3	0.89
6	2	3	1	1.29
7	3	1	3	1.54
8	3	2	1	1.57
9	3	3	2	1.61
K_1	1.04	1.36	1.30	
K_2	1.22	1.20	1.41	
K_3	1.57	1.27	1.12	
R	0.54	0.08	0.29	

Table 4: Influence of different factors on the surface activity of *Sapindus laurifolia* saponins.

	Temperature (°C)			Water hardness (mgL^{-1})			pH		
	25	35	40	50	100	250	6.3	7.0	7.7
Cmc (mgL^{-1})	32.9	24.8	21.6	39.3	56.8	76.9	33.4	60.4	76.8
γcmc	36.3	35.2	34.2	35.9	36.1	37.4	36.5	37.6	38.2

4. Conclusion

When the powder of the pulp was extracted with EtOH (solid-to-solvent ratio = 1:8, w/v) for three times at 60˚C for 3 hours, namely in the best extraction condition, the largest yield of saponins (1.63 g saponins will be extracted from 10 g raw material) was obtained. The stability test showed that the *Sapindus laurifolia* saponins can maintain surface activity at water con-ditions under which people normally use detergent. It is proved that *Sapindus laurifolia* saponins are quality non-ionic active agent. Thus, we can conclude that this technology for saponins extraction from *S. laurifolia* Gaerth. is efficient and environ-mentally friendly.

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