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# A GREENER SUSTAINABLE BIOSURFACTANT: EVALUATION AND COMPARISION OF SAPONIN RICH EXTRACT OF *SAPINDUS MUKOROSSI* OBTAINED FROM DIFFERENT EXTRACTION METHOD

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## ABSTRACT

This study evaluates and compares the saponin-rich extracts obtained from the pericarp of *Sapindus mukorossi* using various extraction methods, including aqueous, ethanol, methanol, ethyl acetate, and enzymatic techniques. The qualitative analysis revealed the presence of key phytochemicals such as alkaloids, flavonoids, saponins, and glycosides, with saponins being present in all extracts. The yield and concentration of saponins were highest in aqueous and 70% ethanol extracts, achieving 31.5% yield and 52.0 mg/ml concentration, respectively. The emulsification index demonstrated significant surfactant properties, with maximum emulsification observed at elevated temperatures. The study further examined the impact of extraction parameters, such as temperature, time, and material-to-solvent ratio, on saponin yield. Results indicated optimal conditions for maximum yield and emulsification at specific temperature and solvent ratios. Additionally, fermentation studies highlighted a decrease in saponin concentration over time, suggesting a need for optimized fermentation processes. The findings establish *Sapindus mukorossi* as a promising source of biosurfactants, with potential applications in various industries, including cosmetics and food.

**Keywords:** *Sapindus mukorossi*, saponins, biosurfactants, extraction methods, emulsification, phytochemicals, sustainable agriculture, environmental applications, qualitative analysis, fermentation.

## INTRODUCTION

Biosurfactants are naturally occurring surface-active agents produced by various organisms, including bacteria, fungi, plants, and animals. Unlike synthetic surfactants, which often pose environmental hazards, biosurfactants are biodegradable, non-toxic, and environmentally friendly. These properties make them increasingly appealing in various industries, including agriculture, pharmaceuticals, and environmental remediation (Rani *et al.,* 2021). Among the different classes of biosurfactants, saponins glycosides composed of hydrophobic aglycones linked to hydrophilic sugar moieties have gained considerable interest due to their potent surface-active properties (Nawaz *et al.,* 2018).

Saponins exhibit amphiphilic characteristics, enabling them to lower surface tension and stabilize emulsions, thus enhancing the solubility of hydrophobic compounds in aqueous solutions (García-Ochoa *et al.,* 2000). One of the most prominent sources of saponins is the fruit of *Sapindus mukorossi*, commonly referred to as soapnut or soapberry. This plant has been used traditionally for its natural cleansing

properties and has been the subject of scientific studies aimed at exploring its potential as a sustainable biosurfactant (Jabbar *et al.,* 2020). *Sapindus mukorossi* contains high levels of saponins, making it an attractive candidate for applications ranging from household detergents to pharmaceutical formulations.

The extraction of saponins from *Sapindus mukorossi* can be performed using various methods, each yielding different concentrations and properties of the bioactive compounds. Traditional extraction techniques, such as maceration and percolation, may be simple and cost-effective but often result in lower yields and longer processing times. In contrast, modern extraction methods, such as ultrasonic-assisted extraction and supercritical fluid extraction, have been developed to improve efficiency and reduce extraction times while minimizing the use of hazardous solvents (Mishra *et al.,* 2021). These methods leverage advanced technologies to enhance the solubility and mass transfer of saponins, making them more efficient for large-scale applications.

Understanding how different extraction techniques affect the yield and functional properties of saponin-rich extracts is crucial for their industrial applications. The choice of extraction method can significantly influence the quality, stability, and efficacy of the biosurfactants produced. For instance, extracts obtained through ultrasonic-assisted extraction may have improved emulsifying and foaming properties compared to those obtained through traditional methods. Such variations highlight the need for systematic studies comparing these extraction techniques to identify the most effective methods for isolating saponins.

This study aims to evaluate and compare the effectiveness of saponin-rich extracts from *Sapindus mukorossi* obtained through various extraction methods, including traditional and modern techniques. The focus will be on analyzing their surface-active properties, such as emulsification, foaming capacity, and surface tension reduction. The findings will provide valuable insights into the potential applications of these biosurfactants in diverse fields, including food, cosmetics, and bioremediation. Ultimately, this research will contribute to the advancement of sustainable practices in the extraction and utilization of plant-derived biosurfactants.

## MATERIALS AND METHODS

### Collection of plant material and other reagents:

The sample of *Sapindus pericarp* used in this work was procured locally and verified by our guide. The yeast selected was dry *Saccharomyces cerevisiae*. It was purchased from Angel Yeast Co., Ltd. Analytical; standard Oleanolic acid (purity 98.10%) was supplied by Chengdu Pufei De Biotech Co., Ltd. The standard saponin sample was obtained from our own lab. Besides, other chemicals that are D-glucose, Diatomite (90%), Vanilin (99%), and Sulfuric acid (98%) were all used of analytical Research grade.

### Preparation of plant material

Since the shell/ pericarps of Sapindus fruits act as the source of surfactants, soapnuts were crushed in a mortar and the seeds were removed. Before the test, these Sapindus

pericarps of *S. mukorossi* was crushed and dried at 60°C in shade for 24 hrs. The crushed Sapindus powder was then screened through an 80-molecular mesh sieve for further phytochemical evaluation and extraction usage. 500ml n-hexane was used to de-fat the powdered plant material in Soxhlet apparatus (5 h).

**Phytochemical Evaluation**

Five extraction solvents i.e. water, ethanol, 70% ethanol (30% water), methanol, and ethyl acetate were used to prepare the extract of powdered material. 10 gm of material was suspended with 30ml of each of solvent in a covered tube. The mixture was kept under constant shaking in water bath at 40°C for 30 min. The extract was stored in air tight container, in refrigerator until use.

Different qualitative chemical tests were performed for each of the solvent extracts with specific consideration for saponin surfactant. The test sperformed to detect various phyto-constituents present in Sapindus pericarp of *S. mukorossi* are as follow.

1) **Alkaloids (Mayer's test):** To 2ml of the extract, 1ml of dilute HCl is added and a few drops of Mayer's reagent were added along the slide of the test tube. The formation of yellow colour indicates the presence of alkaloids.
2) **Flavonoids (NaOH test):** The extract (2ml) was dissolved in 2ml of 2% NaOH and further observed for the formation of intense yellow colour which becomes colorless on addition of few drops of dil. HCl indicates the presence of Flavonoids.
3) **Phenol (Lead acetate test):** The extract (2ml) was dissolved in 5ml of distilled water and 3ml of 10% lead acetate solution. The formation of white precipitate indicates the presence of Phenolic compounds.
4) **Saponin (Foam test):** 1ml of extract was added to distilled water and shaken vigorously. The formation of honeycomb like foam that stayed for 10 to 15minutes indicates the presence of Saponin.
5) **Glycosides (Keller Killani test):** To 1ml of filtrate, 1.5ml of glacial acetic acid was added. Further to this 1 drop of 5% ferric chloride and concentrated H2SO4 along the sides of test tube. Appearance of reddish brown indicates the presence of Glycosides.
6) **Carbohydrate (Molisch's test):** To 2ml of extract 2 drops of alcoholic α-napthol solution was added. Further concentrated $H_2SO_4$ 1ml, along the sides of the test tube was added. The violet ring formation at the junction indicates the presence of Carbohydrates.

Extraction methodology

There are various methods for the extraction of saponins which been extensively researched and well documented. The conventional methods for saponin extraction include maceration, and Soxhlet extraction. These extraction techniques are mainly simple and do not require sophisticated experimental settings (Rai *et al.,* 2021). Underneath we are now describing the extraction methods employed in present study.

### Evaporative extraction of surfactant

The evaporative extraction may be described as the leaching process in which the solvent, i.e., water, is directly in contact with the solid (soap nut) from which the desired solute, i.e. the saponin is to be extracted with simultaneous concentration of the extract through the evaporation of water (Basu *et al.,* 2015; Treybal; 1981). Around 30gm of defatted prepared powered material of Sapindus pericarps were dissolved in 200 ml of distilled water was taken in a round bottom flask and heated in a heating mantle. The mouth was sealed with cotton to restrict the passage of vapour and a thermometer was inserted inside the flask. The temperature was allowed to rise in the flask and after the desired temperature was achieved, the solution was heated under steady state for the required time. The step was carried out for different extraction time and temperature. The solution was cooled and filtered to remove the remaining pulp from the solution and the filtrate was analysed using FTIR spectroscopy and was tested for the determination of surface tension, CMC and the E-24 index.

The Total Saponin Content (TSC) was calculated as a percentage.

**Total yield of saponins (%)** = (Weight of saponins / Weight of sample) x100

### Extraction of surfactant using Soxhlet's apparatus

The Soxhlet extraction is also a leaching process which allows the indirect contact of the condensate of solvent vapour with the solid (kept in a thimble) and the condensate of the vapour was returned repeatedly to the solvent tank. As a result, fresh condensate of solvent is always used for extraction. This was equipped with a re-boiler at the bottom and a condenser at the top to condense the vapour and drip it down to the thimble (Singh *et al.,* 2019).

The thimble was stuffed with 30 gm of prepared soap pulp powder. The thimble is a porous bag made from either a firm filter paper or cellulose. 200 ml of distilled water was taken in the still pot to start the extraction process. Extraction temperature was fixed at 100°C (boiling point of water). During each cycle, a portion of the non-volatile compound, i.e., soap pulp was dissolved in the solvent until the solution in the distillation flask became concentrated. Finally, the extract was allowed to cool and various analysis was carried out with this concentrated solution.

The saponins enriched total content was calculated as a percentage.

**Total yield of saponins (%)** = (Weight of saponins / Weight of sample) x100

### Extraction of Sapindus by maceration

Maceration is a simple extraction method that involves soaking the plant prepared raw material in a coarse or powder form in a solvent of interest at room conditions for at least three days with intermittent agitation (Le *et al.,* 2023). Here also 30gm of prepared plant material was dissolved in 200ml of purified water. The first extract of *Sapindus pericarps* was crudely filtered through a gauze and the residue obtained was continuously extracted with the same procedure. Subsequently, the product solutions were combined and thoroughly mixed to obtain the Sapindus saponins extract. The product is concentrated frequently by the

use of vacuum evaporation. The saponins total content was calculated as a percentage.

**Total yield of saponins (%)** = (Weight of saponins / Weight of sample) x100

**Enzymatic extraction of saponin**

The enzymatic extraction process was conducted in 200ml aqueous mediaby using enzyme cellulose with 30gm of prepared soap nut pericap, and the solid-liquid separation was carried out by adding to the centrifuge for 15 minutes, and the supernatant was taken and stored at 4°C for further analysis. Factors, such as solid-liquid ratio, enzyme concentration, and reaction time will be analysed. The total saponin content was calculated as a percentage.

**Total yield of saponins (%)** = (Weight of saponins / Weight of sample) x100

**Purification of saponins extract by fermentation**

This involves two steps: activation of Yeast and then fermentation.

**Yeast Activation:** Before inoculation, 3 g of the *S. cerevisiae* dry yeast was rehydrated in 15 mL of distilled water and 15 mL of saponin extract medium. The yeast solutions were activated for 30 min in a water bath at 35$^{o}$C and used for further fermentation (Le *et al.,* 2023).

**Fermentation Method:** The activated yeast was inoculated into 150 mL of saponin extract medium. The containers were sealed and incubated at room temperature (25–35$^{o}$C), away from direct sunlight. Then, the fermented extract was autoclaved and filtered with filter aid powder to obtain the Sapindus fermentation product. Fermentation parameters such as time and inoculum amount were investigated to determine the purity of the obtained extracts ( Nguyen *et al.,* 2023).The total saponin content was calculated as a percentage.

**Total yield of saponins (%)** = (Weight of saponins / Weight of sample) x100

**Quantification & Evaluation of Saponin**

**Determination of total saponins content**

The total saponins were determined according to the vanillin-sulfuric acid assay (Hiai *et al.,* 1976). Analytical standard Oleanolic acid was used as the saponin standard.

For this assay, Vanillin reagent (8%, w/v) was prepared by dissolving Vanillin (800 mg) in 10 ml of 99.0 % ethanol (analytical grade). 72% (v/v) sulphuric acid was prepared by adding 72 ml of sulphuric acid (analytical grade, 95%, w/w) to 28 ml of distilled water. For standard saponins solution, 50 mg Oleanolic acid saponins (Sigma-Aldrich) was dissolved in 80 ml of methanol and 20 ml water was added to it. The final concentration of saponins in the solution was 0.5 mg/ml of 80% methanol. Pipetting was done immediately (Le *et al.,* 2023).

Sample measurement: 0.3 mL of vanillin 8% reagent and 3 mL of sulfuric acid 72% reagent (added slowly on the inner side of the wall) were respectively added into 0.3 mL of sample diluted several times. The solutions were evenly mixed using a vortex shaker. After mixing the content in each tube, these were left as such for 3 min. Then these were warmed on water bath at 60$^{o}$C for 10 min then cooled in ice- -cold water for 10 min. The absorbance was determined at 544 nm by using a UV-Vis spectrophotometer (model

Labindia 3000+). Total saponin content (TSC) in the extract was calculated based on a standard calibration curve using Oleanolic acid saponin standard and expressed in mg Oleanolic acid saponin equivalent per millilitre of extracts (mg Ole per mL) (Uematsu *et al.,* 2000).

$$Y(\%) = \frac{W_s}{W_p} \times 100\%$$

Where: Yi (%) is the extraction yield of total saponins Ws (g) is the weight of total saponins in the extract. Wp (g) is the weight of the *Sapindus pericarp*.

**Determination of Surface Tension and CMC**

As mentioned earlier, saponins are characterized by surfactant properties that allow them to lower the surface tension of aqueous solutions. This feature is a direct result of the saponin structure, since they consist of two parts with different solubility in water, which in effect form an amphiphilic molecule. This property thus can be evaluated. With slight modification in the process as describe by Yan C-H using Du Nouytensionmeterfrom Sigma, the surface-active properties of saponins extracted from the pericarp of soapnutwas studied ( Sochacki *et al.,* 2022; Yang *et al.,* 2020).

By plotting the surface tension curve using the values of surface tension measured at different surfactant concentrations, Critical Micelle Concentration (CMC) was determined with the onset of constancy in surface tension in spite of change in concentration.

**Determination of emulsification index (E-24 index)**

E24 index was determined following a standard protocol (Cooper *et al.,* 1987). 2 ml of extract corresponding to different extraction time and temperature was mixed with 2 ml of paraffin liquid in different test tubes. The tubes were placed in vortex and spun for 2 min for vigorous mixing. The resulting mixture, which was allowed to stand overnight, separated into an emulsified layer (hydrophobic phase) and the remaining aqueous layer (hydrophilic phase). Finally, emulsification index (E-24) was measured as the ratio of the height of emulsified layer to the total height.

**Emulsification Index (E − 24)** = (Height of emulsified layer/Total height of the solution) × 100

**RESULTS AND DISCUSSION**

Table 1 demonstrates the presence of various phytochemicals across different extraction methods. The consistent presence of saponins in all extracts indicates their abundant nature in *Sapindus mukorossi*, which aligns with previous studies that emphasize their role as effective biosurfactants (Jabbar *et al.,* 2020). The detection of flavonoids and alkaloids in certain extracts suggests potential synergistic effects that could enhance the biological activity of the saponins.

The results presented in Table 2 illustrate that aqueous and 70% ethanol extracts yielded the highest percentages of saponins (30.2% and 31.5%, respectively). This observation aligns with literature indicating that polar solvents effectively extract saponins due to their amphiphilic nature (Mishra *et al.,* 2021). Conversely, the lower yields from methanol

and ethyl acetate extracts underscore the importance of solvent choice in optimizing extraction processes.

Table 3 provides valuable data on the emulsification index and yield of saponins at varying temperatures. The highest emulsification index (69.56%) was observed at 10 minutes, indicating that saponins can effectively stabilize emulsions, making them suitable for applications in food and cosmetic formulations. Interestingly, the yield increased with temperature, peaking at 90°C, which suggests that heat may enhance the extraction of saponins, possibly by breaking down cell walls and facilitating the release of these compounds.

The findings in Table 4 highlight the interplay between temperature, extraction time, and material-to-solvent ratios in determining saponin yield. The optimal yield was achieved at 80°C, with a 1:7 material-to-solvent ratio, emphasizing the necessity of fine-tuning extraction conditions for maximum efficiency. These results corroborate previous findings that higher temperatures and appropriate solvent ratios can lead to enhanced extraction yields (Rani *et al.,* 2021).

The results in Table 6 reveal a gradual decline in saponin concentration with extended fermentation time, potentially due to microbial metabolism consuming saponins as a carbon source. However, the total sugar content also decreased, suggesting that fermentation affects both components in tandem. This finding indicates that while fermentation can be beneficial for certain metabolic processes, it may not be conducive for maximizing saponin yield over extended periods.

The data from Table 7 show that varying the inoculum amount affects both saponin and sugar concentrations. As the inoculum increased, saponin levels decreased, which may imply that a higher microbial load competes for resources, reducing the availability of precursors necessary for saponin biosynthesis. This observation points to the need for optimized fermentation conditions to balance microbial activity and metabolite production.

The absorbance values presented in Table 8 indicate a linear relationship between concentration and absorbance, affirming the reliability of UV-Vis spectrophotometry for quantifying saponin concentrations. This method provides a valuable tool for the standardization of saponin extracts in future studies and applications.

The surface tension results in Table 9 demonstrate the surfactant properties of the saponin extracts, with surface tension values decreasing as concentration increased. The observed reduction in surface tension indicates that the extracts can effectively reduce interfacial tension, which is crucial for applications in emulsification and stabilization of formulations (Nawaz *et al.,* 2018).

Table 10 summarizes the extraction yields of various methods, showing that Soxhlet extraction (37.0%) yielded the highest percentage, followed closely by enzymatic extraction (36.5%). These results affirm the efficiency of these methods compared to others like maceration and fermentation, which yielded significantly less. This highlights the potential for optimizing extraction processes to enhance the recovery of valuable saponin compounds.

**Table 1: Qualitative detection of secondary metabolites from *S. mukorossi* pericarp different extracts**

| Phytochemical | Test Applied | Aqueous extract | 70% ethanol extract | Ethanol extract | Methanol extract | Ethyl acetate extract |
|---|---|---|---|---|---|---|
| Alkaloids | Mayer's test | + | + | - | + | + |
| Flavonoids | NaOH test | + | + | + | - | - |
| Phenolic | Lead acetate test | + | + | - | + | - |
| Saponins | Foam test | + | + | + | + | + |
| Glycosides | Keller Killani test | + | + | + | - | + |
| Carbohydrates | Molisch's test | - | + | - | - | + |

Here: + sign indicates the presence and – sign indicates the absence

**Table 2: Effect of different extraction solvents on % yield and concentration of saponin**

| Solvents | % Yield | Concentration of saponin (mg/ml) |
|---|---|---|
| Water | 30.2 | 50.8 |
| 70% Ethanol | 31.5 | 52.0 |
| Ethanol | 29.0 | 49.0 |
| Methanol | 25.0 | 43.0 |
| Ethyl Acetate | 20.0 | 36.0 |

**Table 3: Emulsification index and Yield at 60$^{o}$C experimental Temperature**

| Time (min) | Emulsification index (%) | % Yield of Saponin at 60$^{o}$C | % Yield of Saponin at 80$^{o}$C | % Yield of Saponin at 90$^{o}$C |
|---|---|---|---|---|
| 5 | 44.83 | 14.72 | 19.14 | 23.78 |
| 10 | 69.56 | 22.84 | 27.48 | 30.09 |
| 15 | 59.40 | 20.50 | 26.10 | 34.26 |

**Table 4: % Yield at different temperature, time and material to solvent ratio**

| % Yield | Temperature | % Yield | Material to Solvent ratio | % Yield | Time |
|---|---|---|---|---|---|
| 16.9 | 50 | 14.8 | 1/4 | 14.2 | 30 |
| 18.7 | 60 | 17.7 | 1/5 | 16.4 | 60 |
| 20 | 70 | 20.7 | 1/6 | 19.4 | 90 |
| 20.9 | 80 | 20.2 | 1/7 | 21.8 | 120 |
| 20.4 | 90 | 19.7 | 1/8 | 21.4 | 150 |

**Table 5: % Yield at different time and solid-liquid ratio**

| Time (min) | Solid-liquid ratio (w/v) | | |
|---|---|---|---|
| | 5 | 10 | 15 |
| 60 | 107.70 | 77.96 | 65.27 |
| 120 | 110.40 | 82.04 | 67.53 |
| 180 | 115.8 | 89.35 | 67.90 |

**Table 6: Effect of Days of fermentation on the Saponin and total sugar content**

| Concentration of Saponin (mg Ole/ml) | Concentration of total sugar (mgGlu/ml) | Time in Days |
|---|---|---|
| 17.11 | 42.86 | Standard |
| 17.04 | 38.4 | 1 |
| 16.91 | 35.17 | 2 |
| 16.65 | 28.82 | 3 |
| 16.61 | 23.04 | 4 |
| 16.05 | 20.06 | 5 |
| 14.82 | 19.65 | 6 |
| 14.17 | 13.54 | 7 |

**Table 7: Effects of inoculum amount on saponins and total sugar content**

| Concentration of Saponin (mg Ole/ml) | Concentration of total sugar (mgGlu/ml) | Innoculum amount (%) |
|---|---|---|
| 16.79 | 39.66 | Standard |
| 16.63 | 34.51 | 0.5 |
| 16.26 | 28.62 | 1 |
| 16.22 | 25.04 | 1.5 |
| 16.19 | 22.21 | 2 |
| 15.71 | 22.08 | 2.5 |
| 15.98 | 21.74 | 3 |

**Table 8: UV-Vis Absorbance of standard Oleanolic acid**

| S. No. | Concentration (µG/ml) | Absorbance |
|---|---|---|
| 1 | 25 | 0.125 |
| 2 | 50 | 0.200 |
| 3 | 75 | 0.275 |
| 4 | 100 | 0.350 |
| 5 | 125 | 0.425 |
| 6 | 150 | 0.475 |
| 7 | 175 | 0.575 |

**Table 9: Surface tension values from Soap nut extract**

| Concentration (Kg/m3) | Surface Tension (mN/m) |
|---|---|
| 1 | 54.8 |
| 4 | 51.7 |
| 8 | 49.0 |
| 11 | 48.5 |
| 15 | 47.0 |
| 20 | 46.5 |
| 23 | 45.0 |
| 30 | 45.0 |
| 35 | 45.0 |
| 40 | 45.0 |

**Table 10: Comparison of extraction yield**

| S. No. | Methods of Extraction | % Yield |
|---|---|---|
| 1 | Evaporation extraction (EE) | 35.0% |
| 2 | Soxhlet extraction (SE) | 37.0% |
| 3 | Maceration extraction (ME) | 21.4% |
| 4 | Enzymatic extraction (EnE) | 36.5% |
| 5 | From fermented extract (FE) | 20.1% |

## CONCLUSION

The comprehensive evaluation of saponin-rich extracts from *Sapindus mukorossi* indicates that extraction methods and conditions significantly influence yield, phytochemical composition, and surfactant properties. The findings suggest that optimizing these parameters can enhance the functional application of saponins as natural biosurfactants in various industries. Future research should focus on developing eco-friendly extraction techniques that maximize yield and efficacy while minimizing environmental impact.

## DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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