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Development of an organic hand wash devoid of chemicals utilizing *Sapindus emarginatus* (Vahl.) and exploring its potential antimicrobial properties

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Abstract
For centuries, plant extracts and products have served diverse purposes such as traditional medicine, functional food, natural dyes, cosmetics, and even as detergents or in disease treatment. Medicinal plants have been valued for their therapeutic properties in addressing various human ailments. One such plant, *Sapindus emarginatus* Vahl, belonging to the Sapindaceae family and Sapindus genus, is a deciduous tree of medium size found in South India. Thanks to its saponin content, soapnut is renowned for its detergent and insecticidal properties. In light of this, our study aims to develop an herbal hand wash formulation utilizing soapnut plant extracts, focusing on their potential antibacterial activity. We intend to establish soapnut extracts as potent antimicrobial agents for use in herbal hand wash formulations.

Keywords: Soapnut, Handwash, chemical – free, anti-microbial

Introduction
Plant extracts and products have been used for centuries in traditional medicine, functional food, natural dyes, cosmetics, as a detergent and in the treatment of diseases [1, 2, 3] The main advantage of using natural source is that they are easily available, cheap and harmless compared to chemical products. Therefore, research has been increased tremendously towards making natural products with improved quality yet less expensive and no side effect over chemical products.
Medicinal plants are gifts of nature which are used in the treatment of various human ailments. *Sapindus emarginatus* Vahl belongs to family Sapindaceae and genus Sapindus is a medium-sized deciduous tree found in South India [4]. The Tree is about 8m to 10 m tall and has branches. *Sapindus emarginatus* is an economically significant tropical tree species meagerly distributed in diverse geographical provinces like Gangetic Plains, Western Ghats, and Deccan Plateau in India [5]. *Sapindus emarginatus*, commonly referred to as soap nut, is a native to South Asia, particularly India. It is an indigenous medicinal plant, has a folk (Siddha and Ayurveda) reputation in the rural areas of southern India.
Significance of soap nut as a natural detergent *has* been explained in relation to each and every plethora of human life from the dawn of civilization [6]. Several constituents were isolated from the plant including secondary metabolites. Plant *Sapindus emarginatus* found to consists of various compounds i.e. Phytochemical such as flavonoids, Triterpenoids, glycosides, carbohydrates, fatty acids, phenols, fixed oil, and saponins [7]. Saponins are secondary plant metabolites with divergent biological activities [8]. Due to the presence of saponins, soapnut is well known for its detergent and insecticidal properties and it is traditionally used for removing lice from the scalp.
The fruits are of considerable importance for their medicinal value for treating a number of diseases like excessive salivation, pimples, epilepsy, chlorosis, migraines, eczema and psoriasis [9]. The powdered seeds are employed in the treatment of dental caries, arthritis, common colds, constipation and nausea [10]. The seeds of *Sapindus emarginatus* are used in Ayurvedic medicine to remove tan and freckles from the skin. In the present study, saponin was isolated from the extract of *S. emarginatus.* The emergence of bacterial resistance to the currently available antimicrobial drugs necessitates further research in the discovery of new safe and effective antimicrobial agents. In the present study, we have intended to formulate herbal hand wash using soapnut plant extracts with potential antibacterial activity and thereby establishing them as a potent antimicrobial agent in the formulation of herbal hand wash [11].

Materials and Methods

Raw material

The pericarp of *Sapindus emarginatus was* collected from the forest of Forest College and Research Institute. The collected fruit of this tree was separated using wooden hammer the pericarp and seeds are separated, the separated pericarp shade dried and soft powdered by using an electric grinder.

Extract preparation

13g of the soft powdered pericarp was used for the solvent extraction process. the 13g powdered pericarp was filled in the thimble of Soxhlet apparatus using methanol and petroleum ether as a solvent. The process of extraction continued for 12 hours at 55 ºC. The solvent extract collected in air-tight containers was preserved in refrigerated condition at 5 ºC for further use. Before completion of this process, the initial weight of pericarp as taken after completion of the extraction remaining residues are oven dried and weighed the difference of weight is calculated (initial weight - oven dry weight) the weight difference shows that amount of saponin extract.

Other ingredients preparation

100 g of *Bixa orellana* seeds were collected from the field of forest college and research institute. The neem leaf and tulsi leaf and neem flowers were also collected. The collected raw material soft powdered by using an electric mixer and is sieved through the 100mm mesh sieve, the fine powder was collected and stored in airtight containers for further use.

The Jasmine flowers collected from the field, to extract the concentrate, pure alcohol is used to Soxhlet apparatus was extracted oil and when the alcohol evaporates, the oil was left behind. This oil used to add up the fragrance of hand wash.

Observing the yield of saponin from fruit by different extraction methods

To observe the yield of saponin from different extraction methods *viz*., Normal water, water bath, cold water, Autoclaving, soxhlet apparatus. 10 gm of soapnut pericarp powder is taken in all the cases and 250ml of water is added and observation was made for their saponin yield in the case of Normal and cold water. In the case of Autoclaving sample was kept at 120 º c for 15 min at 15 lbs pressure. In the water bath, the sample was kept for 90 minutes. The difference in weight gives the yield of saponin.

Antimicrobial studies

The Nutrient agar medium was prepared and the hand is washed in sterile water. These hand wash water samples are collected and Standard serial dilution and plating method was followed to enumerate the microbes. Sterilized filter paper discs were placed on the medium and sample with ethanol and petroleum ether extracts of *Sapindus emarginatus* pericarp loaded on the discs. The plates were incubated and examined for the presence of a zone of inhibition which is indicated by a clear zone surrounding each filter paper.

Agar well diffusion method was also carried out and the diameter of zone of inhibition surrounding the discs and well were measured.

Results and Discussion

Yield of saponin from the extract using various solvents

The yield of saponin differed with the solvents used. The highest yield of saponin was observed, on using ethanol extract. The Initial weight of the sample (soapnut pericarp powder) taken was noted and after extraction of saponin, the remaining residues were oven dried and weighed. The difference in weight (initial weight - oven dry weight) was calculated to estimate the yield of saponin content. Final results showed that the saponin yield was high from ethanol extract 5.48 g (Table 1).

Table 1: Yield of saponin from the extract using various solvents

S. No	Pericarp powder	Initial Weight Pericarp powder	Oven dry weight	Saponin Yield	Percentage %
1.	Petroleum ether extract	13g	10.5g	2.5g	19.23
2.	Ethanol extract	13g	7.52g	5.48g	42.15

Studies on antimicrobial activity of soap nut extractives & hand wash by agar well diffusion method

The antibacterial activity of the ethanol solvent extracts summarized in the table 2. The studies showed that the zone of inhibition on bacterial plates was high around 1.2 cm at concentration of 15 µl of saponin extract.

Table 2: Studies on antimicrobial activity of soap nut extractives & hand wash by agar well diffusion method

S. No	Concentration of Hand wash (µl)	Zone of inhibition (cm)
1.	11	0.8 ± 0.1
2.	12	0.8 ± 0.1
3.	13	0.9 ± 0.1
4.	14	0.9 ± 0.1
5.	15	0.11 ± 0.1

Agar well diffusion method - bacteria - ethanol extract

The studies show that using soap nut pericarp powder ethanol extract the zone of inhibition on bacterial plates showed the higher inhibition zone is 1.4 cm (Table 3).

Table 3: Agar well diffusion method for bacteria using ethanol extract

S. No	Concentration of Ethanol extract (µl)	Zone of inhibition (Cm)
1.	11	0.7 ± 0.1
2.	12	0.8 ± 0.1
3.	13	0.8 ± 0.1
4.	14	0.9 ± 0.1
5.	15	0.13 ± 0.1

Agar well diffusion method - fungi - hand wash

The studies showed that using soap nut hand wash the zone of inhibition on fungal plates exhibited higher inhibition zone of 1.3cm (Table 4).

Table 4: Agar well diffusion method for fungi

S. No	Concentration of Hand wash (µl)	Zone of inhibition (Cm)
1.	11	0.8 ± 0.1
2.	12	0.10 ± 0.1
3.	13	0.10 ± 0.1
4.	14	0.12 ± 0.1
5.	15	0.13 ± 0.1

Agar well diffusion method - fungi - ethanol extract

The studies show that using soap nut pericarp ethanol extract

the zone of inhibition on fungal plates exhibited higher inhibition zone of 2.5cm. (Table 5).

Table 5: Agar well diffusion method for fungi using ethanol extract

S. No	Concentration of solution (µl) Ethanol extract	Zone of inhibition (Cm)
1.	11	0.9 ± 0.1
2.	12	0.9 ± 0.1
3.	13	0.11 ± 0.1
4.	14	0.12 ± 0.1
5.	15	0.25 ± 0.1

Yield of saponin from the extract using various solvents

Table 1 shows that the yield of saponin differs from the solvent used for an extraction, among the two solvents the petroleum ether and ethanol, the ethanol solvent recorded the highest yield of saponin than that of petroleum ether extract. The yield percentage is being 19.23% and 42.15 recovery percentage of saponin very high in ethanol solvent extraction. The yield is 42.15%.

Studies on antimicrobial activity of soap nut extractives and hand wash

Table 2,3,4,5 shows that study conducted to test the antimicrobial effect of the soap nut pericarp powder extract and the final product (Hand Wash). The ethanol extract against bacterial culture was found to have the inhibition zone in the Petri plates around the well, the well loaded with ethanol extract of 15 µl formed zone of inhibition. The zone measures up to 1.4 cm (Table 3). The extract against the fungal culture was found to have the inhibition zone in the Petri plates around the well 2.5cm (Table 5). The soap nut hand wash against the bacterial culture was found to have the inhibition zone in the Petri plates around the well. The well loaded with soap nut hand wash of volume showed 15 µl the zone of inhibition 1.2 cm (Table 2) the hand wash against the fungal culture was found to have the inhibition zone in the Petri plates around the well measuring about 1.3cm (Table 4).

Conclusion

The studies on formulation of chemical free organic hand wash from *Sapindus emarginatus* showed that it contains the antimicrobial activity and good foaming properties because of the secondary metabolites present in the soapnut (Saponin). This herbal hand wash formulation possess unique aroma and potential antibacterial activity.

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