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Short Communication

ANTIBACTERIAL ACTIVITY OF PLANT BIOSURFACTANT EXTRACT FROM *SAPINDUS MUKOROSSI* AND *IN SILICO* EVALUATION OF ITS BIOACTIVITY

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ABSTRACT

Objective: Natural biosurfactants can replace synthetic surfactants and find applications in cosmetic products. Saponin extracts from *Sapindus mukorossi* fruits have emulsifying properties and have traditionally been used in hair care treatments. Their utility can be enhanced by studying their antimicrobial activity against common skin and other microorganisms.

Methods: Aqueous biosurfactant extracts were prepared from fruits of *S. mukorossi*. Concentrated and diluted extracts were tested for antimicrobial activity against *Micrococcus luteus, Brevibacterium linens, Bacillus subtilis, Staphylococcus epidermidis, Escherichia coli* and *Pseudomonas fluorescens* by the well diffusion method and measuring the zone of inhibition. *In silico* biological activity of different saponins present in *S. mukorossi* was studied using the software Prediction of Activity Spectra for Substances (PASS).

Results: Concentrated extracts were most active against all the target microorganisms. Gram positive organisms were inhibited more than Gram negative ones. Diluted extracts produced comparable inhibition zones, suggesting that the extent of dilution does not affect the antimicrobial activity further. *In silico* evaluation showed that major saponin types (Sapindoside B, Sapinmusaponin A, Sapinmusaponin F and Sapinmusaponin N) had antibacterial activity with probable activity to probable inactivity (Pa>Pi) values less than the threshold level of 0.7.

Conclusion: Biosurfactant (saponin) extracts from *S. mukorossi,* can be included in herbal care products not only for their emulsifying properties, but also for their antimicrobial effect. While *in silico* study showed less than threshold level of antibacterial activity, the combination of all these saponin types together probably contributed to the synergistic antibacterial activity.

Keywords: *Sapindus mukorossi,* Biosurfactant, Saponin, Antimicrobial.

Saponins are glycosides and have predominantly been studied from plant sources with some from animal sources [1]. While structurally diverse, they share the properties of hemolysis and foaming [2]. Surface activity, resulting in foaming, is due to the saponins. They show amphiphilic characteristics with sugar moiety and hydrophobic sapogenin. Their surfactant properties make them interesting for applications wherein they can potentially replace synthetic surfactants. Saponins find applications in pharmaceutical, food and cosmetic industries for their emulsifying properties [3]. Due to increased awareness of environmental safety, there has been growing interest in such natural surfactants from microbial and plant sources in recent times [4, 5]. Saponins have been traditionally used from *Sapindus mukorossi*, Quillaja bark, *Balanites aegyptica* and *Fagonia indica* [6-8].

The soap nut tree or *Sapindus* commonly grows in tropical and sub-tropical regions. The extract from the fruits (ritha) has been traditionally used in India for washing and hair care products. The pericarp of the fruits of *Sapindus* contains 6-10% saponins [9]. While many studies have reported the structural and functional properties of purified saponin, Ghagi *et al.*, [10] have shown that emulsification properties of the crude saponin extract is similar to that of purified extracts, thereby potentially bringing down production costs.

The biosurfactant extracts from *Sapindus* can be used in place of synthetic surfactants in products such as shampoos, soaps and other cosmetics. Being natural, apart from eco-friendly aspects, it is important to also understand their antimicrobial activities. Such characteristics would enhance their utility. Hence, the main objective of the present study was to evaluate the antimicrobial properties of plant biosurfactant extracts prepared from fruits of *S. mukorossi.* To our knowledge, comprehensive studies on the effect of biosurfactant from *S. mukorossi* for activity against common skin microorganisms have not been carried out. Our report presents the results of a study on its antibacterial activity against such skin-inhabiting and other bacteria. Further, *in silico* studies were performed on saponins from this plant to evaluate their antibacterial and other bioactivity potential.

The fruits of *S. mukorossi* were soaked in water overnight (a ratio of 400g fruits to 1L water) and then boiled till it became soft. Aqueous extract was prepared from this softened fruit using a hydrostatic pressure machine. Concentrated form of the extract as well as 1:1 and 1:2 dilutions were prepared with water as diluent and used for studying antimicrobial activity.

Agar-well diffusion method was used for this purpose. Mueller-Hinton agar was prepared, sterilized and poured into petri dishes. Six target organisms (obtained from Microbial Type Culture Collection-MTCC, Chandigarh) were used-*Micrococcus luteus* (MTCC-106), *Brevibacterium linens* (MTCC-268), *Bacillus subtilis* (MTCC-121), *Staphylococcus epidermidis* (MTCC-435), *Escherichia coli* (MTCC-1679) and *Pseudomonas fluorescens* (MTCC-2421). The target organisms (100 µl) were spread plated and 40 µl of each sample was loaded in triplicates in wells. Sodium Dodecyl Sulfate (SDS), as synthetic surfactant, at a concentration of 0.1% was used as positive control. The plates were incubated at 37^{o}C overnight and observed for zones of inhibition. The diameters of the clear zones were measured.

To predict the biological activity profile of different saponins present in *S. mukorossi,* Prediction of Activity Spectra for Substances (PASS) software was used [11]. *S. mukurossi* contains a mixture of different saponins with Sapindoside B (oleanane type) as one of the major constituents [12]. The structures of Sapindoside B and other saponins such as Sapinmusaponin A (dammarane type), F (tricullane type) and N (oleanane type), obtained from PubChem or other literature, were used as input for PASS as per instructions and their biological activity predicted.

All experiments were performed in triplicates and experiments repeated thrice. The data were expressed as mean±standard deviation. Data was subjected to one way analysis of variance

(ANOVA) and means compared using Duncan's Multiple Range Test at significance level of P<0.05 using software COSTAT.

Concentrated and diluted *S. mukorossi* bio surfactant extracts were tested for antibacterial activity. As seen from fig. 1, concentrated extracts produced maximum zones of inhibition against all the target microorganisms (1.5-1.87 cm), statistically significant at p<0.05. Inhibition of Gram positive organisms was more as compared to Gram negative ones. The maximum zone of inhibition amongst Gram positives was seen against *B. subtilis* and *E. coli,* amongst Gram negatives. Diluted (1:1 and 1:2) extracts produced comparable inhibition zones, suggesting that the extent of dilution does not affect the antimicrobial activity further.

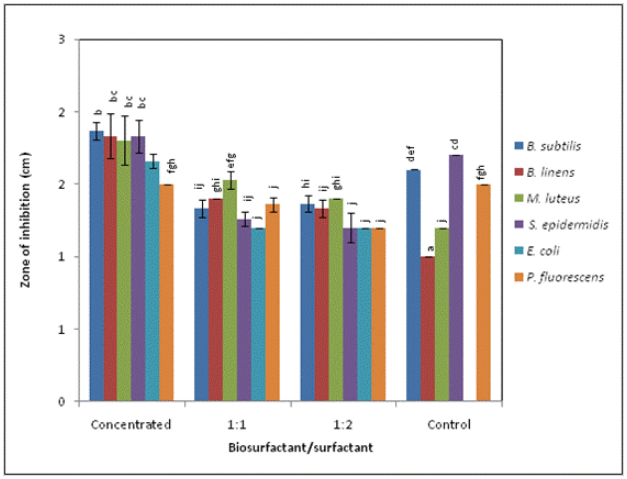

Fig. 1: Antibacterial activity of concentrated and diluted biosurfactant extracts of *S. mukorossi* against Gram positive and Gram negative microorganisms. Letters indicate statistical significance at p<0.05

It was observed that concentrated extracts inhibited the target organisms ranging from 100-180% of control SDS (fig. 2). It was notable that positive control (0.1% SDS) did not inhibit *E. coli* while the plant bio surfactant did.

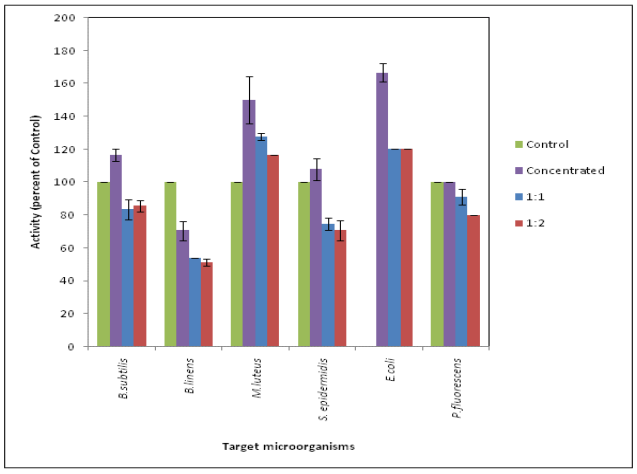

Fig. 2: Antimicrobial activity of concentrated and diluted biosurfactant extracts of *S. mukorossi* as percent of control (0.1% SDS)

Ethanol and chloroform extracts of *S. mukorossi* have been shown to inhibit *Helicobacter pylori* [13]. Hot aqueous, cold aqueous, acetone, methanol and ethanolic extracts were shown to be active only against *Saccharomyces cerevisiae* amongst different organisms causing dental caries [14]. Reports have shown that while ethanolic extracts of plants of *B. aegyptica* were effective against *Klebsiella pneumonia*, aqueous extracts were less active and only against *Salmonella typhi* [15]. A 100 mg ml^{-1} aqueous leaf extract showed the least activity against *S. typhi* as compared to acetone extract [16].

PASS predicts the biological activities based on the chemical structure of compounds. The PASS predicted results of values where the probable activity to probable inactivity (Pa>Pi) is considered for each compound showing different biological activities, Pa being probable activity and Pi probable inactivity [11]. The results showed that Sapindoside B, Sapinmusaponin A (dammarane type), Sapinmusaponin F (tricullane type) and Sapinmusaponin N (oleanane type) were predicted to have antibacterial activity with Pa>Pi values of 0.630, 0.627, 0.535 and 0.686 respectively. These are less than the Pa>Pi threshold level of 0.7; other predicted activities of higher threshold levels were those of membrane integrity antagonist, hepatoprotectant, chemopreventive, immunostimulatory and several other activities. *Sapindus* saponins have been documented to have the ability to inhibit adherence of protozoan parasite to cells [17] and exhibit activity based on attachment to membrane. Antineoplastic activity has also been documented against HeLa, Hepa59T, NCI and Med cell lines [18]. This confirms that prediction based by using PASS match the actual bioactivity. In our study, while antibacterial activity has shown lower threshold level, it is possible that the combination of all these saponin types together contributed to synergistic antibacterial activity.

In this study, the aqueous extracts of fruits of *S. mukorossi* have shown an antibacterial effect against various skin and other microorganisms. Since the prepared biosurfactant extracts are commonly used in herbal preparations meant for the skin, this study presents useful data on the utility value of including plant biosurfactants in such formulations.

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CONFLICT OF INTERESTS

Nidhi Maheshwari uses *S. mukorossi* biosurfactant extracts in the herbal cosmetic formulations manufactured and sold by Ishan Herbotech International, India.

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