Antimicrobial activities of soaps containing *Senna alata* leaf extract

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Abstract

Senna alata is an important ethnomedicinal plant and is often used traditionally to treat skin diseases. Hence, it can be a potential attractive ingredient for natural skincare products. In this study, we determined the potential of producing antimicrobial soaps by using *S. alata* leaf extract, either aqueous crude extract or essential oil, as the key ingredient and also virgin coconut oil as the base. Although *S. alata* essential oil is not feasible yet to be considered for soap production due to its poor extraction yield, the aqueous leaf crude extract had shown promising potential. Our antimicrobial assays showed the aqueous extract exhibited antifungal activity but did not show any antibacterial activity under the conditions tested. Similarly, the prototypes of *S. alata* soap containing the aqueous extract also showed an antifungal activity against *Saccharomyces cerevisiae*. Therefore, the potential use of *S. alata* for antimicrobial soaps warrants further consideration and study.

Index Terms: antimicrobial, *Senna alata*, medicinal plant, soaps

1. Introduction

Senna alata (L.) Roxb. (synonym *Cassia alata*) or candlestick is considered an important ethnomedicinal plant, as it has been used traditionally to treat fungal infections such as ringworm, and to relieve other skin diseases such as itchiness and eczema. It can also be used to treat constipation and intestinal worm infestation.[1] The plant has been previously reported to have antibacterial and antifungal activities.[2-6] Furthermore, it is also reported to have antioxidant, anticancer, anti-allergic and laxative activities.[7-10]

S. alata has the potential to be used as an attractive ingredient for natural soaps, as natural and organic skincare products have been gaining in popularity. However, many of the commercially available natural soaps in the market have not been scientifically proven for their ethnomedicinal claims. Thus, it is vital for any skincare product(s) to be scientifically validated. In this study, we presented prototypes of *S. alata* soaps with scientific validation on their antifungal activities (no antibacterial activities were detected).

2. Experimental approach

Sample collection and preparation

S. alata leaves were collected from Jalan Madang, Brunei Darussalam in March 2015. The samples were ground into powdered form after being air-dried for three weeks.

Extraction of aqueous crude extract

Ultrasound extraction was employed.[11] Powdered samples were mixed with distilled water in the ratio of 1 g to 10 ml. The mixture was ultrasonicated (Soltec Sonica Ultrasonic Cleaner) for 30 mins at 25°C. It was subsequently centrifuged and filtered to remove solid residues, and the filtrate was used directly for antimicrobial assays and soap preparation.

Extraction of essential oil

Hydrodistillation was used to extract *S. alata* essential oil.[12] Powdered samples (100 g) were

boiled in distilled water, and the distillate was collected. To obtain the essential oil, 10% hexane was subsequently added. The resulting oil-containing solvent was collected and gently evaporated to remove hexane, leaving behind the essential oil.

Soap preparation

Prototypes of *S. alata* soap were prepared as described elsewhere [13], to contain virgin coconut oil (as the soap base) and sodium hydroxide in the ratio of 1 g to 0.178 g, respectively. The latter was initially dissolved in distilled water before mixing with the former and also the aqueous extract in different volumes. The mixture was heated with continuous stirring until it became thick. It was subsequently poured into a moulding tray and allowed to solidify. For antimicrobial assays, the solidified soaps were dissolved at 100 µg/ml with distilled water.

Antimicrobial assays

Antimicrobial activities were determined by agar-well diffusion method.[14] Four bacterial strains were studied, *Bacillus subtilis* ATCC-11774, *Escherichia coli* ATCC-11775, *Pseudomonas aeruginosa* ATCC-27853, and *Staphylococcus aureus* ATCC-29213, and one fungal strain, *Saccharomyces cerevisiae*. An overnight nutrient broth culture of the microorganism was uniformly spread onto a Mueller-Hinton agar plate. The agar was bored to create wells with 4-mm diameter, in which the extract was introduced for testing. For positive and negative controls, streptomycin sulfate and distilled water were also tested, respectively. The plate was incubated at 37°C for 18 hours. Antimicrobial activity was detected by a zone of clearance around the well. The diameter of this zone of inhibition was measured.

3. Results and Discussion

S. alata aqueous crude extract was successfully obtained, which had a brownish colour and a mild tea-like aroma, whereas the *S. alata* essential oil had an aroma similar to green tea. However, the percentage yield of extraction was low (<1%) under the conditions tested, suggesting higher starting plant material and/or different extraction technique might be required for viable extraction of essential oil. Alternatively, other parts of the *S. alata* plant could also be tested such as the flowers, roots or seeds.

Under the conditions tested, the aqueous extract of *S. alata* leaf extract did not show any antibacterial activity against Gram-negative bacteria (*E. coli* and *P. aeruginosa*) and Gram-positive bacteria (*B. subtilis* and *S. aureus*) (data not shown). However, it showed an antifungal activity against *S. cerevisiae* with a zone of inhibition of 21 ± 1 mm (mean ± standard deviation, SD of 4 replicates). Similarly, the prototypes of *S. alata* soap containing different fractions of the aqueous extract also did not exhibit any antibacterial activity but also showed an antifungal activity against *S. cerevisiae* (***Table 1***). Although the study could be improved further by also testing other important fungal species, it nicely complementes the ethnomedicinal uses of *S. alata* as antifungal remedy.[1]

Table 1. Antimicrobial activities of *S. alata* soaps. Antibacterial activities were not detected for *E. coli*, *P. aeruginosa*, *B. subtilis* and *S. aureus* (data not shown). Negative control did not show any detectable activity as expected. [a] Concentration is calculated as the volume to volume fraction of the aqueous extract in the soap. [b] Mean values (±SD) of four replicates were presented.

Concentration of *S. alata* aqueous extract (%)[a]	**Zone of inhibition for *S. cerevisiae* (mm)[b]**
0	41 ± 3
3	45 ± 5
6	41 ± 1
20	39 ± 1
40	40 ± 2
60	42 ± 2
80	38 ± 3

Our statistical analysis using one-way ANOVA did not find any significant differences ($P>0.05$) between the means for the different extract concentrations. This means increasing the aqueous extract concentration in the soap did not show any detectable improvement in the antifungal activity. Although the size of the inhibition zone could indicate the potency of the plant antimicrobial, the zone of inhibition test

itself is considered as a qualitative method. Moreover, the soap with 0% aqueous extract also showed similar zone of inhibition compared to the other concentrations, suggesting the observed antifungal activity of these soaps could be contributed solely by or in synergy with virgin coconut oil and/or sodium hydroxide, which were also the other components of the soaps. Virgin coconut oil has been reported to have antimicrobial activities.[15]

In light of the possible synergy, virgin coconut oil should also be further studied. In view of making the soap, it would not only be commercially attractive but also might increase the bioactive potency of any soap that contained different combinations of plant extracts or oils. Thus, it should be taken into consideration when designing future soaps.

A lack of detectable antibacterial activity in this study does not necessarily mean the absence of potential antibacterial in *S. alata*, as the outcomes of the test could be condition-dependent. The use of low concentration of aqueous crude extract could be a limiting factor, and hence, for future studies, freeze drying or lyophilisation could be considered to enable analysis with concentrated aqueous extract. Apart from water, the use of other solvents to generate different *S. alata* extracts could also be considered for future analyses, as different solvents would generally extract a variety of different phytochemicals.

Fractionation of the crude extract by column chromatography could also be considered for the isolation of active fraction(s) containing specific bioactive compound(s), which could be used directly in the soap making instead of the whole crude extract itself. However, in view of a commercial local production, this would only escalate the cost of the soaps but low-priced products are more attractive and competitive.

Finally, other vital tests such as toxicity test should also be taken into account before *S. alata* soaps could be made available in the market, as it might cause unwanted side effect.[16] The effect of *S. alata* leaf extract on the natural microbiome present on the skin surface should also be studied to gain more insight into its antimicrobial efficacy. The prototypes of *S. alata* soaps presented here arguably warrant further consideration and study.

4. Conclusion

Aqueous crude extract from *S. alata* leaves had shown a promising potential to be used for commercial antifungal soap production.

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