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Development of a herbal soap using selected medicinal plants and evaluation of its antimicrobial activity

NM Wijayawardhana, MMDU Cooray, DI Uluwaduge, LDAM Arawwawala and

#WJABN Jayasuriya

*Department of Pharmacy and Pharmaceutical Sciences, Faculty of Allied Health Sciences,
University of Sri Jayewardenepura, Sri Lanka*

*Department of Medical Laboratory Sciences, Faculty of Allied Health Sciences,
University of Sri Jayewardenepura, Sri Lanka*

Industrial Technology Institute, Colombo 07, Sri Lanka

#banukie@sjp.ac.lk

Abstract: Soaps are used as a modality for topical application of medicinal plants used in the treatment of skin infections. In herbal soaps, natural bioactive constituents with a variety of therapeutic activities are incorporated into basic soap medium. Natural herbs are the main ingredients of herbal soap which are safer and beneficial than the commercial soaps. Antimicrobial activity of leaf extracts of *Azadirachta indica*, *Cassia fistula* and flower extract of *Nelumbo nucifera* has been evaluated individually and in combination by a previous study. This study aims to formulate a novel herbal soap using the above combined extract and to investigate its antimicrobial activity. Further, this study evaluates the physicochemical characteristics of the soap. The combined extract used in the formulation of soap was prepared by incorporating aqueous extracts of *A. indica*, *N.nucifera* and ethanol extract of *C.fistula* at a ratio of 4:1:1 according to the previous study. Soap was formulated by including the combined extract with other ingredients into the melted glycerin soap base. The antimicrobial activity of formulated soap was tested by agar well diffusion method against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* and compared with standard drugs. Test was done in triplicate. Formulated soap exhibited

antimicrobial activity against tested organisms with a highest activity against *S aureus*. Physicochemical parameters of soap were evaluated by determining the colour, pH, % free alkali and % of alcohol insoluble matter of the soap. pH at 28 °C was 9.11 and % of alcohol insoluble matter was 24.6% which were within the accepted range. Further studies are pursued to evaluate the safety and efficacy of the formulated herbal soap.

Keywords: Antimicrobial activity, herbal soap, *Azadirachta indica*, *Cassia fistula*, *Nelumbo nucifera*

Introduction

Depending on the recent discoveries done on medicinal plants, the use of herbal medicines has been enormously increased in worldwide. The demand for herbal products is increasing as they are generally safer, cost-effective, and has fewer adverse effects compared to synthetic products. Soaps are used as a modality for topical application of medicinal plants used in the treatment of skin infections (Kareru, P.G., Keriko, J.M., Kenji, G.M., Thiong'o, G.T., Gahanna, A.N. and Makira, H.N., 2010). Natural bioactive constituents with a variety of therapeutic activities are incorporated into basic soap medium in preparation of soap (Wijetunge, W.M.A.N.K. and Perera, B.G.K., 2015). Soaps that are

available in the market are formulated by incorporating one or more plant extracts and claimed for the antifungal or antibacterial property.

Azadirachta indica, *Cassia fistula* and *Nelumbo nucifera* are medicinal plants that are frequently found in Sri Lanka. *C. fistula* which belongs to the family of Fabaceae possesses antioxidant, antitumor, antimicrobial and anti-inflammatory activities. Antimicrobial activity was detected in bark, leaves and flowers of *C. fistula* (Ali, M.A., Sayeed, M.A., Bhuiyan, M.S.A., Sohel, F.I. and Yeasmin, M.S., 2004)

A. indica belongs to the family of Meliaceae, is often known as neem. The plant has been demonstrated anti-bacterial anti-fungal, antiviral, anti-oxidant, anti-malarial activities etc. (Biswas, K., Chattopadhyay, I., Banerjee, R.K. and Bandyopadhyay, U, 2002).

N. nucifera is an aquatic plant in which its flower has shown hypoglycemic, antioxidant, anti-microbial and antihypertensive abilities. Different chemical compounds with different therapeutic activities were isolated from flowers of *N. nucifera* (Gunawardana, S.L.A. and Jayasuriya, W.J.A.B.N., 2019).

Although the antimicrobial activity of leaf extracts of *A. indica* and *C. fistula* and flower extract of *N. nucifera* has been investigated individually, a herbal soap has not been developed using a combined extract of said plants. Antimicrobial activity of the combined extract of leaf extracts of *A. indica*, *C. fistula* and flower extract of *N. nucifera* has evaluated in our previous study (unpublished data). Hence, the present study aims to formulate a novel herbal soap using the above combined extract and to investigate the antimicrobial activity of the formulated soap. Furthermore to evaluate the physicochemical characteristics of the soap.

Methodology

Plant Collection and Authentication.

Fresh leaves of *A. indica*, *C. fistula* and white flowers of *N. nucifera* were collected from Southern and Western Provinces, Sri Lanka. Plants were authenticated at the National Herbarium, Botanical Gardens, Peradeniya, Sri Lanka. The collected plant parts (leaves and flowers) were washed, air-dried and powdered to a coarse powder and stored in air-tight bottles.

Preparation of the extracts

- Preparation of hot ethanol leaf extract of *C. fistula*

Prepared sample of *C. fistula* was added to a round-bottomed flask containing 150 ml of ethanol and boiled for 4 hours. Then the extract was filtered using Whatman 0.45 µm filter paper and the filtrate was concentrated using a rotary evaporator. Stored at 4°C.

- Preparation of hot aqueous extracts of leaves of *A. indica* and flowers of *N. nucifera*

Sample of each plant was added to round-bottomed flasks separately containing 150 ml of distilled water and boiled for 4 hours. Then the extracts were filtered using Whatman 0.45µm filter paper and the filtrate was concentrated using a rotary evaporator and freeze-dried. Extracts were stored at 4 °C.

Preparation of the combined extract

According to the previous study the ratio of the effective combined extract was aqueous extract of *A. indica* (4): aqueous extract of *N. nucifera* (1): ethanol extract of *C. fistula* (1). Hence 1000 mg/ml of aqueous extract of *A. indica*, 250 mg/ml of aqueous extract of *N. nucifera* and 250 mg/ml of ethanol extract of *C. fistula* were combined.

Test microorganisms

Isolates of *Candida albicans*, *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 were obtained from the Department of Microbiology, Faculty of Medical Sciences University of Sri Jayewardenepura.

Formulation of a herbal soap using the combined extract

Herbal Soap was formulated by incorporating the combined extract with other ingredients into the melted glycerin soap base. Volatile oil of *N. nucifera* was added. Then the mixture was stirred for 30 minutes and the melted mixture was poured into molds for solidification

Determination of the antimicrobial activity of the formulated herbal soap

Different concentrations of formulated soap were prepared by dissolving it in 1% DMSO. Then the antimicrobial activity of each solution was tested by agar well diffusion method against *S. aureus*, *P. aeruginosa* and *C. albicans*. Gentamycin and clotrimazole were used as the positive control for bacteria and fungi respectively. Bacterial cultures and fungal cultures were incubated at 37 °C for 24 hours and 48 hours respectively. Antimicrobial activity was determined by measuring the zone of inhibition around the well against each microorganism (Afsar and Khanam, 2016).

Evaluation of physicochemical parameters of the formulated herbal soap

Physicochemical parameters of formulated soap were evaluated by determining the physical characteristics such as colour, odor, etc. pH, Foam height, foam retention time, % of alcohol insoluble matter and moisture matter.

Results and Discussion

Antimicrobial activity of the prepared herbal soap was investigated using the agar well diffusion method. Table 1 represents the diameter of the zone of inhibition for *S. aureus*, *P. aeruginosa* and *C. albicans* at different concentrations of herbal soap.

Table: 1: Diameter of the zone of inhibition for *S. aureus*, *P. aeruginosa* and *C. albicans* at different concentrations of herbal soap

Concentration(mg/ml)	Diameter of zone of inhibition (mm)		
	<i>S. aureus</i>	<i>P.aeruginosa</i>	<i>C.albicans</i>
1000	28±1	25±1	25±1
500	24±1	23±1	25±1
250	22±1	20±1	20±1
125	20±1	19±1	19±1
62.5	18±1	17±1	18±1
31.25	15±1	15±1	15±1
Positive control	31±1	30±1	26±1
Negative control	ND	ND	ND

ND- not detected

According to the results shown in Table 1, herbal soap showed antibacterial and antifungal activity as the combined extract. When consider the antimicrobial activity of individual plant extracts evaluated by our previous study, the herbal soap demonstrate comparatively better activity than individual plant extracts.

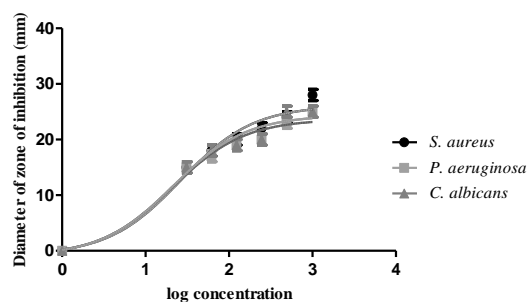


Figure 1: Dose-response curve for formulated soap against *S.aureus*, *P.aeruginosa* and *C.albicans*

Dose-response curve for formulated soap against *S.aureus*, *P.aeruginosa* and *C.albicans* is shown in Figure 1. According to the Figure 1, a dose dependent antimicrobial activity was observed ($R=0.9735, 0.9766, 0.9693$) for each microorganism.

Physical parameters including colour, odor, appearance and chemical parameters including pH, % free alkali, foam height, foam retention and alcohol insoluble matter of formulated soap was evaluated. The results obtained shown in the table 2.

Table: 2 physicochemical parameters of formulated herbal soap

Color	Odor	Appearance	pH	% free alkali	Foam height(cm)	Foam retention(min)	Alcohol insoluble matter (%)	Moisture/Volatile Matter (%)
Dark brown	Fragrant	Good	9.11	1.6	9.5	7	24.6	15.85

Antimicrobial soap was formulated considering its microbiological, physical and chemical properties. The selected herbal extracts used as active ingredients and several additives have been incorporated into the glycerin soap base which has been used as the vehicle for the formulation. Herbal soaps in 50 g in weight were produced by adding plant extracts, distilled water, stearic acid and natural volatile oil of *N. nucifera* into the glycerin soap base. The cleansing property of soap provided by natural oils, alkali and water containing in soap base.

Distilled water was selected as the solvent for preparing plant extracts. Stearic acid functions as a hardening agent when developing solid dosage forms and it also acts as a softener that produces cool sensation. As a fragrance enhancer, volatile oil of *N. nucifera* was added into the formulation. No synthetic bleaching or coloring agents were added to the formulation. As the main aim of the study, the antimicrobial activity of formulated soap was determined against some common skin pathogens. Six concentrations of soap were prepared by serial dilution method. DMSO has

been used as a solvent to dissolve and make different concentrations of soap.

C. albicans has shown slight inhibition only for *N. nucifera* aqueous extract. But there were markedly increased inhibition against *C. albicans* for the soap formulation. The reason behind the above results may be the synergistic activity of a combination of plant extracts used in the formulation of soap or the total sum of effects when compared to individual extracts.

Appearance, color and odor were observed as physical properties of the final product. The dark brown color of the soap arises due to the plant extractions. Fragrant odor was the result of adding the natural volatile oil of *N. nucifera*.

The pH of the soap at 28°C temperature was 9.11. According to the SLS 1220 standard (Sri Lanka accreditation board for conformity assessment), the pH of the soap should be in the range of 4-10. Hence, the pH of the formulated soap was in an acceptable range and safe to use. The increased pH of the soap produces a significant increase in microbial growth.

One of the parameters used to detect the purity of the soap is matter insoluble in alcohol (MIA). MIA value of the formulated soap obtained as 24%. This parameter used to determine the non-soap ingredients known as builders or fillers such as sodium carbonate, sodium silicate and minor compounds such as whitening agents, bleachers in the final product. Higher the MIA value indicates that it contains a high level of impurities which may cause the level of impurities of alkali used for the soap. % MIA range is between 36- 77%

Conclusion

Antimicrobial activity of the formulated herbal soap using the combined extract of *A.indica*, *C.fistula* and *N.nucifera* was considerably higher when compared to the individual plant extracts against *S.aureus*,

P.aeruginosa and *C.albicans*. Therefore this study concludes that the synergism between the constituents or total sum of effects of the combination may cause enhanced growth inhibition of tested microorganisms. Further the value of pH, % free alkali, alcohol insoluble matter and moisture content of the formulated soap were within the accepted range. Clinical trials are recommended to evaluate the safety and efficacy of the formulated soap in future studies.

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