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Anti-microbial evaluation of herbal soap containing neem oil and nalpamaradhi oil

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ABSTRACT

Bacterial and fungal skin infections are most common amongst people, requiring significant attention for treatment, and also to maintain a healthy skin thereafter. The herbs are known to possess various potentials like anti-inflammatory, antibacterial and antifungal properties which are explored for ages and incorporated into various forms, for human use. One such usage is an herbal soap formulation that is used not only for treating microbial infections, but also for using it on a daily basis. The aim and objective of the present study is to formulate an herbal bath soap using Neem oil and Nalpamaradhi oil and explore its antimicrobial properties. Neem and Nalpamaradhi oil were equally mixed for soap making by simple saponification reaction and the formulated herbal bath soap was further subjected to physic-chemical characterizations such as estimation of color, odor, texture, total fatty matter (76.5±0.001), foam test (8 cm), moisture content (2.2±0.005) and pH (6.5±0.001). The formulation was found to be a stable solid without any colour change. The antibacterial and antifungal activities of the formulated soap were performed and result shows that the soap has got considerably good antimicrobial activity. The microbial strains used were of significant importance as the human skin is prone to such infections that arise from blisters, wounds and other eruptions. Hence, the oils were best suited for herbal soap preparation, for treating various skin infections and also for using it as regular bath soap.

Keywords: Neem, Nalpamaradhi, Total fatty matter, Antifungal, Antibacterial, Herbal soap.

1. INTRODUCTION

Plants with medicinal properties are being used as traditional medicine from times immemorial. The extract from the leaves, stem and roots of various medicinal plants have been employed as a natural remedy in curing various ailments and diseases. Even though many plant based products have been replaced by synthetic chemicals, the safety and efficacy of Ayurvedic products could not find their match. The active constituents responsible for such medicinal values are employed topically as creams, soaps, oils and ointments for treating skin related ailments like acne, wounds, eczemas, and ring-worms, as an anti-microbial agent and for cosmetic purposes [1].

Soaps are a very common vehicle for application of these medicinal plants for external use in the treatment of skin diseases [2]**.** Soap is the one of the most effectual cleaning agent in water. Soaps are prepared by the process of saponification reaction. Sodium and potassium salts are used for the preparation of soaps. Soaps are widely used in cleaning and washing of skin. The washing property of soaps are due to the presence of fatty acids that can be obtained from plants and animal sources having both saturated and unsaturated fatty acid chain such as oleic acid, lauric acid, myristic acid, palmitic acid and stearic acid. Many skin irritations like dryness, flaking, redness, itching and rash are more often caused by the chemicals used in commercial soap; some of the chemicals used include petroleum products and chemical fragrances [3]**.**

Almost every part of a Neem tree, *Azadirachta indica* (Meliaceae), is known for its therapeutic values and has been in use as traditional medicine to treat a wide range of human disorders since ancient times. It is an evergreen tree indigenous to South Asia and in

most parts of Indian subcontinent. Antimicrobial activities of neem have widely been recognized [4].

Nalpamaradhi oil is Ayurvedic oil used in the treatment of skin diseases with itching such as eczema. This oil is formulated based on Kerala Ayurveda principles. It is used in the treatment of skin diseases, Herpes, Scabies, Eczema, Dermatitis, allergic skin diseases such as ring worm infestation, Blood impurities. This oil is also used as baby massage oil, to relieve any minor skin conditions and to improve fairness and skin complexion, since it contains Turmeric and sandalwood. This oil is meant to be used for external application only. It is applied over the affected skin parts. Effective on Pittaja skin disorders. There are no side effects with this oil on external application [5].

The present study is based on the formulation of a herbal soap containing Neem oil and Nalpamaradhui oil and evaluation of the soap for physicochemical and antimicrobial properties.

2. MATERIALS AND METHODS

2.1. Purchase of Neem oil and Nalpamaradhi oil

Good quality Neem oil and Nalpamaradhi oil were purchased from Kottaikal Arya Vaidya Sala, Kottaikal, Malappuram, Kerala.

2.2. Soap preparation

Neem oil, 50 g and Nalpamaradhi oil 50 g was weighed into a 500 ml beaker, heated to about 60°C. Saponification was initiated by addition of 20 ml of 23.5 % sodium hydroxide. To the resulting solution, 60 g of Sodium hydroxide pellets dissolved in 100 ml of distilled water was added gradually with constant stirring until completion of saponification. Sodium chloride 8 g dissolved in 30 ml of distilled water was added to the grain soap. The salt was added to separate the spent lye in the bottom, while saponified mass floats on the surface to reduce the soap viscosity and to separate the glycerol water in the bottom. The glycerol water was isolated by siphoning. Therewith, the soap paste was washed again by (5-10%) hot water (90 °C) to reduce excess sodium hydroxide and sodium chloride and any impurities found in the soap paste. The soap obtained was washed with 10 ml of distilled water, filtered using a linen cloth, air-dried, then a small amount of water was added to soften it while heating. The soap was placed in a cast and allowed to dry [6].

2.3. Physicochemical evaluation of herbal soap

organoleptic evaluation

Organoleptic parameters like colour, odour and texture were evaluated manually

2.3.1. Determination of pH

10 g of the powdered herbal soap was weighed and dissolved in distilled water in a 100 ml volumetric flask. This was made up to prepare 10 % soap solution. The pH of the 10 % soap solution was determined using a pH meter [6].

2.3.2. Determination of foaming ability

2.00 g of the soap was dissolved in 50 ml of distilled water in a 100 ml measuring cylinder and shaken vigorously for 2 min. It was allowed to stand for 10 min after which the height of the foam was measured. This was repeated thrice and the mean computed [7].

2.3.3. Determination of total fatty matter (TFM)

The total fatty matter test is carried out by reacting soap with acid in the presence of hot water and measuring the fatty acids obtained. About 10 g of finished soap was weighed and 150 ml distilled water was added and heated. The soap was dissolved in 20 ml of 15 % Sulphuric acid while heating until a clear solution was obtained. Fatty acids on surface of the resulting solution was solidified by adding 7g of bee wax and reheated. The set up was allowed to cool to form cake [6]**.** Cake was removed and blotted to dry and weighed to obtain the total fatty matter using a formula:

$$\text{Percentage TFM} = (A - X)/W \times 100$$

Where;

A= weight of wax+ oil, X= weight of wax, W= weight of soap.

2.3.4. Determination of Moisture content

About 10g of the sample under study were accurately weighed and transferred to a tarred china dish of known weight and kept in a hot air oven at 100 – 105°C for an hour. Then, the sample was weighed along with china dish to deduct the actual weight of tarred china dish. The weight of the content was noted to calculate the percentage moisture content [6].

$$\text{Moisture content} = (\text{Difference in weight/initial weight}) \times 100$$

2.3.5. Anti bacterial activity study

Materials required

Muller Hinton Agar Medium (1 L), Nutrient broth (1L), Standard-Streptomycin, concentration 10 mg/ml

Bacterial strains - Pseudomonas aeruginosa,Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumonia, Escherichia coli

2.3.5.1. Procedure

Agar Well diffusion method was used, petriplates containing 20 ml Muller Hinton medium were seeded with 24 hr culture of bacterial strains such as *Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecalis,* and *Klebsiella pneumoniae* and *Escherichia coli*. Wells of approximately 10 mm was bored using a well cutter and sample of 50, 100, and 200 mg concentrations were added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well. Streptomycin was used as a positive control [8].

2.4. Anti fungal activity study

Materials required

Muller Hinton Agar Medium (1 L), Nutrient broth (1L), Standard drug - Fluconazole, concentration 25mcg/disc

Fungal strains - *Candida albicans, Aspergillus niger.*

Procedure

In order to access the biological significance and ability of the soap sample, the zone of inhibition was determined by Agar well diffusion method. Potato Dextrose Agar plates were prepared and Grown overnight, different species of fungus such as *Candida albicans* and *Aspergillus niger* were swabbed. Wells of approximately 10 mm was bored using a well cutter and samples of different concentration was added; the zone of inhibition was measured after overnight incubation and compared with that of standard antimycotic drug; Fluconazole 25 mcg/disc [8].

3. RESULT AND DISCUSSION

3.1. PREPARATION OF HERBAL SOAP

Herbal soap was prepared by using two medicinally important oils; Neem oil and Nalpamaradhi oil. The picture is shown in figure 1 and figure 2

Figure - 1: Herbal Soap.

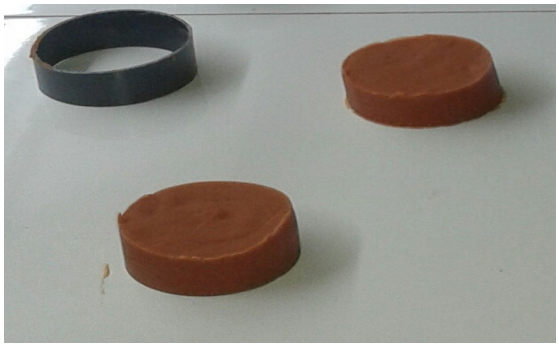

Figure - 2: Herbal Soap along with Mould

3.2. Physicochemical evaluation of herbal soap

Physicochemical parameters like colour, odour, texture, pH, Foaming property, moisture content and Total fatty matter were evaluated and the results were tabulated in table 1.

Table - 1: Physico-chemical evaluation

Parameters	Results
Colour	Dark Brown
Odour	Pleasant odour of Nalpamaradhi oil
Texture	Solid and smooth
pH	6.5±0.001
Foaming index	8cm
Moisture content	2.2±0.005
Total fatty matter	76.5±0.001

3.3. Anti microbial activity study

The antibacterial and antifungal activity of soap sample by well diffusion method against various microorganisms such as *Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumonia, Escherichia coli, Candida albicans* and *Aspergillus niger* were performed and the zone of inhibition was tabulated in table 2 and shown in figure 3 and figure 4

Soaps aid in general body hygiene by physical removal of microorganisms adhering lightly to the skin. The act of washing or scrubbing the body with the soap is expected to lead to a reduction in the microbial load on the skin and this can contribute to a reduction in the incidence of skin infections. Apart from this physical removal of organisms on the skin, the achievement of therapeutic effect of an herbal soap can be due to direct antimicrobial activity on microorganisms present on the skin. These include pathogens of importance in skin and wound infections and commensals implicated in opportunistic infections of the skin in the immune compromised [9].

Table - 2: Antibacterial activity of soap and standard drugs.

Organism	Zone of inhibition (mm) of soap			Zone of Inhibition (mm)
	50 mg	100 mg	200 mg	
Antibacterial				Streptomycin – 200 mcg
Pseudomonas aeruginosa	2.5	2.7	3.1	
Staphylococcus aureus	1.9	2.1	2.8	
Enterococcus faecalis	2.0	2.1	2.6	4.5
Klebsiella pneumonia	2.2	3.2	3.3	
Escherichia coli	2.4	2.6	3.7	
Antifungal				Fluconazole – 25 mcg
Candida albicans	2.6	2.7	3.1	2.1
Aspergillus niger	1.7	2.9	2.3	

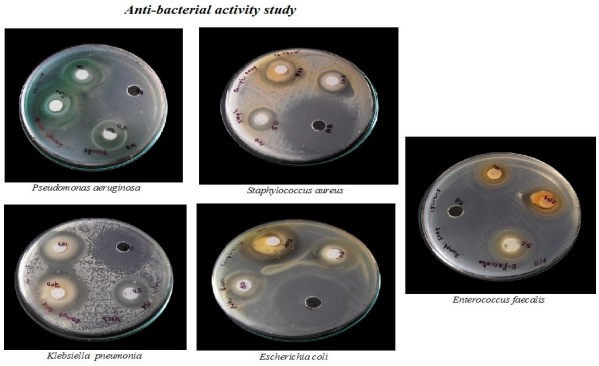

Figure - 3: Anti-bacterial activity study of herbal soap.

Figure - 4: Anti-fungal activity of herbal soap.

Checking the pH of soap is necessary not only for the purpose of improving soaps quality but to regulate the pH level which shall not contribute to the hardness of hands and skin. For the purpose of protecting public health, high pH levels in the 9 to 11 range or low in the 3 to 5 level are considered deleterious to the skin. This is in accordance with NAFDAC regulatory requirements on cosmetics, soaps and detergents [10]. This high value is due to incomplete alkali hydrolysis resulting from the saponification process. It can be overcome by the addition of excess fat or oil or any other superfatting agent to reduce the harshness of the soap. This indicates that, the prepared soap is not corrosive to the skin. As the salt of a weak acid (fatty acid) and strong base (NaOH), soap is alkaline (pH~10) in aqueous solution. Alkaline substances neutralize the body's protective acid mantle that acts as a natural barrier against bacteria and viruses. Healthy skin has a pH 5.4 to 5.9. The alkalinity favours detergency [6].

TFM shows how much fat substance the soap has, i.e., it is the indication of soap quality. If the TFM is more, better the quality of the soap. The lower TFM value is due to presence of unreacted Sodium hydroxide in the mixture. However, dry skin needs soap which is high in TFM of 80%. This re-hydrates the skin making it smooth, and additionally the high oil content within the soap acts as a lubricant throughout the day [6].

The Neem oil and Nalpamaradhi oil based herbal soap demonstrated an excellent antimicrobial activity against the tested microbial skin flora. The herbal soap was active against bacterias like *Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumonia, Escherichia coli* and fungus like *Candida albicans* and *Aspergillus niger.*

The skin carries large numbers of bacteria flora, mainly Gram-positive picked up from the various objects with which it comes in contact, of these natural flora, *Staphylococcus aureus* commonly found on the hands, face and in deep layers of the skin is perhaps the most widely encountered and very undesirable. *Staphylococcus aureus* is ubiquitous and are not easily eliminated especially in the deeper skin layers, sweat gland, sebaceous gland, and the hair-follicles by routine washing and scrubbing even with some antiseptic soap. Thus, the potency of the this herbal soap against *Staphylococcus aureus* is very remarkable and could be harnessed in the containment of the organism implicated as the commonest etiologic agent of boils, carbuncles, breast abscess, infantile-impetigo [11].

Candida albicans which normally inhabits part of the respiratory, gastrointestinal and female genital tracts is also important [12]. In this study, the herbal soap inhibited the growth of Candida cells. *Candida albicans* is known to be inherently resistant to many antimicrobial agents. Although it is a natural human body flora, it also causes some opportunistic infections in debilitated, immunocompromised patients as well as patients on prolonged antibiotics and immunosuppressants [13].

Thus, the formulated soaps have shown good antibacterial and antifungal activities. Hence can be used as a biopharmaceutical product in the treatment for bacterial and fungal skin infections as well, along with its usage as normal herbal bath soap

4. CONCLUSION

Neem oil and Nalpamaradhi oil were selected for the study. The Herbal Soap was prepared through cold saponification method by standard procedure using Neem oil, Nalpamaradhi oil and alkali (Sodium Hydroxide).

Physicochemical parameters such as Color, odour, texture, pH, Total Fatty Matter, Moisture content, Hardness and Foam test were evaluated; these parameters will serve as standardization of the Herbal Soap.

Evaluation of antimicrobial activity such as determination of anti bacterial (*Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecalis,* and *Klebsiella pneumoniae* and *Escherichia coli*) and antifungal activity (*Candida albicans* and *Aspergillus niger*) were performed on the formulated herbal soap and the result shows the soap has got considerably good antimicrobial property

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