International Research Journal of Pure and Applied Chemistry

Volume 24, Issue 5, Page 64-70, 2023; Article no.IRJPAC.105791
ISSN: 2231-3443, NLM ID: 101647669

Antibacterial Properties and Chemical Parameters Determination of Medicinal Soap Produced with *Acalypha wilkesiana* Plant Extracts

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IRJPAC/2023/v24i5826

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/105791

Original Research Article

Received: 25/06/2023
Accepted: 01/09/2023
Published: 13/09/2023

ABSTRACT

In rural communities of the poor developing economies, dermatophytic infections have become pandermic with about 20-25% of the population affected. The present study was carried out to produce medicinal soaps with antibacterial and antifungal activities using leaf extracts of *Acalypha wilkesiana*. We compared the antimicrobial properties of different concentrations of the extract of the plant in soap production and the chemical parameters of the produced soap were determined. The soap containing 15% *Acalypha wilkesiana* showed more activities against clinical strains of Candida albicans and *Staphyloccocus aureus* at all concentrations with zones of inhibition between 6 – 18 mm compared to values obtained against Proteus mirabilis and pseudomonas aeruginosa. However, soap containing 10% of the medicinal plant was not active against all the test

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microorganisms at lower concentrations of 10 and 100 µg/mL but the activity increased with increased concentration of the active ingredient in the soap to 1000 and 10,000 µg/mL. However, the soap containing 5% medicinal plant was not active at any of the concentrations. The chemical parameters determined indicated that Total fatty matter (TFM) 47% is within the acceptable limit. The values obtained for Matter insoluble in water (18%), matter insoluble in alcohol (0.5%), Total free acidity (0.7%), Moisture content (7%), pH(10.5) and % Free alkali as Na_2O (6.19%) were all within acceptable limit. The Foam stability was determined to be 3.98 mins. It is therefore obvious that the soap containing 15% *Acalypha wilkesiana* is more effective against the test microorganisms compared to 10% and could be utilized for the management of skin infections.

Keywords: Acalypha wilkesiana; antimicrobial properties; Candida albicans; moisture content; skin infections.

1. INTRODUCTION

One of the most important things we use in our everyday lives is soap. It has both cleansing and cosmetic properties. Soap embrace all those compounds which result from the reaction of salifiable bases with fats and oils [1]. Fats and oils consist generally of three glyceryl esters, two solid, differing in fusibility, called stearin and palmitin, and one liquid, called olein. Stearin is found most abundantly in fats which are firm and solid and palmitin and olein in the oils. Soap has a distinct if not powerful antiseptic action [1]. Attempts have been made to increase the germicidal value of soap by the addition of various disinfecting agents, especially cresol and mercury iodide. However, the toxicity effects of these chemicals do not make their inclusion in soap formulation very viable hence the reversion to medicinal plants after bacteriological tests came to the conclusion that although the mercury iodide soap had a distinct disinfectant action, for practical purposes it causes cancer [1].

The overall reaction of triacylglycerol saponification is carried out in two steps. Three fatty acid molecules and glycerol are produced by the hydrolysis of ester linkages in first step:

Fat or oil + $3H_2O_3 \rightarrow$ 3fatty acids + glycerol

The second step involves an acid–base reaction where the fatty acid molecules and a base (usually NaOH) are subjected to produce water and salts:

3fatty acids + 3NaOH → 3fatty acid salts + $3H_2O$.

There have been various pharmacological researches on medicinal plants to determine their activities against bacteria and fungi. So many plants came to the attention of researchers because of their use in traditional medicine [2]. Out of about 120 active compounds currently used in modern medicine today, over 80 percent gave a positive correlation between the traditional and modern therapeutic use of the plants. It was reported that about two thirds of the world's plant species with medicinal value come from the developing countries [3].

Acalypha wilkesiana is of the family Euphorbiacacea which is generally used as ornamental. Studies have indicated the presence of phytochemicals such as high presence of carbohydrates, Tannins, Flavonoid. Also a moderate presence of Phlobatannins, Saponins, alkaloids, Cardiac glycosides and minute quanity of Terpenes and Steroids were also reported [4]. It has been widely reported to be used in North Eastern Africa for the treatment of skin infections [4]. The chemoprotective activities of the aqueous extract of the plants in cyclophosphamide-induced toxicity in rats have also been reported [5]. The reported use in traditional medicine of the plant to treat a variety of skin disorders including pityriasis versicolor and seborrheic dermatitis made Katibi *et al*., 2022 [6] carried out an experimental study to determine the spectrum of antifungal activity of 2 variants of the *Acalypha wilkesiana* plant. The antifungal activity of the plant against *Malassezia* sp. and non-malassezia sp. isolated from human skin samples were carried out by Santhanam et al., 2014 [7]. In their study, they concluded that the plant was very active against the test micro-organisms.

The objective of this study was to determine and compare the antifungal activities of soaps prepared with the medicinal plant against the test microorganisms.

2. Materials and Methods

2.1 Chemicals

The reagents used for the study include; Nutrient agar- (Sigma-Aldric), Gentamycin injection

capsules of concentration 80mg/2mL), Dimethylsulphoxide (Sigma- Aldrich). Sodium hydroxide, Sodium sulphate, Soda ash, palm kernel oil. All other reagents were of analytical grade.

2.2 Test Organisms

The organisms used for this study include clinical strains of Candida, Staphyloccocus aureus, Proteus mirabilis and Pseudomonas aeruginosa.

2.3 Collection of Plant Materials

The fresh leaves of the plants were collected from the botanical gardens of Nigeria Natural Medicinal Development Agency, Lagos. The leaves were shade dried and ground to powder using mortar and pestle to fine-sized particles.

2.4 Extraction of Bioactive Materials

The plant sample weighing 200g was extracted with 1 Litre of methanol. The mixture was thoroughly blended for five minutes and then filtered under gravity using filter paper. Rotary evaporator was used to recover the methanol from the filtrate and the extract obtained.

2.5 Preparation of Stock Solution for Antimicrobial Activities

56 g of Nutrient agar was dissolved in 2000 ML of deionized water in a conical flask. The resultant mixture was sterilized in the autoclave for about 15 minutes at 121^{o}C after being thoroughly shaken. From the plant extract earlier obtained, 0.02g and 2.0 ML of DMSO were used in preparing stock of 10,000µg/ML. The stock was then serially diluted to obtain 1000µg/ML, 100µg/ML, 10µg/ML respectively.

3. MICROBIAL ACTIVITY TESTING

3.1 Sterilization of Bottles, Petri-dishes and Experimental Discs

Sample bottles to be used were washed and thoroughly rinsed with distilled water. They were authoclaved at 120^{O}C for 15 mins. The process was repeated for Petri dishes and the experimental discs.

Perforated filter paper of about 160 well rounded standard 0.6cm diameter discs were made. The small sterilized bottles were neatly arranged in quadruplicate with samples to be assayed in different concentration that has been serially diluted from 10,000 ppm, 1000 ppm, 100 ppm to 10 ppm using the Silverton and Baker dilution formula, and each bottle containing 20 discs.

The positive control experimental discs was prepared by adding 0.25ml of gentamycin to 0.75ml of DMSO to make 10ml mark corresponding to 10,000ppm. However, 10ml of DMSO was used for the negative control. The sterilized agar solution were aseptically poured into the petri dishes and thereafter allowed to cool to room temperature. The dishes were inverted and the bottom of each marked into 4 quadrants and the micro-organisms streaked on each plate in a zigzag pattern. Thereafter, the plates were incubated at room temperature for 24 hours.

The plates were examined for growth after 24 hours of incubation, and lack of activity was scored by comparison with the negative control where the tracks should be flourishing. Antimicrobial activity was scored when there is no visible growth on the streaked plates. Activity was scored by writing the lowest concentration which prevented growth usually called the minimum inhibitory concentration (MIC).

3.2 Herbal Medicated Soap Making and Analysis

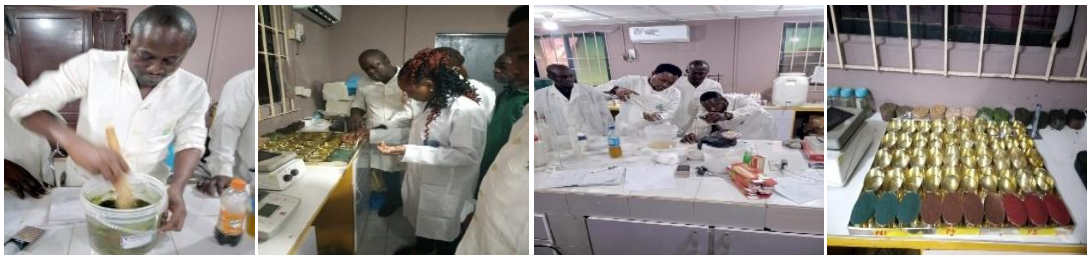
The full boiled method was applied in the production of the soap in this study. Three [3] samples were produced containing 5, 10 and 15% medicinal plant extract respectively while a soap with no medicinal plant was produced as a placebo as shown below in plates 1a-d below.

3.3 Percent Free Alkalinity

Three drops of methyl orange was added to 100ml of the dissolved 10g soap sample and then titration was carried out with 0.1M HCl with the colour changing from yellow to orange when the end point is reached.

3.4 Total Fatty Matter (TFM)

A sample of the soap (10g) was put into a 250cm^3 beaker, 100cm^3 of water was added and the mixture was heated on a water bath until the soap melted. 10cm^3 of H_2SO^4 was added with continued stirring then 5g of candle wax was also added and heating continued until the wax melted. The whole content was then allowed to cool to room temperature.



Plates 1. a-d various stages of production of soap samples

3.5 Free Alkalinity as Na_2O

A sample of the soap was placed in the conical flask and 100ml of neutralized alcohol was added. The flask and the content therein was placed on a water bath and heated until the soap dissolved. 10ml of 10% Barium Chloride solution and 2 to 3 drops of phenolphthalein indicator were added. The whole content was then titrated against NH_2SO_4 until solution became colourless. The free alkali as Na_2O was then calculated (AOAC, 1980).

3.6 Foam Stability

The soap produced was used to form lather in water in a test tube and the time taken for the foam to collapse was determined using a stop watch.

3.7 Moisture Content

This was determined using moisture determining equipment model number Denver Instrument IR 40 of Nigeria Natural Medicine Development Agency, Lagos.

3.8 pH

This was determined using Model 320K Mk 2 pH Meter in the Chemical Laboratory of Nigeria Natural Medicine Development Agency. The pH meter was standardized with buffer solution 4 and 7 after which the sample solution pH was then taken.

3.9 Matter Insoluble in Water

A sample of the soap (2g) was dissolved in 200ml of hot water, and the solution was allowed to cool to room temperature. It was then filtered and the residue dried at 135^oC and then weighed. It was then washed with cold water until the washings are colourless. This was later dried at 135^oC, cooled in a dessicator and then weighed. Increase in weight is reported as water insoluble matter.

3.10 Matter Insoluble in Alcohol

2g of the soap sample was mixed with 10ml alcohol in a 250ml beaker. It was then stirred and heated to boiling point. The hot solution was then filtered and the residue transferred to a beaker where it was dried at 100-105^oC and the increase in weight reported as matter insoluble in alcohol.

4. RESULTS AND DISCUSSION

4.1 Antimicrobial Activities

The results of the antimicrobial assay of the soaps are shown in Tables 1-4. Zones of inhibition were mean triplicate of experiment performed.

Candida albicans at times is classified as a fungus and at times a yeast. The fungus is known to travel the blood stream and usually affect the throat, intestines and heart valves. It is also as a yeast, responsible for some infections such as thrush and virginal yeast infection [8]. As shown in Table 1, 15% and 10% soap formulations were very active against *Candida albicans* at all concentrations though the activity was concentration dependent. However, it was observed that 5% soap formulation and the placebo were not active at all concentrations. The activity of the formulations may probably be due to the prevention of inflammation caused by the organism.

Staphyloccocus aureus is known to be a Gram-positive cocci. It is usually found to cause skin infections. It is usually contracted through infected person, contaminated objects of through inhalation either by sneezing or coughing. *Staphyloccocus* infection is characterized by the formation of abscesses and pus [9]. *S. aureus* is the most common bacterial infections in humans and are the causative agents of multiple human infections, including bacteremia, infective endocarditis and soft tissue infections (e.g., impetigo, folliculitis, furuncles, carbuncles,

Table 1. Zones of inhibition against *Candida albicans* in mm

Soap formulation	10µg/ml	100µg/ml	1000µg/ml	10000µg/ml
15%	6±0.26	8±0.82	11±0.64	15±0.24
10%	4±0.44	7±0.64	9±0.48	12±0.42
5%	-	-	-	-
Placebo	-	-	-	-

Each value is the mean in three measurements ± std in mm

Table 2. Zones of inhibition against *Staphyloccocus aureus* in mm

Soap formulation	10µg/ml	100µg/ml	1000µg/ml	10000µg/ml
15%	8±0.16	9±0.44	13±0.28	15 ±0.62
10%	5±0.22	7±0.82	12±0.28	12±0.42
5%	-	-	-	-
Placebo	-	-	-	-

Each value is the mean in three measurements ± std in mm

Table 3. Zones of inhibition against *Proteus mirabilis* in mm

Soap formulation	10µg/ml	100µg/ml	1000µg/ml	10000µg/ml
15%	3±0.00	7±0.82	9±0.10	12±0.16
10%	-	4±0.54	6±0.12	9±0.32
5%	-	-	-	-
Placebo	-	-	-	-

Each value is the mean in three measurements ± std in mm

Table 4. Zones of inhibition against *Psuedomonas aeruginosa* in mm

Soap formulation	10µg/ml	100µg/ml	1000µg/ml	10000µg/ml
15%	4±0.22	4±1.04	7±0.24	10±1.04
10%	-	3±0.22	5±1.26	9±0.32
5%	-	-	-	-
Placebo	-	-	-	-

Each value is the mean in three measurements ± std in mm

cellulitis, scalded skin syndrome, and others [10]. As shown in Table 2, it is observed that 15 and 10% soap formulations were active at all concentrations though the activity was concentration dependent. This activity is probably due to the fact that the phytochemicals present in the soap formulation were able to alter the mechanism of infection by the bacteria. However, no activity was observed for 5% and the placebo.

Proteus mirabilis is a Gram-negative bacterium and a member of the family *Enterobacteriaceae*. It is capable of causing a variety of human infections and is primarily associated with urinary-tract infections. It is known to grow on agar exhibiting swarming type of growth, spreading across the agar plate and appearing as a series of concentric rings. It was observed in Table 3 that 15% soap formulation was active at all concentrations but 10% soap formulations was not active at the lowest concentration of 10µg/ml. However, for 5% and placebo formulations, there were no activities at all concentrations.

Pseudomonas aeruginosa is a member of Gamma Proteobacteria class of bacteria. It is known to be a gram-negative, aerobic rod belonging to the bacteria family Pseudomonadaceae. Classified as an opportunistic pathogen. It usually initiate infection in compromised health defences [11]. In Table 4, 15% soap formulation was very active at all concentrations however, the activity was less in 10% formulation while there was no activity at all at the lowest concentration of 10µg/ml. Activity was completely absent with 5% and placebo formulations at all concentrations.

4.2 Physicochemical Chemical Analysis

The other chemical parameters determined fall within the ranges specified by the Standards

Table 5. Results for chemical parameters determination

S/N	Characteristics	Specification (% by weight)
1	Total fatty matter	47
2	Matter insoluble in water	18
3	Matter insoluble in alcohol	0.5
4	Total free acidity	0.7
5	Moisture content	7
6	pH	10.5
7	% free alkali as Na_2O	6.19
8	Foam stability	3.98mins

Organization of Nigeria (S.O.N). The Total fatty matter measures the quality of soap, and the range specified by SON is 45-50%. The value of 47% obtained is quite acceptable. Excess free alkali that is incorporated is measured as Na_2O undetected or low free alkali values of the soap immediately after production show that the soap would not be corrosive to the skin. The foam stability of 3.98mins was quite long and good. This is in direct relationship with the moisture content. As the moisture content reduces, the foaming strength increases. The pH value of 10.5 obtained is quite acceptable. If the value is lower than 9, then it is almost neutral and may not be of any medicinal benefit to the skin other than to remove dirt. However, if the value is more than 10.5, the soap will be very corrosive to the skin. The values of 18 and 0.5 obtained for matter insoluble in water and matter insoluble in alcohol respectively were a little bit higher than the specified values by SON. This might probably be due to the medicinal plant incorporated into the soap formulation (Table 5).

5. CONCLUSION

Soft smooth skin is highly desirable, however various microorganisms challenge the integrity of the skin leading to flawed skin which at times is temporary if right medication is applied and permanent if not properly taken care of. The use of synthetic chemical substances as additives in curbing the menace caused by these microorganisms was the earlier solution. These chemicals have hazardous effects on the skin e.g cancer, keloids e.t.c

Natural products however are recognized and accepted by the local population in the developing countries as alternative treatment or therapies, since they are familiar with most of these products and have easy access to them.

In this study, *Acalypha wilkesiana*, a natural herb used pharmacologically proved to have very good medicinal properties, have been included in measured percentages in a produced soap. It is concluded that 10 and 15% formulations were very active against all the test microorganisms but most active against *Staphyloccocus aureus* > *Candida albicans* > *Proteus mirabilis* > *Pseudomonas aeruginosa* . The physicochemical studies carried out on the soap indicated that it can compare favourably with other medicated soap in the market. It is therefore suggested that more studies should be designed in a way to fully utilize this medicinal plant.

DISCLAIMER

The materials used for this research are commonly and predominantly use materials in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ACKNOWLEDGEMENT

Our sincere appreciation goes to late Dr. A Raheem of Spectralab Medical and Diagnostic Services, Sagamu, Ogun State for providing the clinical strains of the microorganisms used.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/105791