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Research Article

Evaluation of Antimicrobia Properties of Soaps Produce with Neem Oil, Pine Oil, Henna and Neem Extract

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Abstract

In certain Northern Nigerian villages, the lower-class residents did not use soap because they lacked the funds to purchase it from the market. This resulted in various skin conditions and major sanitation issues. The goal of the research was to develop and assess soap made from local ingredients which is readily available in most northern Nigeria. Neem oil is well known for its therapeutic qualities and medicinal in nature. It is used to treat a variety of skin conditions. Neem oil and pine oil were utilized in this study to make bar soap, which was then described as the skin problem solution compare and alternative to conventional chemical soap. Analysis was done on the prepared soap's physical characteristics, such as pH and foamability. The sensitivity, minimum inhibitory concentration, and minimum bacterial concentration of the prepared soaps' antibacterial qualities were examined. In terms of sensitivity and minimum inhibitory concentration, the prepared soap's antimicrobial qualities (as they relate to Staphylococcus aureus and Escherichia coli) showed a clear agreement with the World Health Organization's antiseptic soap standards, with the properties of the produced bar soap correlating with those of the commercial soap sample.

Key words: soap; antimicobia; neem; henna; pine

1.Introduction

The human skin is the body's largest organ, covering the entire surface and protecting internal tissues from infection. This is accomplished by forming a physically protective water-resistant layer that prevents bacteria, viruses, fungi, and parasites from entering (Grice *et al*., 2008). Transient bacteria from the environment can be deposited on the skin's surface, causing infections. Such bacteria include Pseudomonas aeruginosa (Fluit *et al*., 2001) and Staphylococcus aureus (Higaki *et al*., 2000). Some friendly bacteria species are known to naturally cover human skin and are referred to as skin flora. This normal flora protects the skin by filling all available spaces, preventing other harmful bacteria species from growing on the body. A wound is defined as a break in the skin's integrity or discontinuity caused by breakage (Al-saimary *et al*., 2013). The high demand for soaps, particularly antiseptic soap, derived naturally from plants and animals, as well as the high cost of available soap made from costly synthesized raw materials, prompted this study to investigate the use of neem extract, a very cheap and readily available plant, particularly in northern Nigeria, which is expected to be medicinally important. Neem leaf extract contains nimbidin, cyclic trisulphide, cyclic tetrasulphide, and polyphenolic flavonoids. These bioactive compounds promote antibacterial, antifungal, and anticancer activity. It is also high in antioxidants, which promote the development of new skin cell tissues. In Ayurvedic medicine, neem leaf is used to treat leprosy, eye problems, epistaxis, intestinal worms, anorexia, biliousness, and skin ulcers. Meanwhile, neem oil contains different types of neem limonoids that can prevent the mutagenic effect. (Alzohairy, 2016; Lakshmi *et al*., 2015; Hossain *et al*., 2013; Biswas *et al*., 2002). When this plant extract is incorporated into soap, it produces high-quality soap that is gentle on the skin. Medicinal plants are a rich source of antimicrobial agents. Plants are used medically in a variety of countries as a source of potent and powerful drugs (Shuaibu *et al*. 2008). When oil or fat molecules react with NaOH in the presence of water, soap and glycerol are produced. The selection of an oil and fat blend for this reaction requires careful consideration in order to produce a product with the desired properties.

2.0 Materials and Methods

2.1 Soap Preparation

Ahmadu Bello University's Chemical Engineering Laboratory was use to conduct the research for the production of medicated soap. The cold process method was used in the study to create the soap. A beaker contained 70 ml of palm kernel oil and 30 ml of neem oil. 50 milliliters of distilled water were combined with 20 grams of KOH and left to dissolve. The solution's concentration was adjusted using a hydrometer

(12.50-12.75). The mixture was used to prepare the lye-water solution. The lye-water solution was added gradually after the oils had been thoroughly combined. After adding the lye-water solution to the oils in a beaker, the mixture was thoroughly stirred until it was homogenized. The mixture of neem and henna extract was mixed with pine oil. After another ten minutes of mixing, the soap was transferred to a mold and left for eight hours.

2.2 Soap characteristics

The following factors are used to characterize soap: pH, foam stability, and antibacterial activity.

2.2.1 PH

One millilitre of natural liquid soap was dissolved in one hundred millilitres of distilled water. A pH metre that had been calibrated was used to confirm the soap solution's pH.

2.2.2 Foam stability test

One milliliter of liquid soap and five milliliters of distilled water were added to a test tube that had been scaled in order to test the foam stability. The reaction tube was shaken vigorously to produce foam, and then the foam's height was measured. The height of the foam was measured ten minutes later.

2.3 Antibacterial activity

2.3.1 Culture Media:

Among the culture media used is Mueller Hinton agar (MHA). In order to test for sensitivity, Muller-Hinson broth (MHB), potato dextrose agar (PDA), nutritional agar (NA), and the minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) were used. To achieve sterility, each medium was prepared according to the manufacturer's instructions and autoclaved for 15 minutes at 121 degrees Celsius.

2.3.2 Determination of inhibitory activity (sensitivity test) of the extract using agar well diffusion method:

The bacterial isolate's standardized inoculate was streaked over sterile Mueller Hinton and potato dextrose agar plates using sterile swab sticks. Each inoculated agar plate has four wells perforated using a sterile corn borer. The concentrations of the produced extract 100, 50, 25, and 12.5 mg/mL were suitably labeled on the well. Extract was added to each well at a volume of roughly 0.2 milliliters. The inoculation plates with the extract were placed on the bench for about an hour to allow the extract to diffuse on the agar. Potato dextrose agar plates were incubated for roughly three to five days at room temperature, while Mueller Hinton agar plates were incubated for twenty-four hours at 37 °C. After the incubation period, the plates were inspected for signs of inhibition, which showed up as a clear zone of inhibition in a region surrounding the wells that showed no growth (Girish, 2008).

2.3.3 Determination of minimum inhibitory concentration (MIC):

Mueller Hinton broth was used as the diluent in the tube dilution method to determine the minimum inhibitory concentration of the extract. In test tubes containing Mueller-Hinton broth, the extract was serially diluted to the lowest concentration that demonstrated inhibition for each organism when the extract was tested during the sensitivity test. All of the tubes that held the broth and extract were inoculated with the standardized organisms. After that, the inoculation tubes were incubated for 24 hours at 37^{o}C. Using turbidity as a criterion, the tubes were inspected or observed at the conclusion of the incubation period to determine if growth had occurred. The minimum inhibitory concentration (MIC) was defined as the lowest concentration in the series that did not exhibit any turbidity or visible signs of growth. Additionally, the outcome was noted (Andrew, 2001).

2.3.4 Determination of minimum bactericidal concentration (MBC):

The minimum inhibitory concentration (MIC) finding was used to determine the extract's minimum bactericidal concentration (MBC). A sterile wire loop was used to dip into the test tube or tubes that did not show turbidity (clear) in the MIC test. A loopful was then taken out and streaked over sterile nutritional agar plates. The plates were incubated at 37 oC for 18 to 24 hours. At the end of the incubation time, the plates were examined or monitored for the presence or absence of growth. This is to determine whether the extract has antimicrobial properties that are bacteriostatic or bactericidal (Andrew, 2001).

3.0 Result and Discussion

Natural soap is produced using the cold process method. Soap is produced by a chemical process called saponification. The soap was infused with pine oil. When fats or oils (fatty acids) come into contact with an alkali, an exothermic chemical reaction known as saponification occurs. The triglyceride units of fats react with potassium hydroxide to form soap and glycerol. Numerous factors affect saponification, as different soap constituents have quite different qualities. Neem, coconut, and palm kernel oils serve as the basis oils for the natural soap used in this study. By adding palm kernel and coconut oils to the recipe, soap prepared with just one oil might not be the most balanced. Every oil adds something special to make a soap that is better balanced. Coconut oil is one of the most popular oils used to produce soap. It offers a unique combination of cleansing, firming, and skin-loving properties. Adding palm kernel oil to the soap has an anti-aging impact since it includes vitamin E, which is great for the skin. To give the soap its scent and antimicrobial qualities, neem seed oil and pine oil are added. Of all the parts of the plant, the oil in neem tree seeds is thought to be one of the most important sources of antibacterial drugs because of its wide range of antibacterial action.

Neem seed oil could be used in place of palm oil to make cosmetic toilet soaps with beneficial medicinal qualities (Mensah, 2011), and the antibacterial qualities of the neem and shea butter oil blends compared favorably to a commercial antiseptic soap containing triclorocarbanilide (Ameh, 2013). According to a prior study, the ideal ratio of neem seed oil to eucalyptus oil for making antibacterial soap was 20:80 (Bello *et al*., 2019). Because of the pine oil, the soap utilized in this study has an earthy, slightly nutty, and piney scent. It has a translucent, yellowish hue. The color deepens with increasing neem oil concentration.

The soap's properties were categorized based on three factors: pH, foam stability, and antibacterial activity. The quality of soap is determined by its physico-chemical properties.

Characteristics	Prepared soap	Commercial soap
Foam height	8 cm	9 cm
pH	9	9
Solubility	Soluble	Soluble
Color	Yellow	Light green

Table 3.1: Physicochemical properties of prepared, neem oil liquid soap and commercial soap

pH

The pH of the soap, which ranges from 4 to 10, meets SNI (2588:2017) standards. The developed soaps fall within the allowed pH range, as shown in Table 3.1 above. A pH of less than 7 indicates greater acidity, whereas a pH of greater than 7 indicates greater alkalinity. Seven is regarded as the neutral pH. Both the commercial control soap and the prepared soap used in this investigation have a pH of 9, as can be seen by comparing their pH values. Prepared soap is naturally alkaline due to the presence of potassium hydroxide components as the base material that undergoes a saponification reaction. (Uzwatania *et al*., 2014).

Human skin has an acidic pH of 5.4 to 5.9, which is important for its ability to protect against microorganisms. The body's protective layer, which acts as a barrier against microbes, is neutralized by alkaline substances like soaps. Furthermore, a highly alkaline pH can harm the acid mantle and the lipid lamellae of the epidermis. By letting allergens and irritants into the skin, this may result in dry skin and increased trans-epidermal water loss. The pH of lsoap is safe for skin (Uzwatania *et al*., 2014).

Foam Stability

The foamability height generated by the soap sample can be explained by the fatty acid composition of the oil used in the soap formulation. It has been shown that soap containing the saturated fatty acids myristic acid and lauric acid has a high foamability height and is fluffy (Phansteil *et al*., 1998). However, as shown in Table 3.1 above, the observed difference in the height of foam created in the soap sample formulation and the commercial soap may have been caused by an addition made to the commercial soap. The height of the foam that was removed from the control sample was nine centimeters. The additions may have contributed to the first produced sample's somewhat lower height (8.5) as compared to the control sample. The height that most closely matched the control data was 8.5 cm.

	Zone of inhibition (mm) at varying conc. (mg/ml) of the soap					
Test organisms	100	50	25	12.5	Commercial soap	Control Cip (10mg)
Staphylococcus aureus,	16	14	11	0	13	22
Escherichia coli	10	0	0	0	0	19

Table 3.2: Determination of inhibitory activity (sensitive test) of the medicated soap on the test organisms

The sensitivity of Staphylococcus aureus and Escherichia coli to different soap concentrations is displayed in Tables 3.2 and 3.3. When tested against Staphylococcus aureus and Escherichia coli, the manufactured soap at concentrations of 50 and 100 showed a greater zone of inhibition than the commercial soap, while the lower concentration of 25 was less effective. Escherichia coli is not impacted by commercial soap, but it is by prepared soap at 100. The table shows that the soap that was made had the strongest inhibitory impact on S. aureus, although it had less of an effect on Escherichia coli. A high value denotes the sensitivity of the bacterium, or its incapacity to multiply while the soap sample is present. This suggests that when the soap sample is at that concentration, it works well against the germs. A low score suggests that a higher dosage of the soap sample is needed to halt development at that concentration.

Test organisms	MIC	MBC
Staphylococcus aureus	25	50
Escherichia coli	0	0

Table 3.3: Determination of inhibition concentration (MCC) and Determination minimum Bacteriocidal concentration (MBC)

Staphylococcus aureus. Escherichia coli had a minimum inhibitory concentration (MIC) of zero, while Staphylococcus aureus had a MIC of twenty-five.

Escherichia coli was unaffected by the minimum bacteriocidal concentration (MBC), which was 50 for Staphylococcus aureus.

4.0 Conclusion

According to this study, neem seed oil and pine oil combined could be used as a natural ingredient in antibacterial bar soap. The resulting bar soap was found to have antibacterial qualities against Staphylococcus aureus and Escherichia coli. This is a brand-new natural soap that is created without artificial coloring, sodium sulfate (SLS), or fragrances derived from pine and neem oils. More research is required to improve the soap's quality.

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