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Research Article

Evaluation of Antibacterial Property of Liquid Soap Made from Blend of Neem and Pine Oil

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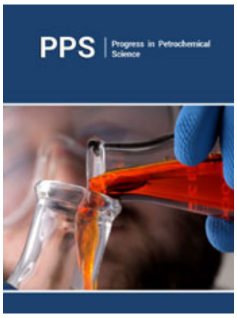

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Abstract

This research used neem oil and pine oil in preparing liquid soap, which were subsequently characterized. The physical properties of the prepared soap, including foamability and pH, were analyzed. The antibacterial properties of the prepared soaps in terms of sensitivity, minimum inhibitory concentration and minimum bacterial concentration were analyzed. The antimicrobial properties of the prepared soap in terms of sensitivity and minimum inhibitory concentration (with respect to *Staphylococcus aureus* and *Escherichia coli*) indicated that the properties observed from the produced liquid soap correlate with those obtained from the commercial soap sample and clearly agree with the standards of the World Health Organization for antiseptic soap.

Keyword: Neem; Pine; Soap; Antibacterial; pH; Foam height

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Introduction

Liquid soap products are available in several kinds. Soap is the term for any type of cleaning product [1]. Soap is made of natural lipids and either sodium or potassium hydroxide. Soap is created when fat and an alkali react to create a fatty acid salt that has purifying properties. Soap deactivates bacteria and viruses and eliminates dirt, stains and grease by upsetting the lipid membrane and intracellular lipids. Numerous research has shown that soap works better than hand rub for hand hygiene [2]. Soap can be divided into two groups according to the kind of alkali that is used: soap bars, also called sodium soap and liquid soap, also called potassium soap. The extensive everyday usage of liquid soap is attributed to its many advantages, which include convenience of use, decreased contamination and a variety of formulas. Liquid soap has gained popularity due to its enhanced look and functionality [3].

A natural liquid soap enhanced with pine oil, neem extract and neem oil can be used as an alternative to the artificial chemicals in soap. An important natural antibacterial ingredient, neem seed oil is derived from the seeds of the neem plant (*Azadirachta indica*) and has been used for millennia in Indian subcontinent ayurvedic medicine. Upadhyay RK et al. [4] discovered the remarkable bactericidal properties of neem oil. According to Subapriya R et al. [5], neem oil has been used to alleviate arthritis-related pain, edoema and inflammation. Neem trees can be found in Indonesia in Bali, East Java, Central Java and West Nusa Tenggara [6]. The primary lipid type included in neem oil is triglycerides. Neem oil has high levels of triglycerides, calcium, vitamin E and vital fatty acids. Because of its high level of vital fatty acids and vitamin E, neem oil penetrates the skin deeply to repair the microscopic fissures created by excessive dryness. According to Sadekar RD et al. [7], oleic acid (52.8%) is the predominant fatty acid in neem kernel oil. It is followed by stearic acid (21.4%), palmitic acid (12.6%), linoleic acid (2.1%), and various lower fatty acids (2.3%).

Neem oil is used in the production of ointments, toothpaste, emulsions, liquors, hair and skin care products, natural and organic cosmetics and medicinal cosmetics [8]. However, neem oil can be extracted mechanically (hot or cold process) or chemically (solvent extraction) using dried neem seeds. The best neem oil with the bulk of phytoconstituents intact is obtained through cold pressing. Ramakrishna G et al. [9] reported that cold press oil had a lighter color and a softer fragrance. Northern and Eastern Europe is home to the Pinaceae family, which includes the *Scots pine* or *Pinus sylvestris*. Eurasia is where it originated. Based on Zafar I et al. [10], the genus *Pinus* is the most common coniferous plant, with over 250 species distributed worldwide. The tree, which is between 25 and 40 meters tall, is made up of evergreen, fragrant, needle-like blue-green leaves that measure 3 to 5cm and are arranged spirally or alternately [11]. According to Maciąg A et al. [11], *P. sylvestris* is the most varied pine species globally, offering a wide variety of uses. The tree is both an ornamental plant that reduces erosion and a raw material utilized in the paper industry [12]. *P. sylvestris* oil is used in many different pharmaceutical applications due to its anti-parasitic, anti-viral, anti-allergenic, antispasmodic, anti-hyperglycemic, anti-inflammatory and expectorant properties [13]. Furthermore, *P. sylvestris* terpenic oil is used as a food additive, preservative, chemical and fragrance industry [13]. The insecticidal and larvicidal properties of *P. sylvestris* oil have also been reported [14]. The goal of this research is to create a natural liquid soap that can replace synthetic body wash by combining neem oil and pine oil. The antibacterial activity and physico-chemical characteristics were assessed to ascertain the liquid soap's quality.

Methods

Soap preparation

Studies on the manufacturing of liquid soap have been conducted in the Chemical Engineering Laboratory at Ahmadu Bello University. The hot process method was used in the study to formulate the liquid soap. Three different oils were heated to 100 °C in a beaker: 50ml of palm kernel oil, 30ml of coconut oil and 20ml of neem oil. The temperature was measured with a thermometer. 50 milliliters of distilled water were mixed with 20 grams of KOH and allowed to dissolve. The solution's concentration was adjusted with a hydrometer (12.50-12.75). The mixture was used to make the lye-water solution. The heated oils were mixed well and turned clear before the lye-water solution was gradually added. The lye-water solution was added to the heated oils in a beaker and the mixture was heated until it became homogenous. The mixture was heated steadily to 120 °C for five to six hours. After an hour of heating, 50ml of distilled water was added and the mixture was constantly stirred with a stick blender. Pine oil was combined with the liquid soap. The liquid soap was carefully sealed and placed into containers.

Soap characteristics

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

pH: One milliliter of natural liquid soap was dissolved in one hundred milliliters of distilled water. A pH meter that had been calibrated was used to confirm the soap solution's pH.

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.

Antibacterial activity

Culture media: Mueller Hinton Agar (MHA) is one of the culture media that are utilized. For sensitivity testing, determining the Minimum Inhibitory Concentration (MIC) and determining the Minimum Bacterial Concentration (MBC), Muller-Hinson Broth (MHB), Potato Dextrose Agar (PDA) and Nutrient Agar (NA) were utilized. Every medium was prepared in compliance with the manufacturer's guidelines and autoclaved for 15 minutes at 121 degrees Celsius to achieve sterility.

Determination of inhibitory activity (sensitivity test) of the extract using agar well diffusion method: Using sterile swab sticks, the standardized inoculate for the bacterial isolate was streaked on sterile Mueller Hinton and potato dextrose agar plates, respectively. Using a sterile cork borer, four wells were punctured on each inoculated agar plate. The well was appropriately labeled with the concentrations of the prepared extract 100, 50, 25 and 12.5mg/mL, respectively. A volume of about 0.2 milliliters was used to fill each well with extract. To give the extract time to diffuse on the agar, the inoculated plates containing the extract were left on the bench for approximately an hour. Mueller Hinton agar plates were incubated at 37 °C for 24 hours and potato dextrose agar plates were incubated for approximately 3-5 days at room temperature. Following the incubation period, the plates were examined for any indication of inhibition, which manifested as a distinct area surrounding the wells devoid of any growth (zone of inhibition) [15].

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 °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 [16].

Determination of Minimum Bactericidal Concentration (MBC): The Minimum Bactericidal Concentration (MBC) of the extract was calculated using the Minimum Inhibitory Concentration (MIC) result. The test tube(s) that did not exhibit turbidity (clear) in the MIC test were dipped into using a sterile wire loop and a loopful was removed and streaked on sterile nutrient agar plates.

For 18 to 24 hours, the plates were incubated at 37 °C. The plates were checked or observed for the presence or absence of growth at the conclusion of the incubation period. This is to ascertain whether the extract has bacteriostatic or bactericidal antimicrobial effects [16].

Result and Discussion

The hot process method was used in the production of natural liquid soap. The chemical process known as saponification is what yields soap. Pine oil was added to the soap. An exothermic chemical reaction known as saponification takes place when fats or oils (fatty acids) come into contact with a base called alkali. This reaction produces soap and glycerol when the triglyceride units of fats react with potassium hydroxide. Saponification is influenced by a wide range of factors and the properties of various soap ingredients vary greatly. The base oils for the natural liquid soap in this study are neem, coconut and palm kernel oils. It's possible that soap made with just one oil isn't the most balanced by incorporating palm kernel and coconut oils into the mixture. Every oil adds something unique to make a soap that is better balanced. One of the most widely used oils to make soap is coconut oil. It provides a special blend of skin-loving, firming and cleansing qualities. Adding palm kernel oil to a liquid soap has an anti-aging effect. Neem seed oil and pine oil are added to the soap to give it an aromatic and antibacterial properties. Given that the oil in the seeds of the neem tree has a broad range of antibacterial activity, it is considered one of the most significant sources of antibacterial medications among all the parts of the plant.

The antibacterial properties of the neem and shea butter oil blends compared favorably well to a commercial antiseptic soap containing triclorocarbanilide [17] and neem seed oil could be used as a substitute for palm oil in producing cosmetic toilet soaps with favorable medicinal properties [18]. The previous study revealed that the best blending ratio for the antiseptic soap was found to be 20:80 of neem seed oil to *Eucalyptus* oil [19]. The soap used in this study smells earthy, slightly nutty and piney thanks to the pine oil. It is transparent yellowish in color. As the neem oil concentration rises, the color gets deeper, most likely as a result of a reaction between the neem oil and the soap paste. Samples of liquid soap did not separate; instead, they stayed stable. Three criteria were used to categorize the soap's qualities: pH, foam stability and antibacterial activity. In actuality, the physico-chemical characteristics of soap dictate its quality.

pH

The liquid soap's pH value, which falls between 4 and 10, satisfies SNI (2588:2017) requirements. As indicated in Table 1 above, the formulated liquid soaps are within the permitted pH range. Anything with a pH of less than 7 is more acidic and anything higher than that is more alkaline. A pH of seven is considered neutral. Comparing the pH of the liquid soap used in this study to that of the commercial control soap, which is likewise 9, reveals that both have a pH of 9. Because potassium hydroxide components are present as the base material in liquid hand soap with a saponification reaction, the soap is naturally alkaline [2]. Alkaline materials, like soaps, neutralize the body's defensive layer, which serves as a barrier against microorganisms. In addition, an extremely alkaline pH may damage the epidermis' lipid lamellae and damage the acid mantle. This could lead to increased trans-epidermal water loss and dry skin by allowing allergens and irritants to enter the skin. Liquid soap has a pH that is safe for skin [2].

Table 1: Physicochemical properties of prepared, neem oil liquid soap and commercial soap.

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

Foam height

The fatty acid composition of the oil used in the soap formulation provides an explanation for the formability height produced by the soap sample. According to reports, soap made with lauric acid and myristic acid both saturated fatty acids have a high formability height and is fluffy [20]. But as Table 2 above illustrates, there might have been an addition made to the commercial soap, which could account for the observed discrepancy in the height of foam formed in the soap sample formulated and the commercial soap. The foam extracted from the control sample measured nine centimeters in height. When compared to the control sample, the height of the first prepared sample (8) was a little low, which could have been caused by the additions. 8cm was the height that was most similar to the data from the control [21].

Table 2: Determination of inhibitory activity (sensitive test) of the medicated soap on the test organisms.

Test Organisms	Zone of Inhibition (mm) at Varying Conc. (mg/ml) of The Soap					
	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

Tables 2 & 3 show how sensitive *Escherichia coli* and *Staphylococcus aureus* are to various soap concentrations. The produced soap at concentrations of 50 and 100 exhibited a larger zone of inhibition than the commercial soap when used against *Staphylococcus aureus* and *Escherichia coli*, whereas the lower concentration of 25 was less effective. Commercial soap does not affect *Escherichia coli*, but liquid soap at 100Conc. has effect on *Escherichia coli*. Although it had less of an effect on *Escherichia coli*, the table indicates that the liquid soap that was produced had the greatest inhibitory effect on *S. aureus*. A high number indicates the microorganism's susceptibility, or its inability to proliferate in the presence of the soap sample. This indicates that the soap sample is effective against the bacteria at that concentration when it is at that concentration. A low value indicates that in order to stop growth at that concentration, a larger dose of the soap sample is required.

The minimum inhibitory concentration test analysis of liquid soap is presented in Table 3. The results indicate that *Staphylococcus aureus* was affected by the sample. *Staphylococcus aureus* has a Minimum Inhibitory Concentration (MIC) of 25, whereas *Escherichia coli* had a MIC of 0. The Minimum Bactericidal Concentration (MBC) was 50 for *Staphylococcus aureus*, with no effect for *Escherichia coli*.

Table 3: Determination of inhibition Concentration (MCC) and Determination Minimum Bactericidal Concentration (MBC).

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

Conclusion

This study demonstrates that neem seed oil and pine oil together could be utilized as a natural component in liquid soap that fights bacteria. It was discovered that the liquid soap generated exhibited antibacterial properties against both *Escherichia coli* and *Staphylococcus aureus*. This is a new product a natural soap made without chemicals like Sodium Sulfate (SLS), artificial colorant or artificial fragrance from neem and pine oils. To raise the caliber of the soap, more research needs to be done.

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