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Production and Investigation of the Antiseptic Properties of Soaps Made from the Barks, Seeds and Leaves Extracts of Neem Tree

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Authors' contributions

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

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ABSTRACT

The main aim of this work is to investigate the antiseptic properties of *Azadirachta indica* (Neem) tree parts (leaves, barks and seeds). The extracts were used in the production of soap samples of various concentrations (20 mg/cm^3, 15 mg/cm^3, 10 mg/cm^3 and 5 mg/cm^3). Inhibitory Activity sensitivity test using Agar-well Diffusion Method was employed to test the antibacterial activities of the soap samples on two bacteria, *Staphylococcus aureus bacteria and Propionibacterium acnes*. The results show that soap samples from the Neem parts exhibited antiseptic properties against the bacteria tested. According to the results, the Neem bark soap produces the highest level of effectiveness across the entire concentration spectrum, followed by the Neem seed soap. The Neem leaves soap produced the lowest level of effectiveness against the two bacteria. The order of effectiveness of the soap samples is: NBRK (Neem barks) > NSED (Neem seeds) > NLVS (Neem leaves). The commercial soap (NRMS) used as a control sample did not exhibit antibacterial activity against the two microbes.

Keywords: Neem; Staphylococcus aureus bacteria; propionibacterium acnes; antimicrobial activities.

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1. INTRODUCTION

Neem (*Azadirachta indica*) which is a word derived from an Indian language that means perfect, completely imperishable is a commonly grown tree that is found mostly in tropical countries such as India, Africa and northern America. It is broad-leaved evergreen that grows up to 30m tall and belongs to mahogany family, called Meliaceae [1,2]. This tree is frequently cultivated in the driest regions of tropical and subtropical countries. .It is known for its therapeutic and ethno medicinal values since prehistoric era [3, 4]. In Nigeria, Neem tree is found growing mostly in the northern part of the country especially in states like Katsina, zamfara, Gombe, Adamawa, Sokoto and Kebbi, where the plant is sometimes found sprouting naturally on its own from scattered seeds or planted from nursery and nurtured into fully grown Neem tree. Neem is an economic viable tree that is widely grown in many towns with the purpose of taming the tide of desertification [5] that is threatening the human existence, especially in the sub-saharan African countries.

Traditionally, the tree has been used for the treatment of many diseases and illnesses, and as natural substance for the control of pesticides and herbicides [5]. It is also used for other agricultural purposes where the seeds are mixed with other substances and converted to natural manures to increase agricultural yields [2].

The bioactive compounds abundant in plants and derivatives are usually recovered from leaves, stem or bark of trees and then used as medicines for the cure of many illnesses, such as headaches, stomach aches, diarrhea, piles, yellow fever, tooth problems [6]. In Nigeria certain parts of the tree, like the leaves, are also traditionally used for bathing new born babies as antiseptic liquid, primarily to enhance the healthy growth and early strength of new born baby. The fruits of the tree are generally consumed orally to drive their succulent, nutritious and medicinal effluents. The seeds are also processed to produce seed oil used as baby lotion oil and also for the production of natural manures for agricultural purposes.

The trunk of Neem trees are mostly chopped into wood logs to serve as a source of burning fuel that provide heat energy for cooking purposes. In some instances the entire trunk is burnt to obtain charcoal that is also used as a fuel source.

Modern researches confirm Neem's curative powers and with the advent of modern scientific investigations the medicinal compounds of Neem hitherto, unknown are being successfully identified. Analysis have shown that Neem parts contain a large number of biologically active compounds including: azadirachtin, meleacin, gedinin, salanin, numbin, valassin and many other derivatives [4]. Meleacin is the substance that provides the bitter taste of Neem tree. Azadiractin, the most important active compound from Neem seeds and other plant parts, has natural insecticidal properties and may be a potential substitute for synthetic pesticides [5].

Substances isolated indicate that each part of the tree produces specific types of compounds with peculiar medicinal and biological properties. Some of the biological activities of these substances have the ability to inhibit the activities of some certain bacterial microorganisms, thereby making them susceptible for use in the development of medicinal or curative agents [7]. For instance, those substances extracted from the seed oil have been found to exhibit anti-inflammatory, anti-arthritis, anti-pyretic, anti-gastric ulcer, spermicidal, anti-fungal, anti-bacterial and diuretic activities, while those extracted from the Neem bark have been found to exhibit anti-inflammatory, immune-modulatory, anti-bacterial and anti-tumour activities. In addition, the compounds extracted from the Neem leaf have been found to exhibit anti-fungal activity [4, 3, 2]

Substances that are active against certain diseases such leprosy, eye problem, intestinal worms, epitasis, chicken pox, piles, cancer, and so on [4, 3, 8] have also been discovered in the Neem parts.

Neem parts have also found application in the production of a wide range of personal care products which include skin care products (such as eczema cream, antiseptic cream and nail care products), hair care products (namely shampoo and hair oils), oral hygiene (toothpaste and Neem twigs), household products (soaps, insect repellants in spray or lotion) and candles [3, 2].

There is a global increase in the knowledge about the medicinal plants and their efficacy as therapeutic aids to fight against ailments as they are from natural source and they contribute towards less environmental effects and other

harmful diseases [9]. Neem (*Azadirachta indica*) tree is one of those therapeutic plants identified in curing various infections and as such the futuristic potential of using the tree in the medicinal and pharmaceutical fields cannot be exaggerated.

Therefore, the objective of this study is to produce antiseptic Neem soaps from three different plant parts and to test their antibacterial properties against two bacteria, *Staphylococcus aureus and Propionibacterium acnes.* Antimicrobial activity of any substance is defined as its ability to either kill bacteria or inhibit the growth of bacteria. Antimicrobial activity is significant with respect to the human body in preventing diseases and skin infections [10].

2. MATERIALS AND METHODS

2.1 Sample Collection

Fresh leaves, seeds and barks were collected from Neem colony in Kalgo town, at the outskirt of Birnin Kebbi, Kebbi State, Nigeria in considerable quantity and open air dried for a period of two weeks under shed to avoid decolonization and depletion of nutrients [11]. The samples were then grinded in mortar and sieved into fine powdered particles as shown in Fig. 1.

2.2 Production of Antiseptic Soaps

2.2.1 Production of leaves powdered extracts

The fresh leaves from the Neem trees were dried under shed to avoid decolonization and depletion of nutrients [11]. The dried leaves were then grinded in mortar and sieved to a fine powdered particles to form the Neem leaves extract.

2.2.2 Production of seeds powdered extracts

The ripe pulps were removed from the seed as soon as possible after collection from the Neem trees. The seeds were then laid out in a thin plastic sheet in the sun to dry out for few days. The dried seeds were then stored in a basket container under plenty of air to stop mould growing. After that, the shells were removed by pounding gently in a big wooden mortar and separated from the seeds through winnowing. Finally, the seeds were grinded using mortar pestle and in the laboratory to produce the powdered extracts.

2.2.3 Production of barks powdered extracts

Fresh Neem-tree barks were cut from the trees with the aid of cutlass in large amount and sun dried directly. The barks were then grinded in the laboratory using mortar and pestle and sieved to fine textured particles.

2.2.4 Fermentation of neem extracts

The first stage in the production of the antiseptic soaps is to ferment the three Neem extracts. The reason why the extracts were fermented is to allow microorganisms, such as yeast and bacteria, to act on the substances to break them into smaller and simpler particles that will produce a soft antiseptic soap. If we use the extracts directly without fermenting, small particles of powdered extracts may remain on the surface of the body after use. The fermentation process was carried out by weighing 70g of each powdered extracts (seed, bark and leaves) and transferring them into three separate 1000 cm^3 beakers. The beakers were labelled as NLVS (leaves), NSED (seeds) and NBRK (barks) respectively. 600ml of distilled water was added to each extract in the beaker and allowed to stand for 48 hours for fermentation to take place. After 48 hours the solutions were then filtered using vacuum filtration machine to obtain clear fermented solutions.

2.2.5 Preparation of caustic soda solution/ lye

Sodium hydroxide (NaOH) exists as a white crystalline pearl. It dissolves readily in water to produce an aqueous solution called Lye. This dissolution process liberates a substantial amount of heat. Caustic soda (34 g) and distilled water (76 g) were weighed according to standard method [12]. The caustic soda was gradually added to the water inside a beaker stirred carefully until it completely dissolved in the water. Then, the mixture was allowed to cool down.

2.2.6 Preparation of oil blend

The oil used for the preparation of the antiseptic soaps is an oil blend of palm kernel oil (P.K.O) and Neem seed oil in the ratio 1:3 (150g of P.K.O oil and 50g Neem seed oil). In order to get a homogeneous solution the oil blend was thoroughly mixed together in the electric mixer for about 30 minutes, and this ensures homogeneous blend of the oils.

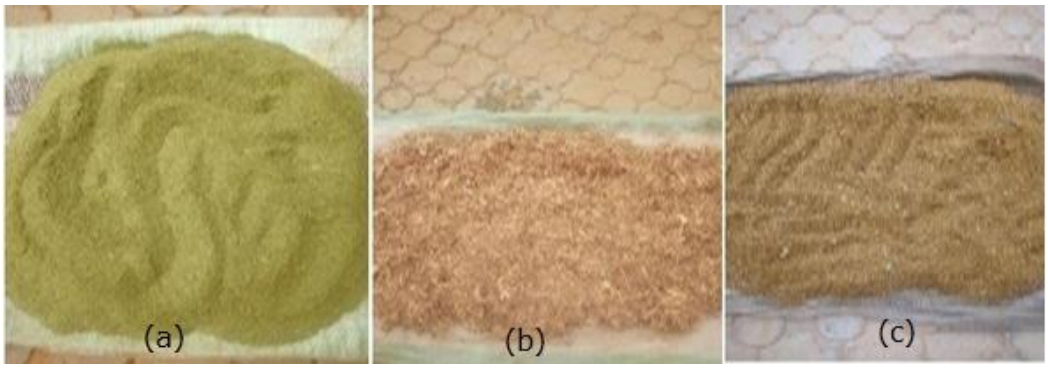

Fig. 1. Grinded Neem a) Leaves, b) Barks and c) Seeds Extracts

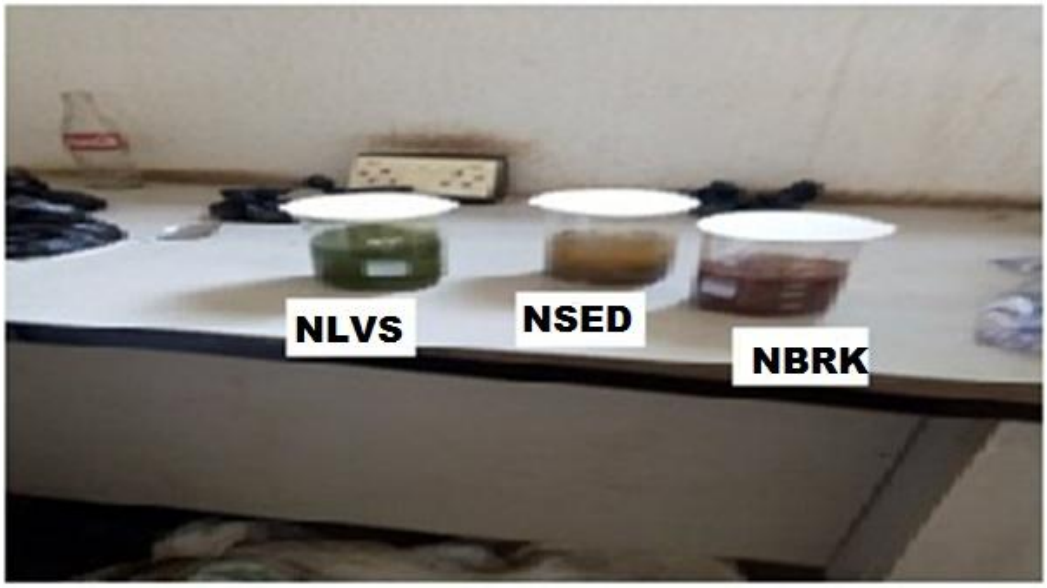

Fig. 2. Pre-fermentation of Neem leaves (NLVS), seeds (NSED) and barks (NBRK) samples

2.2.7 Manufacture of soaps

Oil The blend (200 g) was heated to about 40^0C on the hot plate; this procedure was done to obtain the blend oil and the base at the same temperature. The hot oil was then poured into a plastic mixing container. The caustic soda solution was then gradually poured into the oil.

The mixture was then thoroughly stirred together until a trace level (the point at which the soap mixture starts to thicken and form a stable emulsion) was observed. Immediately after the soap trace 20g solution of the fermented Neem parts (leaves barks and seeds) was added individually and comprehensive stirring continued. The mixture was further stirred until it was thick and the thick viscous soap was quickly poured into the mold. The mold was covered with a blanket for 24 hours to prevent the soap from absorbing moisture and losing its quality after drying. The blanket was removed after 24 hours and the soap was left open to dry. After three days the soap was analysed.

The same procedure was followed to produce the soap samples for extracts from the Neem barks and seeds (NBRK and NSED) using equal quantities by volume of the active ingredients from leaves seeds and barks, equivalent to 15g for the bark sample (NBRK) and 10g for the crushed seeds sample (NSED).

2.3 Bacterial Analysis

2.3.1 Microorganisms tested

The microorganisms tested were clinically isolated, at the Department of Micro-Biology laboratory, Federal University Birnin Kebbi,

Nigeria. The cultured microorganisms used are both bacteria, namely, *S. aureus* (causes pus, a whitish liquid on skin) and *P. acnes* (which causes bruising).

2.3.2 Culture media

The culture media used for the analyses were Mueller-Hinton Agar and potato dextrose agar. The media were used for determination of inhibitory activity (sensitivity test). All media were prepared according to manufacturer's instructions and were sterilized by autoclaving at 121^0C for 15 minutes.

2.3.3 Preparation of soap solutions of different concentrations

Four samples were tested including the three soaps prepared with leaves (NLVS), bark (NBRK) and seeds (NSED) extracts from Neem plant, and a normal market soap (NRM) used as soap control. The samples were prepared by dissolving 5 g, 10 g, 15 g and 20 g in 100 cm^3 of distilled water contained in beakers to make up four different soap concentrations. The samples were allowed to dissolve completely to give a soap solution.

Fig. 3. Production of seeds (a), barks (b) and leaves (c) extracts soaps

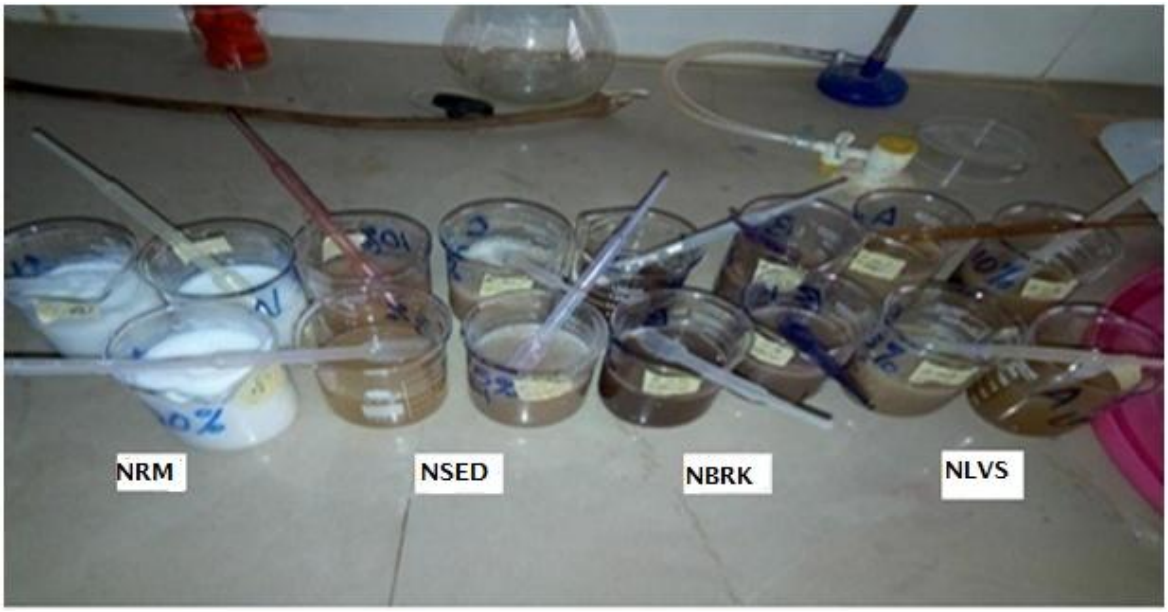

Fig. 4. Soap Solutions of various concentrations

2.3.4 Inhibitory activity through sensitivity tests

The inhibitory activities of the soap samples were determined using well sterile Mueller-Hinton Agar with the aid of sterile swab sticks. Four wells were punched on each of the inoculated cultured plates with a sterile 6mm in diameter cock borer. The wells were properly labelled according to different concentrations of the soap samples prepared. The wells were then filled up with 0.4 cm^3 soap solutions of each concentration. The inoculated plates with the soap samples were allowed to stay on the bench for 1 hour, to ensure that the soap solutions diffuse in the Agar. The Mueller-Hinton Agar plates containing the bacteria isolates were incubated at 35^0C for 18-24 hours.

At the end of incubation period, the plates were observed for any evidence of inhibition, which will appear as a clear zone that was completely devoid of growth around the well (zone of inhibition). The diameters of the zones were measured using transparent ruler calibrated in millimeter (mm) and the results were recorded.

3. RESULTS AND DISCUSSION

Table 1 presents the result of microbial tests carried out on the soaps samples, produced from the three different types of Neem parts (NLVS, NBRK and NSED) and normal commercial soap (NRMS).

The tests were conducted using four different concentrations of the soaps samples (20 mg/cm^3, 15 mg/cm^3, 10 mg/cm^3, and 5 mg/cm^3). These tests showed the effectiveness of anti-microbial action of soap samples, which is indicated by a clear zone around the well. The wider the zone diameter, the higher the activity of soap sample to inhibit the growth of the microbes. The microbes used for the tests are *S. aureus* and *P. acnes*. *S. aureus* is mostly found in public toilets, human genitals where it is transmitted during sexual intercourse, etc. It is a sexually transmitted disease. On the other hand *P. acnes* is a bacteria that is found in the air and on surface of objects.

According to the results, the Neem bark soap sample (NBRK) exhibited the most effective inhibitory behaviour among the three samples, having the highest zone of sensitivity against *S. aureus* (29 mm). This was closely followed by Neem leaves (NLVS) and Neem seeds (NSED).

The results also showed that the effectiveness of the Neem parts to inhibit bacterial growth decreases with the concentration of the soaps. Previous studies carried out on the whole Neem tree has shown that the plant was effective against *S. aureus* activity and a maximum zone of inhibition of 22±3 mm was achieved using a Neem extract of 700 μg in weight[4, 3].

The results on *P. acnes* showed that the Neem barks was the most effective inhibitory substance compared with the other soap extracts (27mm) at concentration 20 mg/ml. This was followed by the Neem seeds (24 mm) and the soap with lowest inhibitory activities was the Neem leaves. The results also showed that the inhibitory activity of the soap extracts against the *P. acnes* decreases with decrease in the concentration of the soap samples.

Table 1. Result of the diameter of zone of the inhibition zones after exposure to the neem soap samples and normal market soap

Concentration (mg/cm^3)	Diameter of Inhibition Zone (mm)			
	NLVS	NBRKS	NSED	NRMS
a) *Staphylococcus aureus*				
20	25±1.30	29±1.29	25±1.25	0
15	23±1.70	27±1.30	20±1.10	0
10	22±1.31	26±1.41	18±1.29	0
5	19±1.31	25±1.50	16±1.25	0
b) *Propionibacterium acnes*				
20	23±1.42	27±1.70	24±1.10	0
15	22±1.29	25±1.51	21±1.25	0
10	20±1.51	23±1.26	19±1.50	0
5	18±1.26	22±1.54	17±1.31	0

NLVS= Neem leaves soap, NBRK= Neem barks soap; NSED= Neem seeds soap; NRMS= Normal Market Soap.

The results show that sample NRMS, which is the normal commercial market soap, was not effective in inhibiting the activities of either the *S. aureus* or *P. acnes* microorganisms as demonstrated by the absence of zone of inhibition on the tested samples at all concentrations.

Analysis of variance for the means of antibacterial activities against the *S. aureus* and *P. acnes* showed that there is a significant difference across the different concentrations ($P<0.05$) in microbial action among the different soap samples tested.

Also, analysis of variance showed that there were no differences ($P>0.05$) between the means of the antibacterial activities across the same concentrations of the soaps samples both for the *S. aureus* bacterium and *P. acnes* tests.

Analysis of the variance of the zone of inhibitions using the combined results of the two microorganisms showed that there were no differences ($P>0.05$) between the means of the zone of inhibitions on the microorganisms used.

Also, the analysis showed that there are significant differences ($P<0.05$) for the means of the antibacterial activities among the different concentrations of the soaps used.

Furthermore, analysis of the variance also revealed that the influence of the type of microorganisms used on the antibacterial activities of the soap samples is not dependent ($P>0.05$) on the concentrations of the soaps used.

4. CONCLUSION

This investigation showed that antiseptic soaps can be produced from Neem plants and the tree is largely available nationwide in Nigeria. The studies revealed that almost all the essential parts of the plant are potent against the bacterial actions of the two forms of microorganisms that are common pathogens available in our environment.

From the results of the investigation it was indicated that the order of effectiveness against the activities of microorganisms follows the order: NBRK>NSED>NLVS>NRMS.

The ordinary soaps commonly purchased from the market as commercial soaps do not possess any antibacterial properties because they do not contain Neem extracts. Neem tree, which is a common tree that is widely available, can be used as a source of bioactive molecules with antiseptic properties against many diseases caused by harmful microbes.

To this end, soaps produced from Neem extracts can be used as an effective substrates of antiseptic compounds to control the skin microbiota and protect against pathogenic bacteria, which can cause numerous skin diseases and infections.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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