Original Research Article

PRODUCTION AND INVESTIGATION OF THE ANTISEPTIC PROPERTIES OF SOAPS MADE FROM THE BARKS, SEEDS AND LEAVES EXTRACTS OF NEEM TREE

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ABSTRACT

The main aim of this work is to investigate the antiseptic properties of the *Azadirachta Indica* (Neem) tree parts (Leaves, Bark and Seeds). The Extracts were used in the production of soap samples of various concentrations (20mg/cm^3, 15mg/cm^3, 10mg/cm^3 and 5mg/cm^3). Inhibitory Activity sensitivity test using Agar-well Diffusion Method was employed to test the antibacterial activities of the soap samples on two micro bacterial organisms, *Staphylococcus aureus* bacteria and *Propionibacterium acnes*. The test results show that soap samples from the Neem parts exhibited antiseptic properties against the microbial bacteria. According to the result, the Neem stem soap produces the highest level of effectiveness across the entire concentration spectrum. This was 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: NSTM>NSED>NLVS. The commercial soap used as a control sample did not exhibits any antibacterial activities against the two microbes.

Keywords: Neem, *Staphylococcus aureus* bacteria, *Propionibacterium acne*, Microbial activities.

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1.0 INTRODUCTION

Neem (Azadirachta indica) which is a word derived from an Indian language that means perfect, complete 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. Frequently cultivated and naturalized throughout the drier regions of tropical and subtropical countries. It is known for its therapeutic and ethno medicinal values since prehistoric era[2]. 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 for the purpose of taming the tide of desertification that is threatening the very existence of humanities 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. 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.

Culturally extract from the plant are customarily extorted from either the leaves, stem or bark of the tree and then used as medicines for the cure of many illnesses, such as; headaches, stomach aches, diarrhoea, piles, yellow fever, tooth problems to mention but a few. 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 the 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 production of natural manures for agricultural purposes.
The trunk of Neem is also converted to wood logs with the intention of using it as a domestic source of burning fuel that provide the heat energy for cooking purpose The entire trunk is burnt to obtain charcoal that is used as a fuel source.
Modern research also confirms Neem's curative powers and with the advent of modern scientific investigations the hidden medicinal compounds of Neem hitherto, unknown became known. Analysis have shown that Neem parts contains a large number of biologically active compounds ranging from azadirachtin, meleacin, gedinin, salanin, numbin, valassin and many other derivatives [1]. 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 [11].
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 are their ability to inhibit the activities of some certain bacterial microorganisms, thereby making them susceptible for use in the development of medicinal or curative agents [4]. 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 and substances extracted from the Neem leaf have been found to exhibit anti-fungal activity [1,2]
Substances that are active against certain diseases such leprosy, eye problem, intestinal worms, epistaxis, chicken pox, piles, cancer, and so on [1, 2, 3] have also been discovered from the neem parts.
Neem parts have also found application in the production of wide range of personal care products which include skin care products- including eczema cream, antiseptic cream and nail care; hair care products - shampoo, and hair oils; oral hygiene- toothpaste and Neem twigs; household products- soaps, insect repellants (spray and lotion) and candles [2].
There is a global increase in 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 [5]. 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 the level of their antibacterial actions against the microbial activities of certain micro-bacterial organisms. 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].

2.0 MATERIALS AND METHODS

2.1 Sample Collection

Fresh leaves, seeds and barks were collected of one of the tree from the 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[7]. They were then grinded in mortar and sieved to fine powdered particles as seen in figure 1 below.

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Fig. 1. Grinded Neem Extracts of Leaves; Stem and Seeds.

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should use the extract directly without fermenting we realize that small particles of the powdered extracts will be on the body after use.

2.2 Production of Antiseptic Soaps

2.2.1 Fermentation of Neem Extracts

The First stage in the production of the antiseptic soaps is to ferment the three Neem's extracts. The reason why the extracts were fermented is to allow microorganisms, such as yeast and bacteria, to acts on the substances to break them into smaller and simpler particles that will produce a clean and clear antiseptic soap. If we

The fermentation process was carried by measuring 70g of each powdered extracts (seed, stem and leaves) and transferring them into three separate 1000ml beakers. The beakers were labelled as NLVS (leaves) NSED (seeds) and NSTM (stem) 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.

Fig. 2, Pre-fermentation of Neem Samples

2.2.2 Preparation of Caustic Soda Solution/ Lye

Sodium hydroxide (NaOH) exits as a white crystalline pearl. It dissolves readily in water to give aqueous solution called Lye. It liberates substantial amount of heat in the course of dissolution. 30.34g of Caustic Soda and 76g of distilled water were weighed according to standard method [9]. The Caustic Soda was gradually added to the water inside a beaker stirred carefully until it completely dissolved in the water. It was allowed to cool down.

2.2.3 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 30minutes, and this ensures homogeneous blend of the oils.

2.2.4 Manufacture of Soaps

200g of the blend oil was heated to about 40^0C on the hot plate; this was done in order to have equal temperature with the base. 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 was observed. Immediately after the trace, 20g solution of the fermented Neem (leaves, stem and seed) was added individually and comprehensive stirring continues. The mixture was further stirred until it was thick and the thick viscous soap was quickly poured into the mould. The mould was covered with 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 stem and seeds (NSTM and NSED) using equal quantities by volume of the active ingredients from leaves, seeds and stems., equivalent to 15g for the bark (sample NSTM) and 10g for the crushed seed (sample NSED).

Fig. 3. Production of Seed, Stem and Leaves Extracts Soaps

2.3 Bacterial Analysis

2.3.1 Test Organisms

The test organisms were clinically isolated, at the department of Micro-Biology laboratory, Federal University Birnin Kebbi, Nigeria. The cultured microorganisms used include both bacteria and fungi, which are Staphylococcus aureus bacteria (causes pus, a whitish liquid on skin) and Priopionibacterium acnes (which causes bruising)

2.3.2 Culture Media

The culture media used for the analyses were Mueller Himpton 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, each, from the three Neem parts and the normal market soap namely; NLVS, NSTM, NSED and NRM were prepared by dissolving 5g, 10g, 15g and 20g of the samples in 100ml distilled water contained in beakers to make up four different soap concentrations. The samples were allowed to dissolve completely to give a soap solution.

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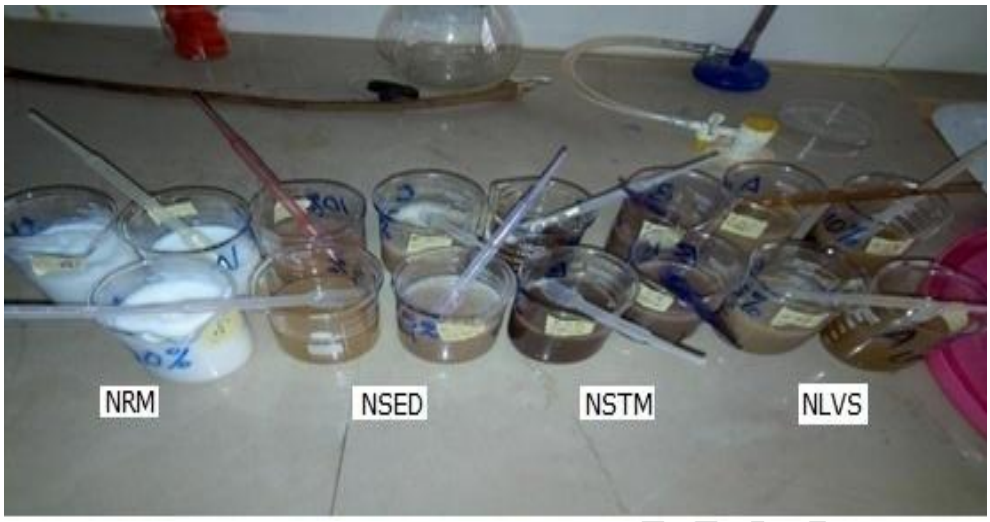

Fig. 4. Soap Solutions of various concentration

2.3.4 Inhibitory Activity (sensitivity) Tests

Determination of inhibitory activity of the soap samples were determined using agar well sterile Mueller Hinton agar (for fungi) with the aid of a 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 labeled 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 were allowed to stay on the bench for 1 hour, to ensure that the soap solution diffuse in the agar. The Mueller Himton 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.

Table 1: Result of the Diameter of Zone of Inhibition for the Neem Samples

Concentration (mg/cm^3)	**Diameter of Zone of Inhibition of Neem Parts (mm)**			
	NLVS	**NSTM**	**NSED**	**NRMS**
a) Staphylococcus. aureus Bacteria				
20	25	29	25	0
15	23	27	20	0
10	22	26	18	0
5	19	25	16	0
b) Propionibacterium acnes				
20	23	27	24	0
15	22	25	21	0
10	20	23	19	0
5	18	22	17	0

NLVS= Neem leaves, NSTM= Neem stem; NSED=Neem seeds; NRMS=Normal soap

3.0 RESULTS AND DISCUSSION

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

The tests were conducted using four different concentrations of the soaps samples (20mg/cm^3,

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15mg/cm^3, 10mg/cm^3, and 5mg/cm^3). These tests showed the levels of anti-microbial action of soap samples, which is signified 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 Staphylococcus aureus bacteria and Propionibacterium acnes. Staphylococcus 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, Propionibacterium acnes is a bacteria that is found in the air and on surface of objects.

According to the results, the Neem stem sample (NSTM) exhibited the most effective inhibitory behaviour among the samples with the highest zone of sensitivity against the S. aureus (29mm). This was closely followed by Neem leaves (NLVS) and the Neem seeds (NSED). The results also showed that the effectively of the Neem parts to inhibition of 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 activities and a maximum zone of inhibition of 22±3mm could be achieved using a Neem extract of 700μg in weight[1, 2].

Result on Propionibacterium acnes showed that the Neem stem is the most effective inhibitory substance compared with the other soap extracts (27mm) at concentration 20mg/ml. This was followed by the Neem seed (24mm) and the soap with lowest inhibitory activities was the Neem leaves. The result also showed that the inhibitory activity of the soap extracts against the Propionibacterium acne decreases with decrease in the concentration of the soap samples.

Statistical analysis of variance for the means of the antibacterial activities against the Staphylococcus aureus and Propionibacterium 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 are no differences (*P>0.05*) between the means of the antibacterial activities across the same concentrations of the soaps samples both for the Staphylococcus aureus bacterium and Propionibacterium acnes tests.

The results show that sample NRM, which is the normal market soap, is not effective in inhibiting the activities of either the Staphylococcus aureus or the Propionibacterium acne microorganisms as demonstrated by the absence of zone of inhibition on the tests samples at all concentrations.

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 use.

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4.0 CONCLUSION

This investigation shows that antiseptic soaps can be produced from Neem plants and the tree is largely available nationwide. The studies revealed that almost all the essential parts of the plant is potent against the bacterial actions of one form 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 microbial activities of microorganisms follows this order NSTM>NSED>NLVS>NSOAP. The ordinary soaps commonly purchased from the market as commercial soaps do not possess any antibacterial properties because they are normally the product of saponification of only oil and alkaline with no medicinal substances added to them. Neem tree, which is a common tree that is widely available, can be used as a form of antiseptic cure against many diseases caused by harmful microbes. As such, Neem tree can be used to serve as an effective control to the skin against the microbial activities of certain form of pathogenic bacteria, which can cause one form of skin diseases or the other.

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