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Effect of Essential Oil Extracted from *Azadirachta Indica* (Neem) Leaves in Formulated Natural Antibacterial Soap

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Abstract

Natural remedy is sustainable in nature and was used in this present study, effects of essential oils from *Azadirachta indica* (neem) leaves in antibacterial soap was investigated. The essential oil was extracted via steam distillation and characterization of oil results are %yield (28%), acid value (16.5±0.5mg KOH/g); iodine value (42.40±2.5mgI/g) and saponification values (139±3.2mg KOH/g). The extract proportions 2, 4, 6, 8 and 10 (mg/ml) respectively were used to formulate the soaps. The results of the soap characterization were: pH (9.3); foamability (3.5cm), color (light yellow). The antibacterial investigation used Ciprofloxacin and ketoconazole as positive controls whereas *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* were clinical isolates of microbes used. Sensitivity test was conducted and an in vitro evaluation of the anti-microbial activity was conducted using ditch-plate techniques. Strains of reference microbes namely *Candida albicans*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* were tested at four different soaps' concentrations (0.0, 2.0, 5.0 and 8.0 mg/ml). ANOVA mean results of zones of inhibition also revealed variability of antimicrobial activity among the natural formulated soaps with strong positive correlation ($r = 0.9912$; $P > 0.01$) between zones of inhibition and soaps' concentrations was evidenced. Blend 8:2 of the formulation exhibited the largest zone of inhibition (40 mm) on S. aureus, followed by Candida albicans with (35 mm) zone of inhibition whereas the least zone of inhibition (1.8 mm) was observed in 2mg/ml extract formulation. The high pH shows it conforms to NAFDEC set range of 9 to 11 pH. Therefore, with local oil, National Economic Transformation is achieved hence, this highly sustainable product is recommended to homes, pharmaceutical companies, health organizations and government in handling topical issues related to skin infections caused by microorganisms.

Keywords: *Herbal soap, Azadirachta indica, Microorganism. Zone of inhibition.*

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Background to the Study

Soap is a mixture of sodium or potassium salts of various naturally occurring fatty acids. It is a substance of ancient origin, the manufacture of which according to Gunstone *et al.*, (1986) has evolved from primitive beginnings into a sophisticated chemical process. Soap making started long ago in Egyptian and Babylonians. Several millennia ago, crude mixtures of animal fats and the alkaline plant ash were found to generate crude soaps which lathered and cleaned effectively (Phanstiel *et al.*, 1998). Through the centuries, there have been other times when people were able to make soap using sodium or potassium salts as is done nowadays. From ancient times Indian, Chinese, Egyptian, Greek, Roman and Syrian medicinal systems documented the use of different plant-based medicine for different diseases (Young *et al.*, 2004). Medicinal properties of neem have been known to Indians since time immemorial. The earliest Sanskrit medical writings refer to the benefits of neem's fruits, seeds, oil, leaves, roots and bark. Each of these has been used in the Indian Ayurvedic and Unani systems of medicine.

Significance of Study

The result of this work will be beneficial to students, household and general public. It will also reduce the skin infections, rashes and inflammations, therefore be of great importance to the public health. Favorably, locally made and natural neem soap can be used to fight against activities of some microorganism. It would help control the transmission of infectious diseases in the environment.

Statement of Problem

There have been problems as a result of bacteria, fungal and viral infections. According to World Health Organization drugs, micro-organisms like *Escherichia coli* and *staphylococcus aureus* are developing resistance to several drugs which pose a severe threat like inflammation and other infections to the general public health. Essential oils from plants are of greater relevance with the current global shift to obtain drugs from plants. According to (Levy, 2001) and (White, 2001), over exposure to medicated soaps might result in antimicrobial resistance and even render an individual more vulnerable to microbial attacks such as opportunistic skin infections (Russell, 1998).

On the other hand, regardless of a wide-spread availability of the so-called medicated soaps; a number of communicable infectious and food-borne diseases as well as poor-hygienic conditions-related health problems are rampant. This can partially be explained by the fact that, occasionally some of these antimicrobial consumer products could have insufficient quantities of antimicrobials. It seems to be more of a marketing phenomenon. Unfortunately, in the long-run may adversely affect the consumers, because overuse of these agents can ascribe to the emergence of drug-resistant microorganisms (Chauncher, 2001). However, serious need for a non-synthetic approach to fight these microorganisms, hence this investigation on Effect of Essential Oil Extracted from *Azadirachta indica* (neem) Leaves in Formulated Natural

Aim and Objectives of the Study

The aim of this research is the effect of essential oils extracted from *Azadirachta indica* (neem*)* leaves in formulated natural bacterial soap herbal soap.

Objectives of study are to

(i) To extract the essential oils from their plants using clevenger apparatus (steam distillation) and soxhlet extractor apparatus (solvent extraction)
(ii) To use the extracted oils to formulate the soaps in single and combined form,
(iii) To conduct microbial sensitivity test of the products and finally
(iv) To characterize the bacterial soap for quality control and assurance

Description of the Plant Azadirachta Indica (Neem) Leaves

Azadirachta indica (neem) is world-renowned medicinal plant, having long history of usage in various ailments in Indian traditional medical system (Ayurveda, Unani, Tibetan), since time immemorial. Each part of neem, including leaves, bark extracts, oil, and products made from neem have medicinal properties. Neem products have been proved antihelminthic, antimicrobial, and act as a contraceptive and sedative agent (Ganguli, 2002). Although neem has been proved antimicrobial in nature (Aarati *et al.*, 2011), to the best of our knowledge, its role has not been evaluated in biofilm-associated infections. Therefore, this study was undertaken to evaluate the role of neem in bathing soap.

Medicinal Values of Neem

It is claimed that neem provides an answer to many incurable diseases. Traditionally neem products have been used against a wide variety of diseases which include heat-rash, boils, wounds, jaundice, leprosy, skin disorders, stomach ulcers, chicken pox, etc. Modern research also confirms neem's curative powers in case of many diseases and provides indications that neem might in future be used much more widely (El Astal *et al.*, 2005). Another investigation found that quercetin, an antibacterial compound, exists in neem leaves. Other studies have shown that the polysaccharides in neem reduce the inflammation and swelling that occur in arthritis. Not only does neem help reduce inflammation; it also has pain suppressing properties. Neem can also help create a balance in the immune system, directly affecting the progression of arthritis.

Cancer

Throughout Southeast Asia neem has been used successfully by herbalists for hundreds of years to reduce tumors. Researchers are now supporting these uses. Neem has been tested on many types of cancers, such as skin cancers, using neem-based creams and lymphocytic cancer, using the herb internally. In India, Europe and Japan scientists have found that polysaccharides and limonoids in neem bark, leaves and seed oil reduced tumors and cancers and were effective against lymphocytic leukemia (Ahmad *et al.* 1998).

In another study, one researcher used an extract of neem leaves to prevent the adhesion of cancer cells to other body cells. If cancers can't stick to other cells, the cancer can't spread throughout the body and is more easily destroyed. Neem's success has been noticeably remarkable with skin cancers. A number of reports have been made by patients that their skin

cancers have disappeared after several months of using a neem-based cream on a daily basis. Injections of neem extract around various tumors have shown sizable reduction in a few weeks' time.

Dental

People in both India and Africa have used neem twigs as tooth brushes for centuries. Neem twigs contain antiseptic ingredients necessary for dental hygiene. Neem powder is also used to brush teeth and massage gums. In Germany many researchers have shown that neem extracts prevent tooth decay and periodontal disease (Kallel *et al.*, 2008). Infections, tooth decay, bleeding and sore gums have all been treated successfully with daily use of neem mouth rinse or neem leaf extract added to the water. Some people have reported a total reversal of gum degeneration after using neem for only a few months.

Diabetes

Because neem is a tonic and a revitalizer, it works effectively in the treatment of diabetes, as well. More than a disease that requires change of diet, diabetes is the leading cause of blindness in people ages twenty-five and seventy-four; it also damages nerves, kidneys, heart and blood vessels; it may even result in the loss of limbs.

Rheumatism

Neem leaves have anti-inflammatory activity, similar to that in drugs such as phenylbutazone and cortisone. They can relieve pain and reduce acute pain edema. For rheumatism, tropical applications of a warmed neem cream that contains neem oil and perhaps a mild neem tea will help lessen pain.

Vitiligo

Vitiligo is believed to be an autoimmune disorder that causes patches of skin to lose their color. It occurs in about five percent of the human population regardless of race, but most commonly in dark-skinned people. The two most common treatments are exposure to sunlight (or PUVA) or corticosteroid old drugs, but these are not always effective.

Neem has been highly successful against harmful fungi, parasites, and viruses. Although it can destroy these, it does not kill off beneficial intestinal flora nor produce adverse side effects. Neem is toxic to several fungi that attack humans, including the causes of athlete's foot and ringworm and Candida, which cause yeast infections and thrush. In fact, neem extracts are some of the most powerful Antifungal plant extracts found in the Indian pharmacopeia that are used for these conditions. The compounds gedunin and nimbidol, found in the tree's leaves, control the fungi listed above. One of neem's stronger advantages is its effect upon the skin in general. It has been most helpful in treating a variety of skin problems and diseases including psoriasis, eczema and other persistent conditions. According to a report from the National Research Council's Ad Hoc Panel of the Board on Science and Technology for International Development, neem preparation from the leaves or oils can be used as general antiseptics. Because neem contains antibacterial properties, it is highly effective in treating epidermal conditions such as acne, psoriasis and eczema. It is also used for treating septic sores, infected burns, scrofula, indolent ulcers and ringworm. Stubborn warts can be cleared

up when a high-quality neem product is used. Unlike synthetic chemicals that often produce side effects such as rashes, allergic reactions, or redness, neem doesn't seem to create any of these results.

Materials and Methods

Collection and Preparation of Plant Materials

The fresh leaves of *Azadirachta Indica* (Neem) Leaves were collected from farms around Oko, Anambra State, Nigeria in March 2019. The plants were identified by a plant Taxonomist/Botanist Dr S.I Okeke of Science Laboratory Technology Department Federal Polytechnic Oko, Anambra State.

Materials/ Apparatus

Instrument and glassware used for this work were the facilities of the Science Laboratory Federal Polytechnic Oko, while the chemicals and reagents were of analytical grade and standard.

Methods

The collected plant materials *Azadirachta Indica* (Neem) Leaves were oven dried at 80°C for 72 hours and ground to powder. The powdered sample was kept in a clean specimen bottle or container and stored in a freezer till used for analysis.

Extraction of Plant Material

The powdered plant material was extracted by steam distillation method via Clevenger apparatus and solvent extraction method with acetone for 3 hours. The oils crude and essential were used in single and combined doses in the ratios of 2:8, 4:6, 6:4, 8:2, 0:0 (blank),0:5 and 5:0 and 5:5, respectively.

Formulation of Herbal Soap: Cold process soap making was used

This process requires a large stainless bowl, rubber gloves, molds, cardboard, plastic bag, Azadirachta Indica (Neem) Leaves oils, natural dye, distilled water and fragrances. The cold process gets its name from the general low temperature that is used to mill this type of soap.

Eight samples of blends, single and blank doses of the active ingredients from Azadirachta Indica (Neem) Leaves were used in the formulation of the soap. The soap samples were prepared separately using each dose of the oil samples. The caustic soda solution and oils were mixed in the ratio of (1:2) the hydrometer gauge was used to check the density of the solution. The oil phase solutions were gradually transferred into the water phase with constant stirring of the solution in one direction to form a homogeneous mixture, hence stable emulsion of the products was achieved (Mabrouk, 2005).

Determination of Percentage Yields of the Product

The products in emulsion form were transferred to molds to solidify at room temperature. The yields of the products were obtained by measuring the mass in grams using top loading balance.

Determination of pH the Products
This was achieved using pH meter by making 2% solution of the product and the results were recorded.

Antimicrobial Analysis of the Product
Antimicrobial susceptibility profile of the herbal soap blends on microbes was carried out. This was achieved using different concentrations of soap suspensions (0.0, 2.0, 5.0 and 8.0) mg/ml, respectively. Ciprofloxacin and ketoconazole were positive controls whereas *pseudomonas aeruginosa, staphylococcus aureus, Escherichia coli* and *candida albicans* were clinical isolates of microbes used. The results are recorded as (--) which denotes no inhibition; (ND) which denotes not done and (ZI values) denotes zone of inhibitions.

Sensitivity Test
Sensitivity test was an *in vitro* evaluation of the anti-microbial activity using ditch-plate techniques. Ciprofloxacin and ketoconazole positive controls were used whereas *staphylococcus aureus, Escherichia coli* and *candida albicans* were clinical isolates of microbes used. Strains of reference microbes namely *Candida albicans*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* were tested at four different soaps' concentrations (0.0, 2.0, 5.0 and 8.0 mg/ml). ANOVA means results of zones of inhibition obtained and recorded.

Results and Discussion
The result of characterization of the oils showed that *Azadirachta Indica* (Neem) Leaves yielded 12.18 % and 10.50%; for essential oils and crude extract. Their pH was 6.0 and 5.8 for essential oils and crude extract respectively (Table 1). The result of characterization of the oil shows high yield of the oil as a source of income generation and can be used in the production of soaps, creams and ointments. The extracted oils were used in the formulation of antibacterial soap in varying ratios of both oils. Low acid values of the oil is an indication of low susceptibility to oxidative rancidity and very good to inflammations and rashes.

Table 1: Result of the Characterization of the Oils

Bio oils	% Yield	pH	Colour	Mass of sample	Acid value	Sapon value
Crude extract	10.50	6.0	light yellow	200g	4.72mgKOH/g	65.10 mgKOH/g
Essential oil	12.28	5.80	Colourless	200g	2.30mgKOH/g	18.55 mgKOH/g

The result of the antimicrobial susceptibility profiles of herbal formulated soap blends on tested microbes shows strains of reference microbes namely *Candida albicans*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* at four different soaps concentrations (0.0, 2.0, 5.0 and 8.0 mg/ml). ANOVA mean results of zones of inhibition also revealed variability of antimicrobial activity among the natural formulated soaps with strong positive correlation ($r= 0.836$; $P<0.01$) between zones of inhibition and soaps' concentrations (Table 2). There is evidence of synergistic effects of *oils essential and crude* ratios 8:2 soap. This is indicated by exhibition of largest zone of inhibition (40 mm) on *S. aureus,* followed by *Candida albicans* with

(35 mm) zone of inhibition whereas single formulations gave the least zone of inhibition (7.2mm and 4.2 mm) respectively for *essential and crude oils*, respectively. The result of the sensitivity test shows determination of minimum inhibitory concentration (MIC), Minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC). All the media was prepared according to manufacturer's instructions and sterilized by autoclaving at 121^{O}C for 15 mins. Results from the incubation of herbal soap suspensions (30 µl) with the strains of reference microorganism-inoculated-agar plates depict a variability of antimicrobial efficacy (Table 2). The ANOVA mean results of zone of inhibition revealed variability of antimicrobial activity among the natural formulated soaps with positive correlation (P<0.01) between zone of inhibition and the tested blend soap formulations. Synergistic effects of (*essential oils and crude extracts)* ratios 8:2 soap exhibited the largest zone of inhibition (40) on S.aureus followed by *candida albicans* with (35mm) zone of inhibition. Blends 6:4, 4:6, 5:5 and 2:8 were equally effective (P<0.05) against *S. aureus* (Table 2). Whereas blend 5:0 and 0:5 exhibited the least zone of inhibition on the tested bacteria. *C. albicans* was the least susceptible showing very small zone of inhibition (2.7mm) at the highest assayed concentration (8 mg/ml) as depicted on (Table 2). This shows that majority of the assayed synergist soaps have satisfactory antibacterial activity, though its antifungal activities were not really highly effective with exception of blend (8:2) for Essential oils and crude extracts.

Table 2: Antimicrobial Susceptibility Profiles of Antibacterial Soap

S/N	Blends	Conc.(mg/ml)	Mean Diameter Zone of Inhibition(mm)			
			P.aureginosa	***E.coli***	***S.aureus***	***C.albicans***
1	8:2	2.0	10.3	12.1	23.8	19.8
		5.0	15.2	20.3	22.7	27.6
		8.0	20.2	27.0	40.0	35.0
2	6:4	2.0	7.1	13.6	13.6	-
		5.0	14.1	14.0	22.7	-
		8.0	15.5	27.4	33.2	11.8
3	4:6	2.0	8.5	7.2	8.1	-
		5.0	11.4	9.7	12.0	4.8
		8.0	16.6	14.0	15.9	8.0
4	2:8	2.0	-	3.3	4.5	6.0
		5.0	4.2	5.1	8.0	7.1
		8.0	6.5	8.7	11.5	9.0
5	0:5	2.0	5.8	11.6	4.2	-
		5.0	11.0	12.6	7.1	7.2
		8.0	13.4	17.7	12.7	12.0
6	5:0	2.0	-	0.5	-	-
		5.0	3.6	5.9	2.3	-
		8.0	6.9	7.5	4.0	2.7
7	5:5	2.0	7.9	12.3	13.0	-
		5.0	12.6	17.8	17.4	-
		8.0	14.8	21.3	24.3	6.8
8	Ketaconazole	15µ/disc	ND	ND	ND	12.5
9	Ciprofloxacin	15µ/disc	26.4	33.0	28.8	ND
10		0.0	0:0	0:0	0:0	0:0

Blend essential and crude oils (-) denotes no inhibition; ND denotes Not Detected

The result of the characterization of the product shows that the pH of the products is slightly alkaline with the selected blend value of pH 7.5. This is an indication of the product in a stable form. This value is in line with the pH of standard l soap (8.0) being slightly alkaline too (Table 3). Also, the antimicrobial susceptibility profiles of herbal soap were carried out and result proved less effective for *essential oils* only blend (5:0), slightly effective for *crude extract* only blend (0:5); and highly effective for essential oils and crude extract blend (8:2) (Table 3). This is in line with work by (Nwambete and Lyombe, 2001) when they studied antimicrobial analysis of medicated soaps. It is an indication of the combined action of the essential oils and crude extracts in the formulation of the antibacterial soap. They possess antibacterial and anti-inflammatory properties.

Table 3: Result of Characterization of the Product (Herbal Soap)

S/N	Soaps	pH	Foamability	Skin Reaction	Antimicrobial
1	*Essential oils* only	8.4	Good	none	less effective
2	*Crude extract* only	7.9	Good	none	mildly effective
3	Essential and crude oils	8.2	Very good	none	highly effective
4	Standard	8.0	Very good	none	highly effective

Conclusion

From the results obtained they proved that with *Azadirachta indica* leaves essential oils and crude extracts gave an effective, cheap and environmentally friendly antibacterial soap. This can be a source of income generation for economic recovery. The soap oils produced from all the blends of the oils exhibited varying levels of zone of inhibition against bacteria and even fungi, however soap of blend of ratio *azadirachta indica* 8:2 essential oils and crude extract gave the highest zone of inhibition. The antimicrobial effect exhibited by the soap in this study signifies the potential of the soap as a topical therapeutic and protective agent.

Recommendations

Therefore, we recommend this product to homes, pharmaceutical companies, health organizations and government in handling topical issues related to skin infections since it is sustainable. It is friendly to the body with no adverse effects to man and environment due to absence of common synthetic antibacterial active ingredients triclosan, tri chloroxylenol and trichlorocarbanilide. This research will be beneficial to the general public in control and treatment of diseases. This is also an awareness that cheap, easily available biomaterial can help in income generation and national security.

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