

Full Length Research Paper

Synthesis and characterization of antiseptic soap from neem oil and shea butter oil

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In this work, neem oil and shea butter oil were mixed in various proportions and used in preparing soaps which were subsequently characterized. The combination of Neem oil to Shea butter oil considered were 100:0, 80:20, 60:40, 50:50, 40:60, 20:80 and 0:100 (wt:wt). The physical properties of the prepared soap including hardness, foamability and pH were analyzed. The antibacterial properties of the prepared soaps in terms of sensitivity, minimum inhibitory concentration and minimum bactericidal concentration (with respect to *Staphylococcus aureus* and *Bacillus subtilis*) indicated that the optimal anti-bacterial property of the developed soap was obtained with the exclusive use of shea butter oil.

Key words: Neem oil, shea butter, antibacterial property.

INTRODUCTION

Antiseptic soap, sometimes called antibacterial soap or anti-fungal soap, is a regular soap in liquid or solid form. According to Osborn and Grobe (1982) antibacterial soaps can remove 65 to 85% bacteria from human skin. Contemporary commercial antimicrobial soaps contain synthetic chemicals such as triclosan, triclorocarbanilide and chloroxynolol, most of which are thought to be carcinogenic, mutagenic and or generate allergic reactions. Triclosan (2,4,4'-trichloro-2'-hydroxydiphenyl ester) has been used in soaps, shampoo and fabrics, as an antimicrobial agent. Though these compounds are considered to have low toxicity, their 2-hydroxy isomers have been shown to undergo thermal and photochemical ring closure to form polychlorinated dibenzo-p-dioxins which are highly toxic (Okumura and Nishikawa, 1996). In addition, it is thought that bacteria could develop resistance to triclosan and this could lead to development of resistance and change in microbial community structure (Aiello et al., 2007). These shortcomings of antiseptic soap (treated with synthetic antiseptics) generate the need to develop more environment friendly toilet soaps.

Shea butter oil is a soft paste of melted fat with a milky colour in solid form and brownish when melted and is obtained from shea tree (*Butyrospermum parkii*). It contains fatty acid triglyceride, phytosterol and a high amount of unsaponifiable matter; from 2.5 to 15% (Eka, 1997). Shea butter oil contains cinnamic acid, a substance that helps protect the skin from harmful ultra-violet rays (Tella, 1979). Shea butter fat finds uses in soap making, in cosmetics and in traditional medicine in rural areas (Alander, 2004; Maranz et al., 2004). In Nigeria, a traditional medicated soap is produced from a mixture of vegetable oils (palm kernel oil and shea butter) that make the soap to have antimicrobial properties recognized in the traditional African households (Getradeghana, 2000; Aliyu et al., 2012). Due to its richness in food nutrients, the shea butter oil has found use as baking fat (Akhter et al., 2008).

Neem oil, extracted from the seed of neem plant (*Azadirachta indica*) is reported to contain natural organic antimicrobial agents, largely used in the Indian sub continent in traditional/folk medicine. Upadhyay et al. (2010)

reported neem oil to be highly bactericidal. Neem oil has been used in the treatment of inflammation, pain and swelling that occur in arthritis (Subapriya et al., 2005). When properly used, the oil combats vaginal infection and sexually transmitted diseases and kills lice (Abdel-Ghaffar and Semmler, 2007). It is used in the treatment of skin diseases such as scabies (Heukelbach and Feldmeier, 2006), ringworm and athlete's foot (Khan and Wassilew, 1987) and also in the treatment of phytophthora infestans, (Mirza et al., 2000) etc. Mak-Mensah and Firempong (2011) prepared toilet soap using neem oil and suggested that due to the phytoconstituents in neem oil and the favorable chemical characteristics of the soap, it can be used as medical and cosmetic toilet soap as also reported by Warra (2012).

Neem plant is known to be abundant in countries such as Nigeria and India and the oil makes up 50% of the kernel. Upadhyay et al. (2010) reported neem oil to be highly bactericidal. This work was aimed at preparing soap using varying combinations of neem oil and shea butter oils, studying the properties of the prepared soap (especially the antibacterial) and comparing the properties with those of a commercial antiseptic soap.

MATERIALS AND METHODS

Procurement of neem oil

Cold pressed neem oil was obtained from National Research Institute of Chemical Technology (NARICT), Zaria. Unrefined Share butter oil was also obtained in Mando market, Kaduna, Nigeria. A commercial antiseptic soap was also procured for use in comparison. The commercial antiseptic was made up of the following ingredients: sodium palmitate, sodium palm kernelate, water, talc, glycerine, perfume, trichlorocarbanilide, sodium chloride, sodium olefin sulfonate, PEG-7 Amodimethicone, cyclodextrine, trideceth-10, acetic acid, tetrabutyl ammonium bromide, tetra sodium EDTA, tetrasodium etidronate, CI 77891, CI 42090 and dye.

FTIR analysis of neem and vegetable oil

The oil samples were analyzed using FTIR (8400S Shimadzu, Japan). The range was 340 to 4700 cm^{-1} and the matrix used was KBr.

Soap making (cold process)

Seven different combinations of neem oil and shea butter oil were prepared by mixing them in the ration 0:100, 20:80, 40:60, 50:50, 60:40, 80:20 and 100:0, respectively. Each combination weighed 100 g. Soap samples were prepared from each combination using the cold process. Using neem oil to shea butter oil ratio of 0:100, the oil was heated to 70°C and cooled to 60°C. 25.52 g of NaOH was weighed and dissolved in 76 g of water and cooled to 60°C. The NaOH solution was added to the oil while stirring until the mixture traces (raised lines of soap appear when the stirrer is run across the surface, and it is noticeably thicker). The tracing mixture was poured into the mould. This was repeated in turn for each neem oil – shea butter blend.

Characterization of soap

All analysis reported here was done for eight soap samples: the seven prepared and the commercial antiseptic soap samples.

Hardness test

To determine the hardness of the soap, a needle (6.4 cm in length; 1 mm in diameter) to which a lead fishing weight (130 g) was attached was lowered unto the soap, the distance into which the needle penetrates the soap, after 30 s, was recorded as a measure of its hardness. This was repeated thrice for each soap sample and the mean and standard deviation computed.

Foamability test

2.00 g of the soap was dissolved in 50 ml of distilled water in a 100 ml measuring cylinder and shaken vigorously for 2 min. It was allowed to stand for 10 min after which the height of the foam was determined. This was repeated thrice for each soap sample and the mean computed.

pH analysis

The pH values of the soaps produced were analyzed using a pH meter (Kent EIL 7055). 2.0 g of the produced soaps were dissolved in 50 ml of deionised water and the pH determined using the meter. This was done twice for each soap sample and the mean computed.

Determination of soap sensitivity, minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)

The antibacterial properties of the soap samples was studied using three clinical isolates of bacteria, *Staphylococcus aureus*, *Bacillus subtilis* and *Eschericia coli*, obtained from Department of Microbiology, Ahmadu Bello University, Zaria, Nigeria. From the slant culture of the test organisms, a colony was obtained and suspended into sterile distilled water in a bijou bottle. This was standardized using Mcfarland turbidity standard scale Number 1 which corresponds to 10^6 cfu/ml. 10 g of each soap sample was weighed and dissolved in a sterile bijou bottle containing 10 ml of distilled water, and stock solution (1000 mg/ml). The stock solution of the soap was used to prepare various concentrations of each soap sample (500, 250, 125 and 62.5 mg/ml).

Sensitivity of the soap samples

The agar well diffusion method was used. 20 ml of freshly prepared Muller-hinton agar was poured into sterile Petri-dishes and the agar was allowed to solidify (gel). Using sterile syringe, 0.2 ml of each of the standardized organisms was inoculated into two Petri-dishes. Four (4) wells each of diameter 6 mm were made into each agar plate using a cork borer and the plate were labeled. Unto each plate, 0.2 ml of appropriate soap dilution was placed in appropriate wells, that is, 500, 250, 125 and 62.5 mg/ml, respectively. The plates were left for about 1 h for the soap to diffuse into the agar and then were incubated at 37°C for 24 h. After incubation, the plates were observed for evidence of inhibition which will appear as a clear zone completely devoid of growth around the well (zone of inhibition). The diameters of the wells were measured using a calibrated ruler in millimeters. The experiment was performed in duplicates and the mean of the zone of inhibition computed.

Table 1. Functional groups identified in neem oil.

Frequency	Functional group
1449.55	C=H
1739.85	C=O
2929	CH _{stretch}

Table 2. functional groups identified in shea butter.

Frequency	Functional group
1449.55	C=H
1739.85	C=O
2929	CH _{stretch}

Table 3. Nomenclature of neem – shea butter soap samples.

Parameter	S1	S2	S3	S4	S5	S6	S7	S8
Ratio (N:S) %	100:0	80:20	60:40	50:50	40:60	20:80	0:100	0:0

N:S, Neem to ratio shea butter; S1 – S7, neem – shea butter soap; S8, commercial soap.

Table 4. Hardness of the soap samples.

Sample	S1	S2	S3	S4	S5	S6	S7	S8
Depth of needle penetration, cm	2.64±0.152	4±0.123	3.46±0.152	3.32±0.084	4.28±0.084	4.06±0.089	2.82±0.148	0.62±0.045

S1 – S7, neem – shea butter soap; S8, commercial soap.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of solution

These were determined using tube dilution method (Talaro and Talaro, 2002; Betts et al., 2012). Muller-hinton broth was the media used. A serial dilution of the soap was made to obtain 125, 62.5, 31.25, 15.75 and 7.87 mg/ml. For the determination of MIC, a loopful (0.01 ml) of each test organism suspension was inoculated into the labeled tubes and incubated at 37°C for 24 h. A tube containing Muller-hinton broth, but without an organism was used as control. The tubes were then examined for the presence or absence of growth using turbidity as criterion. The lowest dilution in the series showing no growth of the test organism was considered to be the MIC. For MBC determination, a sterile wire loop was dipped into the tubes that did not show inhibition of growth and streaked on a sterile nutrient agar plate. The plates were then incubated at 37°C for 24 h and observed for growth. The experiment was performed in duplicates to ascertain the reproducibility of the observed MIC and MBC results.

RESULTS AND DISCUSSION

FTIR analysis

Figures 1 and 2 show the FTIR analysis of the vegetable oils. Tables 1 and 2 correspondingly present the functional groups identified in the oils.

Tables 1 and 2 present the functional group identified in the corresponding FTIR spectra of Figures 1 and 2. The functional group (C=O, C=H and CH_{stretch}) identified in Tables 1 and 2 are typical of vegetable oils. Table 4 shows the extent of penetration of the loaded needle on the soap samples (Hardness). From the result obtained soaps S1 and S7 WERE harder while the commercial soap (S8) was the hardest. Amongst the prepared soap samples S1 to S7, the soap samples prepared using pure neem oil (S1) and pure shea butter oil (S7) were found to be hardest and their degree of hardness were close (2.64 and 2.82 cm of needle penetration, respectively). This implies that combining the oils in soap formulation resulted in a lowering of hardness. S1 and S7 were not as hard as the commercial antiseptic soap (S8).

Table 5 shows the foamability of the various soap samples. As shown, the commercial antiseptic soap (S8) exhibited the highest foamability (with soap solution + foam height of 21.22 cm). It may also be observed from Table 5 that variable neem oil/shear butter oil ratios did not significantly affect foam height (the average of the height of S1 to S8 shown in Table 5 being 14.306 with a standard deviation of 1.055). Formulation of shampoos and detergents usually requires good foamability though

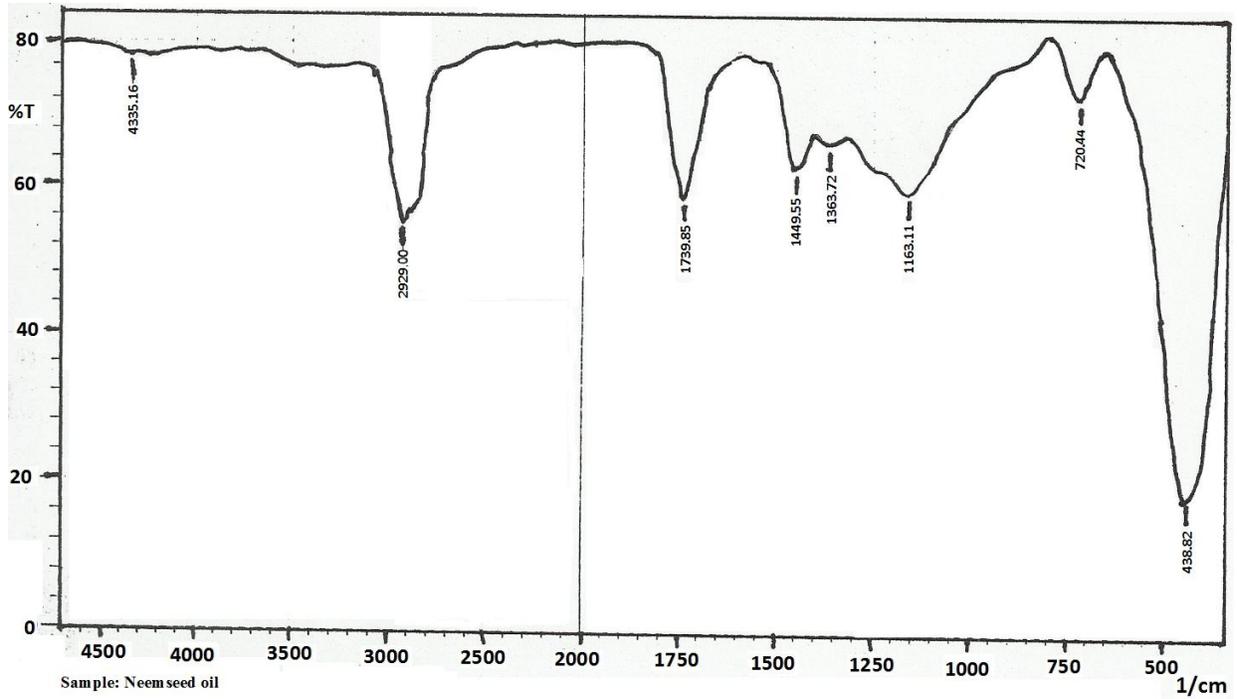


Figure 1. FTIR analysis of neem oil.

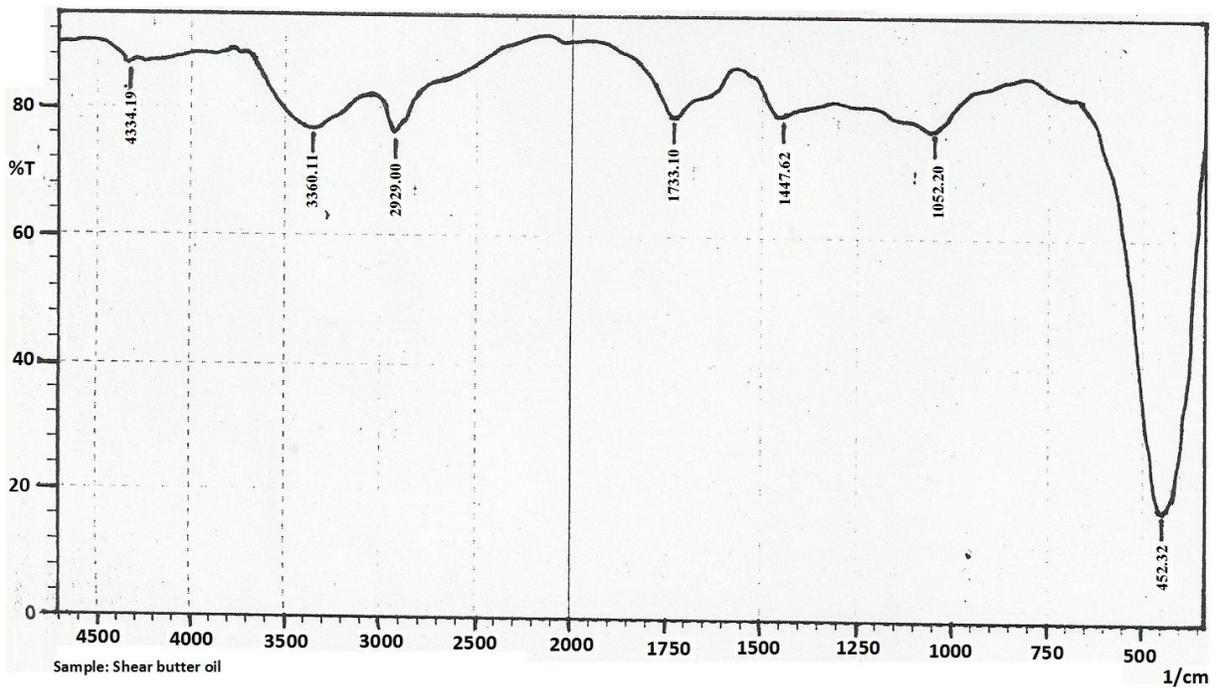


Figure 2. FTIR analysis of shea butter oil.

this can sometimes run against other desirable properties such as rinsability. From Table 6, the pH of all soap samples fell within the recommended range for bathing soap of 9 - 11 (Mak-Mensah and Firempong, 2011).

Table 7 shows the sensitivity of the three microorganisms (*S. aureus*, *B. subtilis* and *E. coli*) to various concentrations of the soaps. The commercial antiseptic soap (S8) recorded the highest zone of inhibition for all soap

Table 5. Heights of the foam by various samples dissolved in water.

Soap	S1	S2	S3	S4	S5	S6	S7	S8
Soap solution+ foam height (cm)	15.56	13.44	13.44	13.44	15.98	14.14	14.14	21.22

S1 – S7, neem – shea butter soap; S8, commercial soap.

Table 6. pH of the neem – shea butter soap samples.

Soap	S1	S2	S3	S4	S5	S6	S7	S8
pH	9.99	9.76	9.67	9.35	9.54	9.26	9.11	9.41

S1 – S7, neem – shea butter soap; S8, commercial soap.

Table 7. Sensitivity of microorganism to various concentrations of soap.

Test organism	Mean zone of inhibition, mm															
	Concentration of S1 (mg/ml)				Concentration of S2 (mg/ml)				Concentration of S3 (mg/ml)				Concentration of S4 (mg/ml)			
	100	50	25	12.5	100	50	25	12.5	100	50	25	12.5	100	50	25	12.5
<i>S. aureus</i>	17	13	10	9	13	10	8	R	16	14	12	10	18	15	13	11
<i>B. subtilis</i>	14	12	10	R	11	9	R	R	13	11	9	R	16	14	12	10
<i>E. coli</i>	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R

Test organism	Concentration of S5 (mg/ml)				Concentration of S6 (mg/ml)				Concentration of S7 (mg/ml)				Concentration of S8 (mg/ml)			
	100	50	25	12.5	100	50	25	12.5	100	50	25	12.5	100	50	25	12.5
	<i>S. aureus</i>	16	14	12	10	17	13	11	10	20	18	15	13	24	21	19
<i>B. subtilis</i>	14	12	10	R	15	3	11	9	18	15	12	10	22	19	17	16
<i>E. coli</i>	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R

S1 – S7, Neem – shea butter soap; S8, commercial antiseptic soap; R, resistance.

concentrations considered when used against *S. aureus* and *B. subtilis*. The microorganisms (*S. aureus* and *B. subtilis*) were similarly susceptible to all prepared neem/shear butter oil soaps (S1 to S7) but the zones of inhibition were generally lower than those for S8. It may also be observed that the zone of inhibition decreased with decreasing soap concentration. It may be inferred from Table 7 that the commercial antiseptic soap exhibited the greatest inhibitory effect on *S. aureus* and *B. subtilis* and that the extent of inhibition was for all soap solution and was concentration dependent. The table also shows that the inhibitory effect on *B. subtilis* improved with increasing shea butter concentration as S6 and S7 showed inhibition against the organism for all soap concentration considered which was not the case was for S1 to S5. For *E. coli*, the same effect of resistance for all soap samples was recorded.

Table 8 shows a general increase in the antibacterial properties of the soap solutions with increasing shea butter oil content. The soap sample prepared with neem/shear butter oil ratio of 0:100 (that is S7) exhibited the greatest antibacterial effect (against *S. aureus* and *B.*

subtilis) and also recorded similar MIC with the commercial antibacterial soap (S8). The table also shows that all soap solutions were unable to inhibit *E. coli*.

Table 9 shows that the soap prepared from neem/shear butter oil ratios of 0:100 (S7) recorded the best antibacterial property (amongst the prepared soap) recording the same MBC as the commercial antiseptic soap (S8). S1 to S6 recorded lower and similar MBC values. This and the results of Tables 7 and 8 indicate that the antibacterial properties of soap prepared from neem/shear butter oil blends was lowered by increasing neem oil concentration. Table 9 also shows that the growth of *E. coli* was not inhibited by any of the soap solutions within the concentration range considered.

Conclusions

Soap was produced using various blends of neem oil and shea butter oil. All soap samples prepared exhibited antibacterial properties of varying degrees. The soap prepared using shea butter oil alone exhibited the highest antibacterial property against *S. aureus* and *B. subtilis*.

Table 8. Minimum inhibitory concentrations (MIC) of the soaps against the test organisms.

Test organism	Observation of turbidity															
	Concentration of S1 (mg/ml)				Concentration of S2 (mg/ml)				Concentration of S3 (mg/ml)				Concentration of S4 (mg/ml)			
	25	12.5	6.25	3.125	25	12.5	6.25	3.125	25	12.5	6.25	3.125	25	12.5	6.25	3.125
<i>S. aureus</i>	-	-	MIC	+	-	MIC	+	+	-	-	MIC	+	-	-	MIC	+
<i>B. subtilis</i>	-	MIC	+	+	-	-	MIC	+	-	MIC	+	+	-	-	MIC	+
<i>E. coli</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Test organism	Observation of turbidity															
	Concentration of S5 (mg/ml)				Concentration of S6 (mg/ml)				Concentration of S7 (mg/ml)				Concentration of S8 (mg/ml)			
	25	12.5	6.25	3.125	25	12.5	6.25	3.125	25	12.5	6.25	3.125	25	12.5	6.25	3.125
<i>S. aureus</i>	-	-	MIC	+	-	-	MIC	+	-	-	-	MIC	-	-	-	MIC
<i>B. subtilis</i>	-	MIC	+	+	-	MIC	+	+	-	-	-	MIC	-	-	-	MIC
<i>E. coli</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

S1 – S7, Neem – shea butter soap; S8, commercial antiseptic soap; R, resistance.

Table 9. Minimum bactericidal concentrations (MBC) of the soaps against the test organisms.

Test organism	Observation of growth															
	Concentration of S1 (mg/ml)				Concentration of S2 (mg/ml)				Concentration of S3 (mg/ml)				Concentration of S4 (mg/ml)			
	25	12.5	6.25	3.125	25	12.5	6.25	3.125	25	12.5	6.25	3.125	25	12.5	6.25	3.125
<i>S. aureus</i>	-	MBC	+	+	MBC	+	+	+	-	MBC	+	+	-	MBC	+	+
<i>B. subtilis</i>	MBC	+	+	+	MBC	+	+	+	MBC	+	+	+	-	MBC	+	+
<i>E. coli</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Test organism	Observation of growth															
	Concentration of S5 (mg/ml)				Concentration of S6 (mg/ml)				Concentration of S7 (mg/ml)				Concentration of S8 (mg/ml)			
	25	12.5	6.25	3.125	25	12.5	6.25	3.125	25	12.5	6.25	3.125	25	12.5	6.25	3.125
<i>S. aureus</i>	-	MBC	+	+	-	MBC	-	+	-	-	MBC	+	-	-	MBC	+
<i>B. subtilis</i>	MBC	+	+	+	MBC	+	+	+	-	-	MBC	+	-	-	MBC	+
<i>E. coli</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

S1 – S7, Neem – shea butter soap; S8, commercial antiseptic soap; +, growth; -, no growth; MBC, minimum bactericidal concentrations.

The antibacterial properties of the neem/shear butter oil blends compared favorably well to a commercial antiseptic soap containing triclorocarbanilide.

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