

Antimicrobial Performance of Cold-Process Soap Formulated from Neem Seed Oil and Palm Kernel Oil Blends

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Abstract : This study evaluated the antimicrobial performance of bar soaps produced by cold-process saponification of neem seed oil (*Azadirachta indica*) and palm kernel oil. Five formulations were prepared: 100% neem oil (S1), 100% palm kernel oil (S2), 50:50 neem/palm kernel oil (S3), 70:30 neem/palm kernel oil (S4), and 30:70 neem/palm kernel oil (S5), with a commercial Tura soap as control. Antibacterial activity was assessed by agar-well diffusion against *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa* at 100, 50, 25 and 12.5 mg/mL. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were evaluated by broth dilution and subculture. The study showed formulation- and organism-dependent activity. S2 produced the largest experimental inhibition zone against *S. aureus* at 100 mg/mL (22 mm), whereas S5 produced the largest zones against *B. subtilis* (26 mm) and *P. aeruginosa* (17 mm). The commercial control remained more active against *S. aureus* and generally had lower MIC/MBC values, although S2 and S5 compared favorably with it against *B. subtilis*. The findings support further development of locally sourced vegetable-oil soaps, but they also show that an in-vitro antimicrobial effect should not automatically be equated with regulated antiseptic performance or clinical efficacy.

Keywords: Neem seed oil; palm kernel oil; antimicrobial soap; saponification; *Staphylococcus aureus*; *Bacillus subtilis*; *Pseudomonas aeruginosa*; agar-well diffusion; minimum inhibitory concentration; minimum bactericidal concentration.

Introduction

Soap is produced when triglyceride-containing fats or oils react with an alkali to form fatty-acid salts and glycerol. The amphiphilic structure of soap enables oily soils to associate with hydrophobic portions of the molecule while the ionic portion interacts with water, supporting removal during washing. Vegetable oils remain important feedstocks because their fatty-acid composition influences hardness, lathering, cleansing behavior and the properties of the final bar.

Recent studies continue to investigate vegetable-oil soaps as locally adaptable cleansing materials and to optimize their physical and biological properties. (4,7)

Neem seed oil is of particular interest because neem contains limonoids and other secondary metabolites with reported biological activity. Contemporary studies continue to report antibacterial effects of neem-derived materials, including activity against *S. aureus* and *P. aeruginosa*. Palm kernel oil is also widely used in cleansing products and provides a distinct fatty-acid profile that can influence soap structure and performance. These characteristics make blends of neem and palm kernel oils attractive for formulation studies. (8,9).

This study investigated five oil compositions and compared them with a commercial Tura soap. The research focuses specifically on the antimicrobial results and interprets them conservatively.

The objective was to determine how oil composition affected inhibition of three bacterial isolates and to compare the experimental formulations with the commercial control using inhibition zones, MIC and MBC observations.

2. Materials and Methods

2.1 Formulation and production

Neem seed oil and palm kernel oil were obtained from sources in Kaduna State, Nigeria. Sodium hydroxide, distilled water and lemon essential oil were used in production. The five formulations are presented in table 2.1.

Table 2.1: Formulations for the oils used in the production.

Code	Neem oil	Palm kernel oil
Sample 1 (S1)	100%	0%
Sample 2 (S2)	0%	100%
Sample 3 (S3)	50%	50%
Sample 4 (S4)	70%	30%
Sample 5 (S5)	30%	70%
Control Sample (C)	Commercial	Commercial

Duplicate bars were reportedly prepared. (Etta 2025; Jacob *et al.*, 2024)

2.2 Cold-process saponification

For the representative S1 preparation, 120 mL neem oil was heated to 70 °C and cooled to 60 °C. Sodium hydroxide (16.08 g) was dissolved in 56.28 mL distilled water and also cooled to approximately 60 °C. The alkali solution was combined with the oil while stirring until trace was reached; perfume was then added and the mixture moulded. The same approach was used for the remaining oil ratios. (6)

2.3 Antimicrobial testing

The test organisms were clinically isolated *S. aureus*, *B. subtilis* and *P. aeruginosa*. Mueller-Hinton agar, Mueller-Hinton broth and nutrient agar were used. Standardized cultures were spread on MHA, four 8-mm wells were made, and 0.2 mL of soap solutions at 100, 50, 25 and 12.5 mg/mL was added. After approximately one hour for diffusion, plates were incubated at 37 °C for 24 h and inhibition zones were measured in millimetres. (1).

MIC was determined by serial dilution in Mueller-Hinton broth followed by incubation at 37 °C for 24 h. The lowest concentration without visible turbidity was taken as MIC. For MBC, material from non-turbid tubes was streaked onto nutrient agar and incubated; absence of growth was interpreted as bactericidal activity. The procedures are conceptually consistent with modern broth-dilution frameworks.(2)

3. Results

The pictures of the produced soap are presented in figure 3.1 to 3.5 and that of the commercial as figure 3.6.



Figure 3.1: Sample 1
(100% neem seed oil)



Figure 3.2: Sample 2
(100% palm kernel oil)



Figure 3.3: Sample 3
(50% neem oil and 50% palm kernel oil)



Figure 3.4: Sample 4
(70% neem oil and 30% palm kernel oil)



Figure 3.5: sample 5
(30% neem oil and 70% palm kernel oil)



Figure 5.6: Control sample
(commercial Tura soap).

Furthermore, Table 3.1 presents the antimicrobial test findings from the clinically isolated organisms

Table 3.1 : Antimicrobial testing

Sample	Concentration (mg/mL)	<i>S. aureus</i> (mm)	<i>B. subtilis</i> (mm)	<i>P. aeruginosa</i> (mm)
S1	100	14	16	15
S1	50	12	14	13
S1	25	–	12	–
S1	12.5	–	–	–
S2	100	22	25	14
S2	50	20	23	12
S2	25	18	21	–
S2	12.5	16	19	–
S3	100	16	20	14
S3	50	14	18	12
S3	25	12	16	–
S3	12.5	10	14	–
S4	100	17	20	15

S4	50	14	16	12
S4	25	12	12	–
S4	12.5	10	–	–
S5	100	18	26	17
S5	50	15	23	13
S5	25	13	21	11
S5	12.5	11	19	–
C	100	34	20	16
C	50	32	18	14
C	25	30	16	12
C	12.5	28	14	–

The experimental formulations showed concentration-dependent inhibition in most cases. S2 produced the strongest experimental inhibition of *S. aureus* at 100 mg/mL (22 mm). S5 was strongest against *B. subtilis* (26 mm) and *P. aeruginosa* (17 mm) at 100 mg/mL. The control produced a 34-mm zone against *S. aureus* at 100 mg/mL.

3.1 MIC and MBC findings

The MIC and MBC findings for the formulated soap samples as well as the control samples are presented in table 3.2.

Table 3.2 MIC AND MBC findings for the clinically isolated organism.

Organism	Formulation	Reported MIC	Reported MBC
<i>S. aureus</i>	S1	50 mg/mL	Not achieved in tested range
<i>S. aureus</i>	S2	12.5 mg/mL	25 mg/mL
<i>S. aureus</i>	S3	25 mg/mL	50 mg/mL
<i>S. aureus</i>	S4	25 mg/mL	50 mg/mL
<i>S. aureus</i>	S5	25 mg/mL	50 mg/mL
<i>S. aureus</i>	Control	6.25 mg/mL	12.5 mg/mL
<i>B. subtilis</i>	S1	25 mg/mL	50 mg/mL
<i>B. subtilis</i>	S2	6.25 mg/mL	12.5 mg/mL
<i>B. subtilis</i>	S3	12.5 mg/mL	25 mg/mL
<i>B. subtilis</i>	S4	12.5 mg/mL	25 mg/mL
<i>B. subtilis</i>	S5	6.25 mg/mL	12.5 mg/mL
<i>B. subtilis</i>	Control	6.25 mg/mL	12.5 mg/mL
<i>P. aeruginosa</i>	S1	25 mg/mL	50 mg/mL

P. aeruginosa	S2	25 mg/mL	50 mg/mL
P. aeruginosa	S3	50 mg/mL	Not achieved
P. aeruginosa	S4	50 mg/mL	Not achieved
P. aeruginosa	S5	50 mg/mL	Not achieved
P. aeruginosa	Control	12.5 mg/mL	25 mg/mL

4. Discussion

The antimicrobial response varied with both oil composition and organism. S2, which contained no neem oil, produced the largest inhibition zone against *S. aureus* among the experimental soaps.

Conversely, S5, which contained 70% palm kernel oil and 30% neem oil, produced the strongest experimental responses against *B. subtilis* and *P. aeruginosa*. This shows that the biological behavior of a finished soap cannot be predicted simply from the presence or absence of neem oil. The findings are compatible with current literature showing that neem-derived preparations can exhibit antibacterial activity.

Recent work has characterized neem seed oil and reported activity against *S. aureus*, MRSA, *E. coli*, *Salmonella* and *B. subtilis*, while other studies have demonstrated antimicrobial effects of neem oil against clinically relevant organisms. Such evidence supports the inclusion of neem in natural-product research, but the activity of the original oil may change during alkaline saponification. (5,8,9) The strong performance of palm-kernel-rich formulations may also reflect the chemical nature of palm kernel oil. Its fatty-acid profile is suitable for cleansing bars, and recent Nigerian work confirms the feasibility of cold-process soap production from palm kernel oil.

The soap matrix itself may contribute to microbial inhibition through alkalinity, surfactant action and disruption/removal of microbial cells, in addition to any bioactive constituents retained from the oils. (6,7). *P. aeruginosa* was comparatively difficult to inhibit.

The study recorded resistance at several lower concentrations and limited bactericidal activity for the blended formulations. This organism's Gram-negative envelope and other physiological features can reduce susceptibility to some antimicrobial agents. The result emphasizes why antimicrobial claims should be based on multiple organisms and standardized testing rather than a single inhibition-zone measurement. (1,2,3)

The commercial control was generally more potent against *S. aureus* and had lower reported MIC/MBC values. This is not surprising because the control was a formulated commercial product rather than a simple plant-oil soap. More importantly, the comparison demonstrates that the natural formulations require optimization if they are intended to compete directly with commercial antimicrobial products.

Current CLSI standards provide updated approaches for susceptibility testing, MIC determination and quality control and should guide future experiments. (1,2,3). The agar-well method also has limitations for soap formulations.

Diffusion through agar depends on viscosity, molecular size, concentration and interactions with the medium. Therefore, a larger zone does not necessarily mean proportionally greater antimicrobial potency. Future studies should include independent batches, standardized inocula, positive and negative controls, and appropriate quality-control strains. (1,2).

Conclusion

The five neem/palm-kernel-oil soaps exhibited measurable in-vitro antibacterial activity against *S. aureus*, *B. subtilis* and *P. aeruginosa*, with substantial differences between formulations and organisms. S2 showed the strongest experimental inhibition of *S. aureus*, while S5 performed best against *B. subtilis* and *P. aeruginosa* at 100 mg/mL. The commercial control was stronger against *S. aureus* and generally showed lower MIC/MBC values. The study therefore supports continued development of locally sourced plant-oil soaps, but the products should be described as experimentally antimicrobial rather than automatically classified as antiseptic or clinically effective.

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All consulted literatures has been duly acknowledged.

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