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DEVELOPMENT AND CHARACTERIZATION OF NANO-ENHANCED SOAP WITH ENHANCED PHYSICOCHEMICAL AND ANTIMICROBIAL PROPERTIES

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ABSTRACT

The development of nano-enhanced soap offers a groundbreaking approach to enhancing the physicochemical and antimicrobial attributes of conventional soaps. This study compared the prepared soap with two commercially available soaps, examining parameters such as physicochemical properties, antimicrobial efficacy, and minimum inhibitory and bactericidal concentrations. The physicochemical analysis indicated that the prepared soap met regulatory standards for pH, solubility, hardness, and total fatty matter (TFM), confirming its suitability for human use. Specifically, the produced soap exhibited the following properties: pH = 6.30±0.02, Hardness = 1.3±0.02 cm, Foamability = 6.9±0.02 cm, Solubility = 80±0.02 s, and Total Fatty Matter (TFM) = 55±2%. Despite slightly reduced foamability due to the absence of foam enhancers, the prepared soap retained effective cleaning capabilities. Antimicrobial testing using the disc diffusion method demonstrated the soap's broad-spectrum activity against Gram-positive and Gram-negative bacteria, as well as fungal species, with efficacy increasing in a concentration-dependent manner. Unlike the two commercially available soaps used for comparison, which each exhibited high activity against specific pathogens, the prepared soap consistently inhibited all tested pathogens. This broad-spectrum activity highlights the superior antimicrobial properties of the prepared soap. It exhibited a minimum inhibitory concentration (MIC) of 50 mg/L and a minimum bactericidal concentration (MBC) of 100 mg/L for all pathogens, outperforming the commercially available soaps. Overall, the prepared soap demonstrated enhanced physicochemical characteristics and balanced antimicrobial efficacy, positioning it as a versatile hygiene product capable of addressing a wide range of microbial infections. Its wide-spectrum activity against all pathogens and superior physicochemical properties underscore its potential applications in both personal care and therapeutic settings.

Keywords: Total fatty matter, Minimum inhibitory concentration, Minimum bactericidal concentration, Soap, Antimicrobial

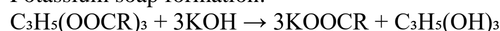
INTRODUCTION

The history of soap dates back thousands of years to ancient Babylonia, where the first soap was a crude mixture of animal fat and lye (an alkali derived from wood ashes) with a greasy texture and unpleasant odor (Jones & Miller, 2017). Over time, soap evolved significantly, incorporating a variety of ingredients to enhance its cosmetic appeal. Modern skin cleansers are far more complex than traditional soaps, containing not only surfactants but also skin-conditioning agents for added benefits (Brown & Lee, 2019). The practice of skin cleansing has been integral to personal hygiene, religious rituals, and therapeutic uses for millennia. The earliest documented bathing rituals are found in ancient Indian texts such as the "Grihya Sutras" (Taylor, 2018). Early cleansing methods included scraping impurities with bones or stones and washing with soapwort plant ash. Ancient Romans, renowned for their aqueduct systems and public baths, did not initially use soap for cleaning; instead, Cleopatra is said to have used essential oils and fine sand as bath abrasives (Smith et al., 2020). The first records of soap-making appear on Sumerian clay tablets circa 2000 B.C., describing a mixture of animal fat and wood ash used for cleaning wool (Johnson et al., 2022). Ancient Egyptians, as recorded in the Ebers Papyrus (1550 B.C.), combined animal oils and ash for bathing. By 600 B.C., the Phoenicians were producing soap from tree ash and animal fat (Jones & Miller, 2017). According to Roman legend, Mount Sapo, where animal sacrifices occurred, lent its name to soap, as rainwater washed fat and ashes down the slopes, creating a cleaning substance discovered by Roman women (Smith et al., 2020). The Roman scholar Pliny the Elder documented the use of soap made from tallow and ashes in his *Naturalis Historia* (77 A.D.), noting its application for hair dyeing by the Gauls (Taylor, 2018). By the 7th century, soap-making had become an established craft in Spain, Italy, and France, with olive oil serving as a key ingredient (Davis & White, 2021). Over time, fragrances and specialized soaps for bathing, shampooing, and laundry were developed. In Britain, the introduction of soap in the 13th century led to deforestation due to the high demand for wood ash, and soap became a luxury item heavily taxed until the tax was abolished in 1853 (Davis & White, 2021). The industrial era brought significant advancements in soap production. In 1791, French chemist Nicolas Leblanc developed a method to produce soda ash from sodium chloride, revolutionizing commercial soap-making (Parker & Green, 2023). During World War II, material shortages spurred the invention of synthetic detergents, which formed the basis for modern skin cleansers (Johnson et al., 2022). In India, soap manufacturing began in 1897, with the establishment of the North-West Soap Company in Meerut. The Tata Group later entered the market, introducing coconut oil-based soaps in the 1930s (Davis & White, 2021). Modern cleansers incorporate surfactants, which reduce surface tension and facilitate cleaning. These surfactants, with hydrophobic and hydrophilic ends, can cause skin irritation by disrupting the stratum corneum, leading to dryness and flaking (Morris et al., 2017). Advances in technology have led to milder formulations with moisturizing ingredients, such as glycerin and oils, to minimize irritation.

Today's cleansers range from Syndet bars, which use synthetic surfactants like sodium cocoyl isethionate for reduced irritation, to liquid body washes known for their convenience and superior emollient deposition (Brown & Lee, 2019). Specialized cleansers address specific skin

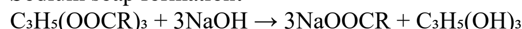
concerns, such as acne, atopic dermatitis, and sensitive skin, while facial cleansers are formulated with mild surfactants to avoid heavy residues. Cosmetic additives like fragrances, colorants, and lather boosters further enhance consumer appeal. This progression reflects the continuous innovation in soap and cleanser development, meeting evolving consumer expectations for health, hygiene, and cosmetic benefits. The production of soap involves a chemical reaction between oils or fats and an alkali, resulting in the formation of soap molecules and glycerol (glycerin) (Singh & Kaur, 2020). Chemically, soap is defined as a water-soluble salt of fatty acids containing eight or more carbon atoms (Davis & White, 2021). Soaps are produced for various purposes, including cleaning, bathing, and medicinal applications. The cleansing action of soap is attributed to the hydrocarbon chain of the fatty acid, which is attracted to oil and grease, and the carboxylic group, which is water-attractive, enabling soap to function effectively with water as a cleaning agent (Johnson et al., 2022). Beyond basic raw materials, additional components are incorporated to enhance soap's properties and applications. Medicated soaps, for instance, include therapeutic ingredients. While potassium and sodium salts are common in soap-making, other metals like calcium, magnesium, and chromium are used to create metallic soaps, which are insoluble in water and serve non-cleaning purposes (Singh & Kaur, 2020). The hardness of soap varies with the metallic element in its salt composition; sodium-based soaps are firmer than potassium-based ones, while soaps made with divalent metals such as magnesium or calcium are water-insoluble (Davis & White, 2021). Soap is traditionally produced through the saponification of triglycerides, which are fats or oils. In this process, triglycerides react with a strong alkali, such as sodium hydroxide or potassium hydroxide, to yield glycerol and fatty acid salts. These reactions are represented as follows:

Potassium soap formation:



$C_3H_5(OOCR)_3 + 3KOH \rightarrow 3KOOCR + C_3H_5(OH)_3$

Sodium soap formation**:**



$C_3H_5(OOCR)_3 + 3NaOH \rightarrow 3NaOOCR + C_3H_5(OH)_3$

Here, R represents the hydrocarbon chain or alkyl group of fatty acids, which are straight-chain monocarboxylic acids typically ranging from C_{10} to C_{20}. Common fatty acids in soap production include saturated types such as palmitic acid (CH_3-$(CH_2)_{14}$-COOH) and unsaturated types like oleic acid ($C_{17}H_{33}COOH$). Oils and fats suitable for soap-making are primarily combinations of fatty acids and alkali, whereas hydrocarbon oils like paraffin are chemically unsuitable for saponification. The soap-making process also yields glycerin as a by-product, and the resulting soap's physicochemical properties such as hardness, lathering ability, pH, and antimicrobial activity depend on the raw materials and production methods (Singh & Kaur, 2020).

Neem, baobab, and black seed oils have long been valued for their unique chemical compositions and potential applications in skincare products. Neem oil is rich in fatty acids, such as oleic acid and linoleic acid, and contains azadirachtin, a compound known for its antimicrobial and anti-inflammatory properties (Biswas et al., 2002). Baobab oil is a source of essential fatty acids, including omega-3, omega-6, and omega-9, and is lauded for its moisturizing and skin-repairing abilities (Nkafamiya et al., 2007). Black seed oil, on the other hand, is abundant in thymoquinone, a bioactive compound with potent antioxidant, antibacterial, and antifungal activities (Ahmad et al., 2013). These oils also possess natural saponifiable properties, making them suitable candidates for soap formulation.

Despite their promising properties, limited studies have explored the integration of these oils into soap formulations, particularly in combination with metal nanoparticles such as silver (AgNPs), copper (CuNPs), and zinc (ZnNPs). While individual studies have demonstrated the antimicrobial, anti-inflammatory, and skin-penetration-enhancing effects of metal nanoparticles (Kim et al., 2012), their synergistic interactions with neem, baobab, and black seed oils remain underexplored. This study seeks to fill this gap by investigating the potential of these oils, combined with nanoparticles, to enhance the antimicrobial activity and physicochemical properties of soap. By doing so, it aims to provide new insights into the development of multifunctional soap formulations for both cosmetic and therapeutic applications.

MATERIALS AND METHODS

Materials

All the solvents and reagents used were of Analytical grade which includes ethanol, sodium silicate, essential oil, distilled water, Hydrochloric acid, filter paper, hand sewing needle, measuring cylinder, Baobab seed oil, Neem seed oil, Black seed oil were also used for the saponification, pH meter, rotary evaporator, water bath were all used, also all glassware were washed, cleaned and dried in an oven at 105°C, Biosynthesized Ag, Cu and Zn nano particles were also used.

Methods

Soap Preparation

The soap-making procedure described by Kumral et al. (2019) was followed with slight modifications to optimize the formulation. The production steps were as follows:

Preparation of Lye Solution

About 100 g of sodium hydroxide (NaOH) was dissolved in 300 ml of distilled water, yielding a lye solution with a concentration of 0.33 g/ml.

Oil and Nanoparticle Blending

About 20 ml of baobab seed oil, 30 ml of neem seed oil, and 30 ml of black seed oil were measured and combined in a mixing container. Metal nanoparticles, including 0.6 mg of silver nanoparticles (AgNPs), 0.6 mg of copper nanoparticles (CuNPs), and 0.4 mg of zinc nanoparticles (ZnNPs), were added to the oil blend. The mixture was stirred thoroughly to ensure uniform dispersion of the nanoparticles.

Mixing of Oils and Lye Solution

About 40 ml of the prepared lye solution (0.33 g/ml NaOH) was gently added to the oil blend with continuous stirring to form a homogenous mixture.

Addition of Additives

About 5 ml of sodium silicate and 5 ml of essential oil were incorporated into the mixture. Stirring continued until the mixture reached the "trace" stage, where it thickens and leaves visible traces on the surface when stirred.

Molding and Initial Curing

The soap mixture was poured into molds, covered to retain heat, and left undisturbed for 24 hours.

Demolding and Final Curing

After 24 hours, the soap was removed from the molds and set aside to cure for a period of 7 days, allowing it to harden and mature.

Characterization
Once the curing process was complete, the soap was subjected to physicochemical and antimicrobial characterization to assess its quality and effectiveness. This method leverages the unique properties of baobab, neem, and black seed oils along with the antimicrobial and physicochemical benefits of metal nanoparticles, resulting in a soap formulation with enhanced properties.

Soap Characterisation
Physicochemical Analysis
The prepared soap was characterised by its pH, foaming ability, solubility and hardness whilst comparing its values with commercial soap samples using standard procedure (Ameh et al., 2013). Commercial bathing soaps A and B were used as standards for comparison.
(a) pH Test: For the pH test, the soap solution was first prepared by weighing about 1g of the soap and dissolving it in 10 mL of distilled water. The solution was then diluted to a total volume of 100 mL to prepare a 1% (w/v) homogeneous soap solution. The electrode of the pH meter was immersed into the prepared soap solution, and the pH value was recorded. The process was repeated for each soap sample.
(b) Hardness Test: The hardness of the soap was determined using a needle penetration method, a hand-sewing needle (4.2 cm in length and 0.5 mm in diameter) was inserted into the soap, the needle was loaded at the top with a 400 g weight on a lever system. The lever was lifted, allowing the weighted needle to penetrate the soap slowly over 30 seconds. The depth of penetration was measured and the test was repeated three times, and the average penetration depth was recorded.
(c) Foamability Test: The foaming ability of the soap was assessed as follows, about 1 g of the soap sample was added to a 100 mL measuring cylinder containing 10 mL of distilled water. The cylinder was shaken vigorously for 2 minutes to generate foam, the cylinder was allowed to stand undisturbed for 10 minutes and the height of the foam was measured and recorded.
(d) Solubility Test: The solubility of the soap was evaluated based on the time required for dissolution, about 1g of soap was placed into a 100 mL measuring cylinder containing 10 mL of distilled water, the cylinder was shaken continuously until the soap completely dissolved. The time taken for complete dissolution was recorded for each soap sample.
(e) Total Fatty Matter (TFM) Test: The total fatty matter content of the soap was determined using the Gerber method, about 2g of soap was dissolved in 50 mL of ethanol then 25 mL of hydrochloric acid was added, and the mixture was heated to near-boiling temperature (~70°C) until the soap dissolved completely. The solution was cooled and filtered to remove impurities. The fatty acids were extracted using ether, which dissolved the fatty matter and the residue of the fatty acids obtained was weighed, the total fatty matter (TFM) was calculated based on the weight of the fatty acids.

Antimicrobial Susceptibility Testing (Well Diffusion Method)
The antimicrobial properties of the soap samples were assessed using the well diffusion method, following the standards outlined by the National Committee for Clinical Laboratory Standards (NCCLS). Soap stock solutions were prepared at concentrations of 500 mg/mL, 250 mg/mL, 125 mg/mL, and 62.500 mg/mL using the serial dilution technique. An additional dilution to 31.25 mg/mL was prepared for extended testing, Mueller-Hinton Agar (MHA) plates were used as the testing medium and Wells of 6 mm diameter were punched into the agar using a sterile borer.Test bacterial cultures were adjusted to a turbidity matching the 0.5 McFarland standard to ensure consistent bacterial density and the agar surface was streaked uniformly with the bacterial suspension using a sterile cotton swab. Then the plates were allowed to air dry for 3–5 minutes to remove excess moisture, each well was carefully filled with 50 µL of the prepared soap solutions (500 mg/mL, 250 mg/mL, 125 mg/mL, 62.50 mg/mL, and 31.25 mg/mL) and the inoculated plates with soap solutions were incubated at 37°C for 24 hours. After incubation, the plates were examined for zones of inhibition around the wells, indicating antimicrobial activity and the diameter of each inhibition zone was measured in millimeters using a metric ruler. The results, measured as inhibition zones, indicate the susceptibility of the microorganisms to the soap formulations (Chatterjee et al., 2014).

MIC Test
The minimum inhibitory concentration (MIC) was determined using broth dilution method (Fadaei et al., 2021). The lowest concentrations of the fractions showing inhibition for each organism were serially diluted in the test tube containing Mueller Hinton Broth and the bacterial strains and fungi strain were inoculated in tubes with equal volume of nutrient broth and fractions, the tubes were incubated at 37 degrees for 24hoours for the bacteria while 48hours for the fungi. Three control tubes were maintained for each strain (media control, organism control, and soap control). The lowest concentration (highest dilution) of the fractions that produced no visible growth (no turbidity) when compared with the control tubes were considered as the MIC.

Determination of MBC and MFC Values
From the antimicrobial susceptibility testing, the test dilution showing no visible turbidity (indication of bacterial growth inhibition) was selected, then an aliquot from the selected dilution was streaked onto freshly prepared nutrient agar plates using a sterile inoculating loop, the plates were incubated at 37°C for 24 hours**.** After incubation, the plates were examined for the presence or absence of bacterial colonies and the highest dilution (lowest concentration) at which no bacterial colony was observed was recorded as the MBC value.
Similar to bacterial testing, the dilution with no visible turbidity from antifungal susceptibility testing was selected and an aliquot from the selected dilution was streaked onto freshly prepared nutrient agar plates, the plates were incubated at 25°C for 48 hours to allow fungal growth. After incubation, the plates were examined for fungal colony growth, the highest dilution (lowest concentration) at which no fungal colony was observed was recorded as the MFC value (Bauer et al., 2019).

RESULTS AND DISCUSSION

Result

Table 1: Physicochemical Analysis of the Produced Soap and Market Soaps in Comparison

Soap Samples	pH	Hardness (cm)	Foamability cm)	Solubility (s)	Total Fatty Matter (%)
Soap P	6.30+0.02	1.30+0.02	6.90+0.02	80+0.02	55±2
Soap A	6.30+0.02	1.20+0.02	9.20+0.02	72+0.02	49±2
Soap B	6.10+0.01	1.30+0.02	8.30+0.02	90+0.02	54±2

Key; Soap P= Prepared soap, Soap A= Market soap, Soap B= market soap.

Table 2: Antimicrobial Activity of Produced Soap (Gram Negative and Gram Positive Bacteria species)

S/N	Concentration (mg/L)	A.baumannii (mm)	E.coli (mm)	K.Pneumoniae (mm)	S.mutans (mm)	B.Subtilis (mm)	S.Lentus (mm)
1	500	20	20	20	19	18	18
2	250	18	18	18	17	16	16
3	125	14	15	16	14	14	14
4	62.50	12	12	14	12	12	12
5	31.25	10	10	12	10	10	10

KEY; NZ = No Zones, E.coli= Escherichia Coli, K.Pneumoniae = Klebsiella Pneumoniae, A.baumannii = Acinetobacter baumannii, S.mutants= Streptococcus mutans, B.subtilis = Bacillus subtilis, S.Lentus= Staphylococcus Lentus.

Table 3: Antimicrobial Activity of Produced Soap (Fungi species)

S/N	Concentration(mg/L)	C.albicans (mm)	A.niga (mm)	F.Oxyporum (mm)
1	500	18	20	20
2	250	16	18	19
3	125	14	16	17
4	62.50	12	14	15
5	31.25	10	12	12

KEY; NZ = No Zones, C.albicans= Candida albicans, A.niga= Klebsiella Pneumoniae, F.Oxyporum = Fusarium oxyporum

Table 4: MIC of the Produced Soap (Gram Negative and Positive Bacteria species)

S/N	Concentration (mg/L)	A.baumannii (mm)	E.coli (mm)	K.Pneumoniae (mm)	S.mutans (mm)	B.Subtilis (mm)	S.Lentus (mm)	S.mutans (mm)
1	400	+	+	+	+	+	+	+
2	200	+	+	+	+	+	+	+
3	100	+	+	+	+	+	+	+
4	50	+	+	+	+	+	+	+
5	25	-	-	-	-	-	-	-

KEY; NZ = No Zones, E.coli= Escherichia Coli, K.Pneumoniae = Klebsiella Pneumoniae, A.baumannii = Acinetobacter baumannii, S.mutants= Streptococcus mutans, B.subtilis = Bacillus subtilis, S.Lentus= Staphylococcus Lentus.

Table 5: MIC of the Produced Soap (Fungi species)

S/N	Concentration(mg/L)	C.albicans (mm)	A.niga (mm)	F.Oxyporum (mm)
1	400	+	+	+
2	200	+	+	+
3	100	+	+	+
4	50	+	+	+
5	25	-	-	-

KEY; NZ = No Zones, C.albicans= Candida albicans, A.niga= Klebsiella Pneumoniae, F.Oxyporum = Fusarium oxyporum

Table 6: MBC of the Produced Soap (Gram Negative and Gram Positive Bacteria species)

S/N	Concentration (mg/L)	A.baumannii (mm)	E.coli (mm)	K.Pneumoniae (mm)	S.mutans (mm)	B.Subtilis (mm)	S.Lentus (mm)	S.mutans (mm)
1	400	+	+	+	+	+	+	+
2	200	+	+	+	+	+	+	+
3	100	+	+	+	+	+	+	+
4	50	-	-	-	-	-	-	-
5	25	-	-	-	-	-	-	-

KEY; NZ = No Zones, E.coli= Escherichia Coli, K.Pneumoniae = Klebsiella Pneumoniae, A.baumannii = Acinetobacter baumannii, S.mutants= Streptococcus mutans, B.subtilis = Bacillus subtilis, S.Lentus= Staphylococcus Lentus.

Table 7: MBC of the Produced Soap (Fungi species)

S/N	Concentration(mg/L)	C.albicans (mm)	A.niga (mm)	F.Oxyporum (mm)
1	400	+	+	+
2	200	+	+	+
3	100	+	+	+
4	50	-	-	-
5	25	-	-	-

KEY; NZ = No Zones, C.albicans= Candida albicans, A.niga= Klebsiella Pneumoniae, F.Oxyporum = Fusarium oxyporum

Table 8: Antimicrobial Activity of Market soap A (Gram Negative and Gram Positive Bacteria species)

S/N	Concentration (mg/ml)	A.baumannii (mm)	E.coli (mm)	K.Pneumoniae (mm)	S.mutans (mm)	B.Subtilis (mm)	S.Lentus (mm)
1	500	16	16	16	12	12	12
2	250	12	14	12	08	08	08
3	125	08	11	08	NZ	NZ	NZ
4	62.50	04	08	04	NZ	NZ	NZ
5	31.25	NZ	04	NZ	NZ	NZ	NZ

KEY; NZ = No Zones, E.coli= Escherichia Coli, K.Pneumoniae = Klebsiella Pneumoniae, A.baumannii = Acinetobacter baumannii, S.mutants= Streptococcus mutans, B.subtilis = Bacillus subtilis, S.Lentus= Staphylococcus Lentus.

Table 9: Antimicrobial Activity of Market Soap A (Fungi species)

S/N	Concentration (mg/L)	C.albicans (mm)	A.niga (mm)	F.Oxyporum (mm)
1	500	14	14	20
2	250	12	18	19
3	125	10	16	17
4	62.50	08	14	15
5	31.25	06	12	12

KEY; NZ = No Zones, C.albicans= Candida albicans, A.niga= Klebsiella Pneumoniae, F.Oxyporum = Fusarium oxyporum

Table 10: MIC of the Market Soap A (Gram Negative and Gram Positive Bacteria species)

S/N	Concentration(mg/L)	A.baumannii (mm)	E.coli (mm)	K.Pneumoniae (mm)	S.mutans (mm)	B.Subtilis (mm)	S.Lentus (mm)
1	400	+	+	+	+	+	+
2	200	+	+	+	+	+	+
3	100	+	+	+	-	-	-
4	50	-	-	-	-	-	-
5	25	-	-	-	-	-	-

KEY; NZ = No Zones, E.coli= Escherichia Coli, K.Pneumoniae = Klebsiella Pneumoniae, A.baumannii = Acinetobacter baumannii, S.mutants= Streptococcus mutans, B.subtilis = Bacillus subtilis, S.Lentus= Staphylococcus Lentus.

Table 11: MIC of the Market Soap A (Fungi species)

S/N	Concentration (mg/L)	C.albicans (mm)	A.niga (mm)	F.Oxyporum (mm)
1	400	+	+	+
2	200	+	+	+
3	100	+	+	+
4	50	-	-	-
5	25	-	-	-

KEY; NZ = No Zones, C.albicans= Candida albicans, A.niga= Klebsiella Pneumoniae, F.Oxyporum = Fusarium oxyporum

Table 12: MBC of the Market soap A (Gram Negative and Positive Bacteria species)

S/N	Concentration (mg/L)	A.baumannii (mm)	E.coli (mm)	K.Pneumoniae (mm)	S.mutans (mm)	B.Subtilis (mm)	S.Lentus (mm)
1	400	+	+	+	+	+	+
2	200	+	+	+	-	-	-
3	100	-	-	-	-	-	-
4	50	-	-	-	-	-	-
5	25	-	-	-	-	-	-

KEY; NZ = No Zones, E.coli= Escherichia Coli, K.Pneumoniae = Klebsiella Pneumoniae, A.baumannii = Acinetobacter baumannii, S.mutants= Streptococcus mutans, B.subtilis = Bacillus subtilis, S.Lentus= Staphylococcus Lentus.

Table 13: MBC of the Market soap A (Fungi species)

S/N	Concentration (mg/L)	C.albicans (mm)	A.niga (mm)	F.Oxyporum (mm)
1	400	+	+	+
2	200	+	+	+
3	100	-	-	-
4	50	-	-	-
5	25	-	-	-

KEY; NZ = No Zones, C.albicans= Candida albicans, A.niga= Klebsiella Pneumoniae, F.Oxyporum = Fusarium oxyporum

Table 14: Antimicrobial Activity of the Market soap B (Gram Negative and Gram Positive Bacteria species)

S/N	Concentration (mg/ml)	A.baumannii (mm)	E.coli (mm)	K.Pneumoniae (mm)	S.mutans (mm)	B.Subtilis (mm)	S.Lentus (mm)
1	500	12	13	12	14	14	13
2	250	08	08	08	12	12	12
3	125	NZ	NZ	NZ	08	08	08
4	62.50	NZ	NZ	NZ	NZ	NZ	NZ
5	31.25	NZ	NZ	NZ	NZ	NZ	NZ

KEY; NZ = No Zones, E.coli= Escherichia Coli, K.Pneumoniae = Klebsiella Pneumoniae, A.baumannii = Acinetobacter baumannii, S.mutants= Streptococcus mutans, B.subtilis = Bacillus subtilis, S.Lentus= Staphylococcus Lentus.

Table 15: Antimicrobial Activity of Market Soap B (Fungi species)

S/N	Concentration (mg/L)	C.albicans (mm)	A.niga (mm)	F.Oxyporum (mm)
1	500	14	14	20
2	250	12	18	19
3	125	10	16	17
4	62.50	08	14	15
5	31.25	06	12	12

KEY; NZ = No Zones, C.albicans= Candida albicans, A.niga= Klebsiella Pneumoniae, F.Oxyporum = Fusarium oxyporum

Table 16: MIC of the Market Soap B (Gram Negative and Positive Bacteria species)

S/N	Concentration(mg/L)	A.baumannii (mm)	E.coli (mm)	K.Pneumoniae (mm)	S.mutans (mm)	B.Subtilis (mm)	S.Lentus (mm)
1	400	+	+	+	+	+	+
2	200	+	+	+	+	+	+
3	100	-	-	-	-	-	-
4	50	-	-	-	-	-	-
5	25	-	-	-	-	-	-

KEY; NZ = No Zones, E.coli= Escherichia Coli, K.Pneumoniae = Klebsiella Pneumoniae, A.baumannii = Acinetobacter baumannii, S.mutants= Streptococcus mutans, B.subtilis = Bacillus subtilis, S.Lentus= Staphylococcus Lentus.

Table 17: MIC of the Market Soap B (Fungi species)

S/N	Concentration (mg/L)	C.albicans (mm)	A.niga (mm)	F.Oxyporum (mm)
1	400	+	+	+
2	200	+	+	+
3	100	+	+	+
4	50	-	-	-
5	25	-	-	-

KEY; NZ = No Zones, C.albicans= Candida albicans, A.niga= Klebsiella Pneumoniae, F.Oxyporum = Fusarium oxyporum

Table 18: MBC of the Market soap B (Gram Negative and Gram Positive Bacteria species)

S/N	Concentration (mg/L)	A.baumannii (mm)	E.coli (mm)	K.Pneumoniae (mm)	S.mutans (mm)	B.Subtilis (mm)	S.Lentus (mm)
1	400	+	+	+	+	+	+
2	200	-	-	-	-	-	-
3	100	-	-	-	-	-	-
4	50	-	-	-	-	-	-
5	25	-	-	-	-	-	-

KEY; NZ = No Zones, E.coli= Escherichia Coli, K.Pneumoniae = Klebsiella Pneumoniae, A.baumannii = Acinetobacter baumannii, S.mutants= Streptococcus mutans, B.subtilis = Bacillus subtilis, S.Lentus= Staphylococcus Lentus..

Table 19. MBC of the Market soap B (Fungi species)

S/N	Concentration (mg/L)	C.albicans (mm)	A.niga (mm)	F.Oxyporum (mm)
1	400	+	+	+
2	200	+	+	+
3	100	-	-	-
4	50	-	-	-
5	25	-	-	-

KEY; NZ = No Zones, C.albicans= Candida albicans, A.niga= Klebsiella Pneumoniae, F.Oxyporum = Fusarium oxyporum

Discussion

Table 1. shows the physicochemical analysis result of the prepared soap and the two market soaps in comparison, the pH results shows that the prepared soap have pH within same range with the two market soaps, and the values obtained are within the limit range of the pH of a bathing soap set by NAFDAC in Nigeria (5.0-6.5), similarly the solubility and hardness of the prepared soap is also within same range with the market soap which is also indicating that the prepared soap possesses good hardness and solubility as it can be compared to other commercial soaps. Moreover, the foamability of the market soap was found to be less than that of the two market soaps which mainly due to the foam enhancers that were used in the market soaps based on the description in their packages, but the prepared soap also shows good foamability. As reported in ISO 21138:2006, a good soap has total fatty matter within range of 40-60% (soap with very high TFM >70% can be too harsh while those with very low TFM <30% may not clean effectively), the prepared soap and the markets soaps in this study all have TFM within range of 40-60%, thus this indicates this prepared soap will clean human skin effectively.

The antimicrobial efficacy of the prepared soap sample was investigated against 3 Gram-negative, 3 Gram-positive bacterial pathogens and 3 fungal species by the disc diffusion method (Table 2 and 3). Different concentrations of the prepared soap was used viz.,500ml/L, 400ml/L, 300ml/L, 200ml/L and 100ml/L. The antibacterial action was found to be concentration dependent and varied for the different microbial strains. The inhibitory effect of the soap sample was found to be highly effective on both the gram-positive and the gram-negative bacterium, as well as on the fungus, the results shows inhibition zones from highest concentration of 500mg/L down to the lowest concentration of 32.25mg/L. These findings goes in hand with other literatures only that these results shows more activity as compared to other works because in others works its either the soap shows more activity on the gram positive or negative and less on fungi or vice versa, e.g Kumar et al.,2019 worked Antimicrobial activity of soap made from Azadirachta indica (neem) against Gram-positive, gram-negative bacteria and fungi and based on his findings the soap shows more activity on the gram positive bacteria and fungi than on the gram negative. Similarly, Singh et al., 2018 worked on the Antimicrobial soap from Aloe vera gel and based on his findings the soap shows more activity on the gram negative bacteria and fungi species than on the gram positive bacteria species. Silva et al., 2020 also labored on Antimicrobial activity of Eucalyptus oil soap against Gram-positive, Gram-negative bacteria and fungi and based on his findings the soap tends to have more activity on the fungi species than on the bacteria species. Similarly, Lee et al., 2020 worked on the Antimicrobial activity of soap containing Tea tree oil against Gram-positive, Gram-negative bacteria and fungi species and the result of his findings shows that the soap have more activity on the Gram-positive bacteria than on the gram negative and fungus. All these findings showing the prepared soap having more effect on either gram negative, gram positive bacteria or fungi are indicating that the soap samples might be antimicrobial but it will cure skin diseases based on the precursor of the disease, that is to say a soap that is more effective on the gram positive bacteria will cure more skin diseases that are caused by gram positive bacteria than those that are caused by gram negative or fungi, and vice versa. While based on the result of the prepared soap in this study its indicating that the soap possesses the ability to cure any diseases that is caused by either gram positive, gram negative or fungi species.

The broth dilution method was used to determine the lowest concentration of the prepared soap that could inhibit the growth of the pathogenic organisms. (Table 4 and 5) shows that the minimum concentration of the prepared soap required to inhibit the growth of the pathogens was 50ml/L for all the pathogens in question. The MBC of the prepared soap that killed 100% of the bacterial and fungal population, which did not exhibit any viable bacterial or fungal growth, was 100ml/L for all the pathogens in question (Table 6 and 7)

A market soap named soap A was used in comparison to the prepared soap and it shows more antimicrobial activity on the gram negative bacteria species than on the gram positive bacteria species and also shows considerable activity on the fungi species (Table 8 and 9). It's MIC that inhibits bacterial and fungal growth was found to be 100mg/L for the gram negative bacteria species and fungi species while it was found to be 200mg/L for the gram positive bacteria species (table 10 and 11). MBC results shows that the minimum concentration that kills the pathogens totally is 200ml/L for the gram negative bacteria species and fungi species while it was found to be 400mg/L for the gram positive bacteria species (table 12 and 13). These goes in hand with was previously reported in other findings like in the work of Singh et al.,2018 on the Antimicrobial soap from Aloe vera gel, based on his findings the soap shows more activity on the gram negative bacteria and fungi species than on the gram positive bacteria species. Thus, this market soap when used for treatment of skin infections it will cure more skin infections that are caused by either gram negative bacteria species or fungi more effectively than it will cure for those infections that are caused by gram positive bacteria species, while the results of the prepared soap in this study shows it possesses the ability to cure both, Hence the prepared soap is considered to be more antimicrobial than this market soap in comparison.

A market soap named soap B was also used in comparison to the prepared soap and it shows more antimicrobial activity on the fungi species than on the gram positive and negative bacteria species (Table 14 and 15). MIC that inhibits bacterial and fungal growth was found to be 200mg/L for the gram positive and negative bacteria species while it was found to be 100mg/L for the fungi species (table 16 and 17). MBC results shows that the minimum concentration that kills the pathogens totally is 400ml/L for the gram positive bacteria and gram negative bacteria species while it was found to be 200mg/L for the fungi species (table 18 and 19). These goes in hand with was previously reported in other findings like in the work of Silva et al., 2020 on Antimicrobial activity of Eucalyptus oil soap against Gram-positive, Gram-negative

bacteria and fungi and based on his findings the soap tends to have more activity on the fungi species than on the bacteria species, and Similarly the work of Kim et al.,2020 on Antimicrobial soap from ginger extract, based on his findings the soap tends to have more activity on the fungi species than on the bacteria species. Thus, this market soap when used for treatment of skin infections it will cure more skin infections that are caused by fungi species than ones being caused by either gram positive or gram negative bacteria species, while the results of the prepared soap in this study shows it possesses the ability to cure both, Hence the prepared soap is considered to be more antimicrobial than this market soaps in comparison.

CONCLUSION

This study explores the formulation and evaluation of an antimicrobial soap incorporating neem seed oil, baobab seed oil, black seed oil, and metal nanoparticles (AgNPs, CuNPs, and ZnNPs). The physicochemical parameters (pH, hardness, foamability, solubility and Total fatty matter) of the prepared soap all met regulatory standards (NAFDAC/SON) ensuring skin safety. The prepared soap being assessed against nine microbial strains, including both bacteria (Gram-positive and Gram-negative) and fungi demonstrated broad-spectrum antimicrobial efficacy, and this is attributed to the active compounds in the oils and the antimicrobial properties of the nanoparticles. The prepared soap is both skin-friendly and effective as an antimicrobial agent, and the inclusion of metal nanoparticles enhances its antimicrobial performance, making it a promising alternative to commercial antimicrobial soaps.

RECOMMENDATIONS

Economic Viability: Conduct a cost analysis to determine the competitiveness of the prepared soap compared to commercial antimicrobial soaps.
Broader Studies: Investigate other underutilized plant seed oils for potential inclusion in soap formulations and examine the synergistic effects of biosynthesized metal nanoparticles with different oils.
Further Evaluation: Extend research to more detailed MIC and MBC analyses for additional microbial strains and explore long-term stability, user acceptability, and shelf-life of the prepared soap.
Scalability: Test the scalability of the production process for potential commercialization.

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