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Production of Antimicrobial Soap from A Blend of Moringa Oleifera Oil and Castor Oil

Ejike D. Ugwuanyi¹, B. Mukhtar² & M.S. Aliyu³

¹Department of Chemical, Biochemical and Environmental Engineering, University of Maryland Baltimore County, Baltimore, Maryland, USA.

²Department of Chemical Engineering, Ahmadu Bello University Zaria.

³Department of Microbiology, Ahmadu Bello University Zaria

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ABSTRACT: Moringa oleifera oil and castor oil were both extracted from their seeds using the mechanical press method. The Moringa oleifera oil and castor oil were then blended in various proportions and used in preparing soaps which were subsequently characterized. The physical properties of the prepared soap including hardness and pH were analyzed. The antimicrobial activity of the soap produced was examined against some clinical isolates of pathogenic organisms (Staphylococcus aureus, Escherichia coli and Candida albicans). The soaps produced from all the blends of oils exhibited antimicrobial activity against E.coli. However, soap of 100 % of Moringa oleifera oil gave the highest zone of inhibition (19 mm) at a concentration of 400 mg/ml. Soap prepared using 100 % castor oil alone gave the highest zone of inhibition against S. aureus (28 mm) and C. albicans (25 mm). Therefore, the therapeutic efficacy of these oils cannot be overlooked hence exploring them becomes essential.

KEYWORDS: moringa oleifera, castor oil, soap, antimicrobial activity, pathogenic organisms

INTRODUCTION

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. Wide scale changes in the global climate system have driven the need for more sustainable means, especially in Africa which ranks as one of the most affected by climate change. The organic or bio soap process is eco-friendlier and prevents Carbon dioxide (CO2) emissions.

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An antimicrobial soap is designed to safely kill germs and cleanse the skin. Soaps with natural ingredients extract are increasing in demand because are considered safer for the skin (Putri, 2018). Antibacterial soaps stand out in their packaging and eliminate 99.9% of microorganisms or bacteria as noted by Santos-Junior et al. (2022). Nonetheless, Rama et al. (2011) observed that antibacterial soaps remove 65 to 85% of the population of microbes deposited on the surface of the skin from environmental sources which can cause infections.

Moringa has developed as a beauty and health supplement. Antioxidants are the main component of moringa, particularly the leaves, which have a high concentration of these compounds. It has antifungal and antibacterial properties in addition to antioxidants. Moringa oleifera oil is of excellent quality similar to olive oil, and is slow to become rancid. It has good antioxidant capacity with potential for industrial, nutritional and health applications (Ogbunugafor et al., 2011). The oil is rich in essential fatty acids, making it an ideal moisturizer and healing and soothing emollient for rough, dry skin and therapeutic massages. The fatty acids in Moringa oleifera oil are oleic acid (70 %), palmitic acid (7.8 %), behenic acid (6.2 %) and stearic acid (7.6 %) (Abdulkarim et al., 2005). Among the several fatty acids in Moringa oleifera oil, the most abundant of the unsaturated fatty acids is oleic acid which is recommended for use in pharmaceutical preparation preferably in skin treatment (Warra, 2011). Various extraction methods such as solvent extraction and supercritical CO2 are employed in obtaining oil from Moringa oleifera seeds. Moringa oleifera oil is non-drying with a pale-yellow consistency. It has various cosmetic values and is used in body and hair care as a moisturizer and skin conditioner. It is useful in removing dirt from the hair and is an efficient natural cleanser.

A good sign of quality for use in the soap and cosmetics industries is the presence of ricinoleic acid, oleic acid, palmitic acid, stearic acid, and dihydroxylstearic acid in castor seed oil. Because castor oil contains hydroxyl acid, it is more viscous, less soluble in hexane, and more soluble in ethanol than other popular vegetable oils (Abdulrasheed et al, 2015). Castor oil is a colourless to very pale yellow liquid with a distinct taste and odour once first ingested. The major component present in castor oil fatty acid chain is the ricinoleic acid which is approximately ninety percent. According to Mutlu and Meier (2010), the oil is applicable in the manufacturing of soaps, lubricants, hydraulic and brake fluids, pharmaceuticals and perfumes. The fatty acid composition of a typical castor oil contains ricinoleic acid (87 - 90 %), oleic acid (2 - 6 %), linoleic acid (1 - 5 %), stearic acid (0.5 - 1 %), palmitic acid (0.5 - 1 %) (Rial et al., 1999). Non-comedogenicity is castor oil's least understood or appreciated benefit. Comedogens are defined as cosmetics or cosmetic ingredients that exacerbate or contribute to acne and are an important factor for dermatologists and consumers alike. To overcome this growing concern, cosmetic manufacturers are formulating products with non-comedogenic emollients. Castor oil and its derivatives are recognized as non-comedogens and emollients (Mutlu & Meier 2010).

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The issue of strains becoming resistant to the majority of synthetic antibiotics is closely related to the need for new antimicrobial agents. This resulted from the widespread use of antibiotics, which makes the majority of antimicrobial drugs available today ineffective at treating certain bacterial infections (Gustavo et al., 2010). There is mounting evidence that suggests using medicinal plants as a substitute for conventional treatments for mild cases of infectious diseases may be appropriate. Additionally, they might be a source of recently developed, reasonably priced antibiotics that pathogenic strains cannot resist. Several studies have provided scientific justification for the widespread use of plants to treat infectious diseases (Talreja et al., 2023).

Safer substitutes must be made accessible due to the possible harm that these artificial antibacterial agents may cause. It becomes vital to look for alternative vegetable oils that are active against certain bacteria, including Escherichia coli, as the majority of vegetable oils used in the formulation of antimicrobial soap have not been able to display an antimicrobial impact on this type of bacteria.

The study examined the extraction and characterization of Moringa oleifera oil and castor oil from their seeds and the determination of antimicrobial soap inhibition against some clinical isolates of pathogenic microorganisms (Staphylococcus aureus, Escherichia coli and Candida albicans) using agar diffusion method.

MATERIALS AND METHODS

Procurement of oil

The mechanical press method of extraction was used in this research work to extract the oils from the seeds. This method of extraction is accomplished by exerting sufficient force on the confined seed to create pressure high enough to rupture the cells and force oil from the seed to escape. The extraction is done by compressing the material in a container that has small perforations, either round or slotted, that allow the liquid component to leave.

Soap making process

Eleven samples containing a blend of Moringa oleifera oil and castor oil in different proportions were prepared by mixing them in the ratio 100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90 and 0:100, respectively. Each combination weighed 100g. Soap samples were prepared from each combination using the cold process. A calculated amount of NaOH (lye) was weighed and a fixed amount of distilled water was added to it. The caustic soda was stirred well using a pestle until it blended with the oil. The caustic soda was poured very gradually into the oil and stirred gently in one direction to enhance the thorough mixing of the solution until the mixture traces. The soap mixture was poured into the mould and it was left for 3-5 days for solidification and proper hardening up.

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Hardness test

The hardness of the soap was measured using a needle (6.4 cm in length; 1 mm in diameter) to which a lead fishing weight (130g) was attached and lowered into the soap, the distance to which the needle penetrated the soap after 30s, was recorded as a measure of its hardness.

pH determination

The pH values of the soaps produced were measured using a pH meter. 2.0g of the produced soaps were dissolved in 50 ml of deionized water and the pH was determined using the meter.

Screening for antimicrobial activity

The susceptibilities of the test organisms to the soaps were assayed using agar-well diffusion method (Ndukwe et. al., 2005; Aliyu, et al., 2012). Clinical isolates of C. albicans, S. aureus and E. coli were obtained from the Department of Microbiology, Ahmadu Bello University, Zaria. The test organisms from growth on nutrient and potato dextrose agar plates incubated at 37° C and room temperature for 18h for the bacteria and fungus respectively were suspended in saline solution and adjusted to match a turbidity of 0.5 McFarland standard. The standardized suspension was used to inoculate the surfaces of Mueller Hinton agar plates and PDA plates using a sterile cotton swab. Different concentrations; 400 mg/ml, 200 mg/ml, 100 mg/ml and 50 mg/ml of the soap were prepared (using sterile distilled water) following serial dilution. Six wells were made on each plate using sterilized corn borer (6 mm) and 0.3 ml of each concentration was transferred into each of the 6 wells. The plates were incubated at 37° C for 24 hours and at room temperature for 48 hours for the bacteria and fungus respectively. The plates were observed for clear zones also referred to as the zone of inhibition around the wells and measured using the transparent meter rule. The entire test was conducted in duplicate.

Minimum inhibitory concentration (MIC)

The soap was dissolved in sterile distilled water and 2 ml each of sterile Mueller Hinton broth and potato dextrose broth were transferred into a set 4 of tubes and 2ml of each concentration (400 mg/ml, 200 mg/ml, 100 mg/ml and 50 mg/ml) of the soap was added to obtain final concentrations of 200 mg/ml, 100 mg/ml, 50 mg/ml and 25 mg/ml respectively. Each test organism was inoculated into the labelled tube except the control; the tubes were incubated at 37° C for 18 hours for the bacteria and at room temperature for 48 hours for the fungus. The MIC was taken as the lowest concentration that prevented visible growth.

Minimum bactericidal concentration (MBC)

The minimum bactericidal concentration and minimum fungicidal concentration were determined according to the National Committee for Clinical Standards (1999). The tubes that showed no visible growth from the test tubes used in the determination of MIC were subculture onto freshly prepared Mueller Hinton agar and PDA at 37° C for 24 hours for the bacteria and at room temperature for 48 hours for the fungus, respectively. The least concentration at which the organisms died was taken as the MBC.

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RESULTS AND DISCUSSION

The soap sample nomenclature as shown in Table 1 gives the blending proportion of the Moringa oleifera oil to castor oil. Table 2 presents the hardness test result for each of the formulated soaps. As evident from the results, the depth of needle penetration increased steadily with an increase in the castor oil content, which connotes a decrease in the hardness of the soap bar. The decrease in hardness of the soap bar can be accounted for, due to the addition of more castor oil in the blend because the castor oil belongs to the group of soft oils. The fatty acid composition of castor oils makes it not to be classified as hard oil. For this reason, castor oil is blended with other hard oils like Moringa oleifera oil to produce a hard soap. Excess of castor oil in any soap formulation gives rise to a relatively soft soap (1.63 cm). The hardness of the soap bar (0.4 cm) when it is solely Moringa oleifera oil was somewhat close to the hardness of the commercial antibacterial soap (0.5 cm).

Table 3 shows the pH of the different soap samples. The pH which is a measure of the degree of alkalinity or acidity of the soap is an important property that must be checked first before application on the skin. A right balance of the pH of every soap must be maintained because a highly acidic soap or alkaline irritates or burns the skin. The pH of all the soap samples fell within the recommended range for bathing soap according to MakMensah and Firempong, (2011) and higher than those found by Ndiaye et al. (2022) which shows a content range of 0.028, to 0.043.

Table 4 shows the susceptibility of the three microorganisms (S. aureus, C. albicans and E. coli) to various concentrations of the soap. The commercial antibacterial soap (Dettol) recorded a somewhat similar zone of inhibition with the formulated soap (S11). The microorganisms (S. aureus and C. albicans) were similarly susceptible to all prepared Moringa oleifera oil and castor oil soaps (S1 to S11). The sensitivity of microorganisms is concentration dependent as seen from the result. It was seen clearly that gram-positive bacteria (Staphylococcus aureus) were more susceptible to the soap (with a maximum inhibition zone of 28 mm) than gram-negative bacteria (Escherichia coli). S. aureus and C. albicans have been incriminated in causing skin infections including boils, thrush impetigo etc. The susceptibilities of these organisms to the soap indicate the therapeutic potential of the soap in the treatment of such diseases. The cell walls of S. aureus which is made up of mainly peptidoglycan is found to be distorted by long-chain fatty acids that are found in vegetable and is present in both Moringa oleifera oil and castor oil, an active ingredient in the soap (Ugbogu, 2006). The effect of long-chain fatty acid leads to disruption of the fungal membrane allowing leakage of macromolecules such as nucleotide, inorganic acid or phosphorylated ammonium compound (Arora, 2004). E. coli being gram gram-negative organism has little peptidoglycan in its cell wall and this may hinder the activity of the active components of the soap (fatty acids and phytochemicals).

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Parameter	S1	S2	S3	S4	S5	S6	S7	S8	S 9	S10	S11
Ratio	100:0	90:10	80:20	70:30	60:40	50:50	40:60	30:70	20:80	10:90	0:100
(M:C)%											

M: C, Ratio of Moringa oleifera oil to castor oil

Table 1: Nomenclature of the prepared soap samples

s/no	Depth of needle penetration (cm)
S 1	0.40
S2	0.43
S3	0.60
S4	1.27
S5	1.30
S6	1.40
S7	1.45
S 8	1.50
S 9	1.55
S10	1.60
S11	1.63
Dettol	0.50

Table 2: Hardness of the soap samples

Sample	S 1	S2	S3	S4	S5	S6	S 7	S8	S 9	S10	S11	Dettol
pН	9.00	9.10	9.00	9.00	9.10	9.10	9.10	9.20	9.00	9.00	9.10	9.10

Table 3: pH of the soap samples

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					Zon	e of inh	ibition	(mm)				
Test Organism	Concentration of S1 (mg/ml)				Concentration of S2 (mg/ml)				Concentration of S3 (mg/ml)			
	400	200	100	50	400	200	100	50	400	200	100	50
E. coli	19	17	10	9	18	15	10	9	17	16	11	8
C. albicans	19	16	14	10	19	17	13	11	21	19	17	15
S. aureus	20	17	16	11	21	19	18	15	23	22	19	17
Test Organism	Concentration of \$4				C	W-1-1-1-1-1-1	ation of	`S5	C	oncentr		S6
220			g/ml)	55	-		g/ml)	100	700		g/ml)	-
-201 VS	400	200	100	50	400	200	100	50	400	200	100	50
E. coli	17	15	11	8	16	14	10	8	15	12	10	8
C. albicans	21	18	16	12	22	20	17	15	23	21	20	16
S. aureus	23	22	21	19	24	22	19	17	25	22	21	17
Test Organism	Concentration of S7 (mg/ml)				Concentration of S8 (mg/ml)				Concentration of S9 (mg/ml)			
	400	200	100	50	400	200	100	50	400	200	100	50
E. coli	15	13	12	11	15	12	11	8	15	11	9	8
C. albicans	23	20	19	16	24	21	19	17	24	22	20	17
S. aureus	25	22	20	18	27	24	21	19	27	25	22	19
Test Organism	Concentration of S10 (mg/ml)				Concentration of S11 (mg/ml)				Concentration of detto (mg/ml)			
ENT ME	400	200	100	50	400	200	100	50	400	200	100	50
E. coli	15	11	9	8	15	10	9	9	17	15	12	11
C. albicans	25	23	20	18	25	23	20	17	24	21	20	17
S. aureus	28	24	23	19	28	25	22	19	27	24	21	19

Table 4: Susceptibility of microorganism to various concentrations of soap

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	Observation of Turbidity												
Test			ntration o	fS1			ntration o	Concentration of S3 (mg/ml)					
Organism			mg/ml)				mg/ml)						
	200	100	50	25	200	100	50	25	200	100	50	25	
E.coli	×	MIC	+	+		MIC	+	+		MIC	+	+	
C.albicans	*	MIC	+	+			MIC	+			MIC	+	
S.aureus	×		MIC	+			MIC	+	•	•	MIC	+	
Test	Concentration of S4						ntration o	fS5	Concentration of S6 (mg/ml				
Organism	esteria.		mg/ml)	200	2633		mg/ml)	3-10	2000	-	200	0000	
	200	100	50	25	200	100	50	25	200	100	50	25	
E. coli		MIC	+	+		MIC	+	+		MIC	+	+	
C.albicans	ž		MIC	+			MIC	+			MIC	+	
S. aureus	3		MIC	+	ũ	•	MIC	+			MIC	+	
Test		0.0000000000000000000000000000000000000	ntration o	fS7		777777	ntration o	f S8	Concentration of S9 (mg/ml)				
Organism	(mg/ml)					(mg/ml)						
	200	100	50	25	200	100	50	25	200	100	50	25	
E. coli		MIC	+	+		MIC	+	+		MIC	+	+	
C.albicans			MIC	+		•	MIC	+			MIC	+	
S. aureus	•		MIC	+	•	٠	MIC	+	•	٠	MIC	+	
Test	Concentration of S10					Concer	tration of	S11	Concentration of dettol				
Organism		(mg/ml)			(mg/ml)		(mg/ml)				
	200	100	50	25	200	100	50	25	200	100	50	25	
E. coli		MIC	+	+		MIC	+	+	1.77	MIC	+	+	
C.albicans		•	MIC	+			MIC	+		•	MIC	+	
S. aureus			MIC	+			MIC	+			MIC	+	

⁺ represents the presence of turbidity at the given concentration which connotes the presence or the activity on the microbial strain.

Table 5: Minimum inhibitory concentrations (MIC) of the soap samples against the microorganisms.

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	Observation of Growth												
Test	(Concentra				tion of S2	Concentration of S3 (mg/ml						
Organism		(mg			(mg								
	200	100	50	200	100	50	200	100	50				
E.coli	MBC	+	+	MBC	+	+	MBC	+	+				
C.albicans	×	MBC	+		MBC	+	11 . 00	MBC	+				
S. aureus	-	MBC	+		MBC	+	((€)	MBC	+				
Test Organism	Concentration of S4 (mg/ml)			3	Concentra (mg	tion of S5	Concentration of S6 (mg/ml						
O'guinoin	200	100	50	200	100	50	200	100	50				
E. coli	MBC	+	+	MBC	+	+	MBC	+	+				
C. albicans	-	MBC	+	-	MBC	+	-	MBC	+				
S. aureus	-	MBC	+		MBC	+		MBC	+				
Test Organism	Concentration of S7 (mg/ml)			9	Concentra (mg	tion of S8 /ml)	Concentration of S9 (mg/ml)						
	200	100	50	200	100	50	200	100	50				
E. coli	MBC	+	+	MBC	+	+	MBC	+	+				
C. albicans	-	MBC	+	-	MBC	+		MBC	+				
S. aureus	*	MBC	+		MBC	+	(3.0)	MBC	+				
Test Organism	Concentration of S10 (mg/ml)			C	Concentrat	ion of S11 /ml)	Concentration of dettol (mg/ml)						
	200	100	50	200	100	50	200	100	50				
E. coli	MBC	+	+	MBC	+	+	MBC	+	+				
C. albicans		MBC	+		MBC	+		MBC	+				
S. aureus		MBC	+	-	MBC	+		MBC	+				

Table 6: Minimum bactericidal / fungicidal concentration of the soap samples against the microorganism

Table 5 presents the results for the minimum inhibition concentration (MIC) of the various formulated soaps. The lowest concentration (highest dilution) of the test agent preventing the appearance of turbidity (growth) was considered to be the MIC. The low MIC value (50 mg/ml) observed for S. aureus and C. albicans for most of the soap formulated is a good indication of high efficacy against this bacterium and fungus. On the other hand, a higher MIC value (100 mg/ml) was obtained for the bacterium E. coli which may be an indication of low efficacy or that the organisms have the potential for developing resistance to the bioactive compounds (Doughari et al., 2007). The MIC and the zone of inhibition are inversely correlated; the more susceptible the microorganism is to the antimicrobial agent, the lower the MIC and the larger the zone of inhibition.

Table 6 shows the result of the minimum bactericidal and fungicidal concentrations of the various soaps. The MBC and MFC demonstrate the lowest level of antimicrobial agent that results in microbial death. This means that even if a particular MIC shows inhibition, plating the bacteria onto agar might still result in organism proliferation because the antimicrobial did not

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cause death. The MBC and MFC value for both the S. aureus and C. albicans was 100 mg/ml by implication the formulated soap with this minimum level of concentration is capable of causing the death of both microbes.

For the E. coli the MBC was somewhat higher with a value of 200 mg/ml which suggests that at this concentration of the formulated soap, the microbe will certainly not survive.

CONCLUSION

Soap samples were produced using various blends of Moringa oleifera oil and castor oil. All the soap samples exhibited antimicrobial properties of varying degrees which provides scientific justification for its medicinal use. The soap prepared using castor oil alone (S11) exhibited the highest antimicrobial properties against S. aureus and C. albicans. The soaps exhibited activity on E.coli, however soap sample (S1) gave the highest zone of inhibition (19 mm). The antimicrobial properties of the Moringa oleifera and castor oil blends compared favourably well to commercial antibacterial soap.

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