FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 4 No. 4, December, 2020, pp 348 – 354
DOI: https://doi.org/10.33003/fjs-2020-0404-492

PHYSICOCHEMICAL AND ANTIMICROBIAL PROPERTIES OF TRANSLUCENT SOAP PREPARED USING *ALOE VERA* GEL AND *ZIZIPHUS JUJUBE* LEAF EXTRACT AS ANTIMICROBIAL SOURCE

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ABSTRACT

In this study, the antimicrobial and physicochemical properties of translucent antimicrobial soap prepared using two medicinal plants, *Aloe vera gel and Ziziphus jujube* leaf extract was evaluated. The results of the physicochemical analysis showed the pH (7.52±0.02), hardness(1.3 cm±0.02), solubility (0.82 g±0.02) and foamability (6.80 cm±0.03) of the prepared soap and these values were within the acceptable limit set by WHO/SON which make the prepared soap skin-friendly. Similarly, the antimicrobial screening was carried out on selected Gram-positive and gram-negative bacteria as well as on fungi species using disc diffusion methods and the results showed varying antimicrobial activity at different concentrations ranging from 62.5-500 mg/ml. However, the susceptibility of test bacteria in terms of the zone of inhibition at 500mg/ml of the soap was observed on *Staphylococcus lentus* (22 mm), *Staphylococcus aureus* (20mm), *Escherichia coli* (15 mm), *Raoltella ornithinolytica* (12mm). The result of antifungal properties was as follows, *Candida albicans* (12 mm), *Trichophyton rubrum* (12 mm), and *Aspergillus nigar* (10 mm) at 500 mg/ml concentration. The results when compared to other antimicrobial soap in the market indicate good quality by inhibiting the growth of both Gram-positive, gram-negative bacteria and fungi. The antimicrobial activities exhibited by the soap in this study could be attributed to the presence of phytochemical constituents in the plant extracts, which signify the potential of the soap as an antimicrobial agent. Therefore, these findings confirmed the efficacy of *Aloe vera* gel and *Zizipus jujube* extract in traditional medicine.

Keywords: Soap, translucent, Antimicrobial, Aloe vera, Zizipus jujube, susceptibility test

INTRODUCTION

Saponification is a chemical reaction that yield soap as the major chemical product (Gunstone, 2004; Scrimgeour, 2005). The response for making soap is a base (usually NaOH or KOH) hydrolysis of triglycerides to form soap and glycerol. The molecules crystallize differently depending on the base used. NaOH produces a harder bar while KOH is used more frequently for liquid soaps. A cleaning soap molecule has a long chain hydrocarbon with a carboxylic acid group on one end, which has an ionic bond with a metal ion, usually sodium or potassium. The hydrocarbon end is nonpolar which is tremendously soluble in Non-polar substances and the ionic end is soluble in water. The cleansing ability of soaps is because of their potential to amalgamate or comingle water-insoluble materials and hold them withinside the suspension of water. This capacity is noted from the molecular arrangement of soaps. When soap is added to water that contains oil or other water-insoluble materials, the soap or detergent molecules surround the oil droplets. The oil is, dissolved withinside alkyl groups of the soap molecules while the ionic end permit it to be dissolved in water. As the end result, the oil droplets are to be scattered throughout the water and can be washed away (Girgis, 1997). An accurate cleaning soap should not contain chemicals that can be disastrous to the environment or cause undue problem to the surrounding. It should dissolve without tediousness and get rid of stains from garments, human pores and skin or any fabric being wiped clean. It should have the capacity to provide sufficient bubbles, deliver a glowing type of cleanliness and fine scent. It must not leave gummed traces on the garments or the body. It should have a wonderful color that is moderate and does not stain and damage the garments or materials (Girgis, 1997). Antimicrobial cleaning soap is designed to soundly kill germs and cleanse the skin. In the antimicrobial cleaning soap formulation, the types of organisms the product should be effectual against and how much time is needed for the product to work have to be observed. Also factors related to cleansing such as foamability, time of foaming, rinse capacity, and skin touch have to be noticed also. According to Osbore and Grube, (1982), excellent antimicrobial soap can take away 65 – 85 % germs from human skin. However, modern-day industrial antimicrobial soaps contain artificial chemical compounds

including triclosan, trichlorocarbanilide and chloroxylenol, majority of which are thought to be carcinogenic, mutagenic and or generate allergic reactions. Triclosan (2, 4, 4 - trichloro-2-hydroxydiphenyl ester) is used in soaps, shampoo and fabrics, as an antimicrobial agent. Though these compounds are taken into consideration to have low toxicity, their 2-hydroxy isomers were proven to undergo thermal and photochemical ring closure to form polychlorinated dibenzo-p-dioxins, which are distinctly toxic (Okumura and Nishikawa, 1996). In addition, it is far widely recognised that microorganism could generate obstinacy to triclosan and this could lead to the development of hostility and change in microbial community arrangement (Aiello *et al*., 2007). The need for brand new antimicrobial source is highly associated with the problem of emergence of strains that are immune to many artificial antibiotics. This has arisen because of the extensive use of antibiotics, which renders most of the contemporary antimicrobial agents ineffective in handling many bacterial diseases (Gustavo *et al*., 2010). There is increasing evidence to prove that medicinal plants may represent an alternative treatment for non-severe cases of infectious diseases. They could also serve as a possible source of new and low cost antibiotics to which pathogenic strains are not immune and several researches provide scientific prove for the popular use of medicinal plants against infectious diseases (Kitula, 2007; Ajibesin et al., 2008; Wu et al., 2008) and safety benefits (Joshi and Pawal, 2015; Sharma et al., 2008). The international dependence on natural products appears to be at the growth. WHO anticipated that approximately 80% of the African population rely directly or indirectly on medicinal plants (Ekor, 2014; Joshi and Pawal, 2015; WHO, 2002). The worldwide demand for herbal medicines was over $60 billion per year with an estimate of a 6.4% annual increase growth rate. This is seemingly because of the contribution of the massive fitness and economic values of herbal products (Inamdar et al., 2008; Sharma et al., 2008; WHO, 2002).

Girgis and Khalil (1997) labored on the physical nature of bathing soap produced from apricot kernel oil and palm stearin of which only the physio-chemical analysis of the soap were carried out. The antimicrobial properties of the soap were not determined.

Eke *et al.* (2004) performed an analysis of locally produced soap using shea butter oil blended with palm kernel oil of which only the physicochemical properties of the soap were tested. Warra *et al.* (2010) run analysis on cold process synthesis and properties of soaps prepared from different triacylglycerol sources (shea nut oil, groundnut oil, tallow) of which the physical properties of the soap were analyzed but the antimicrobial properties remained unidentified.

Shoge, (2011) worked on the quality of soaps using different oil blends of which the physicochemical properties and cost analysis were evaluated for different soaps but their antimicrobial properties were left unchecked. Aliyu et al. (2012) worked on the antimicrobial activity of *Sabulun salo* a local traditional medicated soap of which the soap inhibited *Staphylococcus aureus* and *Candida albicans.*

Garba et al (2012) worked on an analysis of the antibacterial activity of Africa black soap on some selected pathogens of which the soap inhibited *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli.* Ameh et al (2013) reported the synthesis and characterization of antiseptic soap from neem oil and shea butter oil of which the soap hardness, foamability and pH were analyzed. The soap also possessed antibacterial properties inhibiting *Staphylococcus aureus* and *Bacillus subtilis.* From the above-related works it can be deduced that works on antimicrobial property of soap only inhibited the Gram-positive bacteria and fungi, so it becomes necessary to seek out alternative soap formulation that will exhibit antimicrobial property on both the Gram-positive bacteria, Gram-negative bacteria and fungi.

The present study is aimed at producing herbal soap with antimicrobial properties that can inhibit both Gram-positive and negative bacteria as well as antifungal using *Ziziphus jujube* leaf and *Aloe vera* gel by adopting the principle of green chemistry to attain a pollution free environment.

MATERIALS

All the solvents and reagents used were of Analytical grade which includes ethanol, glycerol, Sulphuric Acid, Ethyl acetate, Dilute hydrochloric acid. Pko oil and olive 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^{o}C. Then Aloe vera gel and Ziziphus jujube leaves were used as the antimicrobials.

METHODS

Preparation of Crude Aloe vera Extract

The procedure reported by Noor et al., 2011 for extraction of Aloe vera was adopted, the leaves were washed with tap water and weighed, and then the epidermis of the leaves was peeled off, and then the colorless, solid gel was cut into pieces, then it was lyophilized and grounded , and the lyophilized gel powder was packed into soxhlet apparatus and extracted with 90 % ethanol at 90 °C for 4hrs, then finally it was filtered and concentrated using a rotary evaporator and stored at room temperature.

Preparation of Crude Ziziphus jujube Leaf Extract

The procedure reported by Moody et al., 2014 was adopted with slight modification, the leaves of the *Ziziphus Jujube* were collected from the farm, Dried under shade, the leaves were grinded into a coarse powder using mortar and pestle for further investigation, 2.5 g of the powdered leaves were measured and soaked into 120 ml of ethanol in a sample bottle and leave undisturbed for 48 hours, After 48 hours it was filtered using filter paper and funnel, the filtrate was subjected to evaporation in vacuum to obtain a crude extract of the leaves.

PHYTOCHEMICAL SCREENING

For the qualitative determination of the phytochemicals (Alkaloids, Anthraquinone, Saponins, Tannins, and

Flavonoids) the procedures reported by Thilagavathi *et al.,* 2015 was adopted with slight modification.

Preparation of Translucent Antimicrobial Soap Using Cold and Hot Processes

The procedure reported by Sadeghian et al. 2015 was adopted with little modification. 100 g of NaOH was measured and dissolved into 300 ml of distilled water, which gives a lye solution of 0.33 g/ml of NaOH, then 50 ml of PKO oil was measured and combined with 50 ml of Olive oil, then 50 ml of 0.33 g/ml lye solution was measured and added into the oil gently with continues stirring until a homogenous mixture was obtained. Then 20 ml of antibacterial solution(10 ml aloe vera extract and 10ml Indian jujube extract) was added and 10 ml of sodium silicate and 10 ml of essential oil were also added and stirred gently until it reaches trace, Upon reaching trace the soap solution was poured into mold and covered properly and leaved undisturbed for 24 hours. After 24 hours the soap was removed from the mold and cut into small pieces and poured into a crooked pot and heated until it melts. After the soap has been melted 50 ml of denatured alcohol and 50 ml of sugar solution and 20 ml of glycerin were added and stirred until the mixture turns colorless. The soap was poured into molds again and covered and allowed undisturbed for 8 hours, and after 8 hours it was removed from the mold and allowed to cure for a period of 7 days. After the curing period characterization of the soap was then carried out.

CHARACTERISATION OF THE PREPARED SOAP

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 which include Dettol soap, Skineal transparent soap were used as standards for comparison.

pH Test

The pH values of the soaps were determined using a pH meter (Inolab, WTW, Germany, pH 7310). 1 g of the soaps was weighed and dissolved in 10 mL distilled water and made up to 100 mL mark to afford 1% (w/v) homogeneous soap solution. The electrode of the pH meter was inserted into the solution and the value recorded. The steps were repeated for all the soaps.

Hardness Test

The hardness of the soap was determined by inserting a regular hand-sewing needle (4.2 cm in length and 0.5 mm in diameter) into the soap. The needle was loaded at the pinnacle with a weight of 370 g on the lever system. The lever was raised and allowed to slowly penetrate the soap within 30 s. The process was repeated three instances and the common intensity of the penetration of the needle was measured and recorded.

Foamability Test

Foaming ability test 0.2 g each of the soap sample was inserted into a 100 mL measuring cylinder containing only 10 mL distilled water. The mixture was shaken vigorously to generate foams. After shaking for 2 min, the cylinder was allowed to stand for about 10 min and the height of the foam was then measured and recorded.

Solubility Test

Solubility test 0.2 g of each soap was added to a 100 mL measuring cylinder containing 10 mL of distilled water. The duration of the dissolution of the soap after continuous shaking was recorded.

Antimicrobial Screening of the Prepared Soap

Antimicrobial susceptibility testing was carried out using the well diffusion method according to the standard of the national committee or clinical laboratory standards. Soap stock solutions were prepared at the concentration of 500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml using the serial dilution method. The soap samples were tested on Mueller Hinton Agar plates to detect the presence of antimicrobial properties. Before striking the plates with bacteria, 6 mm diameter was punched on to the media using a sterile borer. All plates were inoculated with the test bacterium which has been previously adjusted to the 0.5 Mcfarland standard solution (reference used to adjust the turbidity of bacterial suspensions so that the number of bacteria will be within a given range to standardize microbial testing). The surfaces of the agar plates were streaked over the entire sterile agar using a sterile swab. The plates were allowed to dry for 3 to 5 minutes to dry the excess moisture. Then 500 mg/ml,250 mg/ml,125 mg/ml,62.5 mg/ml of each test soap solution was dispensed into each well after the inoculation of the plates with the bacteria and placed in an incubator set to 37^0C. After 24hours of incubation, each plate was examined for inhibition zones. A metric ruler was used to measure the inhibition zones in millimeters (Chatterjee *et al.*,2014)

RESULTS

Table 1: Result of phytochemical screening of the plants extract

Plants	Alkaloids	Flavonoids	Saponins	Tannins	Anthraquinones
Aloe Vera	+	+	+	+	++
Indian Jujube	++	+	+	+	-

Key; "+" indicates the presence, "++" indicates more presence **,** "–" indicates not detected.

Table 2: Physicochemical Analysis

Soap Samples	pH	Hardness (cm)	Foamability(cm)	Solubility(g)
Soap A	7.52±0.02	1.3±0.02	6.8±0.02	0.80±0.02
Soap B	7.30±0.02	1.2±0.02	9.5±0.02	0.72±0.02
Soap C	7.10±0.01	1.3±0.02	8.2±0.02	0.92±0.02

Key Soap A= prepared soap**,** Soap B= Dettol soap**,** Soap C = Skineal transparent soap

Table 3: Result of Antimicrobial test (sensitivity) for soap A

Test Organism	**Mean Zones Of Inhibition(mm)**				
	Conc. Of soap A (mg/ml)				
	500	250	125	62.5	32.25
S.L	22	18	15	10	6
S. aureus	20	16	12	9	7
E.coli	15	11	9	8	7
R.O	12	11	10	8	6
T.R	12	6	6	6	6
C.A	12	8	6	6	6
A. nigar	10	10	8	7	6

Table 4: Result of Antimicrobial test (sensitivity) for soap B

Test Organism	Mean Zones Of Inhibition(mm)				
	Conc. Of soap A (mg/ml)				
	500	250	125	62.5	32.25
S.L	14	10	10	10	6
S. aureus	17	13	12	9	7
E.coli	15	11	9	8	7
R.O	14	11	10	8	6
T.R	10	6	6	6	6
C.A	12	8	6	6	6
A. nigar	12	10	8	7	6

Table 5: Result of Antimicrobial test (sensitivity) for soap C

Test Organism	Mean Zones Of Inhibition(mm)				
	Conc. Of soap A (mg/ml)				
	500	250	125	62.5	32.25
S.L	16	12	10	10	8
S. aureus	13	13	12	9	7
E. coli	11	11	9	9	7
R.O	9	9	6	6	6
T.R	11	6	6	6	6

C.A	11	8	6	6	6
A. nigar	14	10	8	6	6

Key: S.L= *Staphylococcus Lantus* ;Gram positive bacteria**,** S. Aureus= *Staphylococcus aureus* ; Gram positive bacteria**,** E.coli.=*Escherichia coli'*; Gram negative bacteria**,** R.O= *Routella Ornithonolytica* ; Gram negative bacteria**,** T.R= *Trichophyton Rubrum* ;Fungi**,** C.A= *Candida Albican* ;Fungi**,** A.nigar= *Aspergillus nigar* ;Fungi

DISCUSSION

From table 1, The preliminary qualitative phytochemical screening of the plants extract revealed the presence of secondary metabolites such as alkaloids, flavonoids, saponin, tannins and anthraquinones most of which were reported by previous literatures to possess antimicrobial properties, alkaloids, flavonoids, saponin, tannins as well as anthraquinones were all detected in aloe vera gel while alkaloids, flavonoids, saponin, and tannins were found present in the Ziziphus jujube leaf extract. And from table 2 , the pH which is a measure of the acidity or alkalinity was determined. The the pH value of the produced soap fell within acceptable range (7–10) that is permitted by a regulating authority especially in Nigeria (Oyedele, 2002). The pH value obtained is also in agreement with literature results (Ogunsuyi and Akinnawo, 2012; Vivian et al., 2014) and also agrees with that of the market soaps that were used as control. Therefore, the obtained pH value indicate that the soap would be less corrosive, skin-friendly and is expected to produce less skin reaction when used. Similarly the other parameters (Foamabilty, solubility and hardness) shows how good the quality of the produced soap is, it can be seen that the market soaps tend to have higher foamability values which is precisely due to the foam boosters that were used in their manufacture as indicated by the manufacturers, while the produced soap does not contain such additives and still shows good quality concerning the foam it produces and the solubility and hardness of the soaps is an indication of the ability of the soaps to last longer when used due to its ability to dissolve slowly in water.

Both market soap and prepared soap were subjected to antimicrobial susceptibility test using bacterial and fungal clinical isolates as test organisms. The bacterial organisms are gram-negative and gram-positive bacterial species and some fungal organisms as shown earlier. Antimicrobial agent if in contact with any organism that is susceptible to it at a concentration cidal or static to the organism should make the population of the organism to reduce gradually until such a time that the medium may become sterile. The microorganisms (*S. aureus* and *C. albicans*) were similarly susceptible to the prepared soap(Soap A). The bacteria (*E. coli*) was also susceptible to the prepared soap but showed a higher resistance inhibition when compared with the other microorganisms used in this study. It may also be observed that the zone of inhibition decreased with decreasing soap concentration of which we can affirmatively claim that the sensitivity of microorganisms was concentration-dependent. On the other hand, 62.5mg/ml l soap concentration showed higher colony growth of *E. coli, C.albicans* and *Staphylococcus aureus*, whereas 500 mg/ml soap concentration inhibited the growth of all the microorganisms. It was seen clearly that gram-positive bacteria (*Staphylococcus aureus*) were killed at a low concentration of soaps than gram-negative bacteria (*Escherichia coli*). The findings of this study showed that the the prepared soap have an antimicrobial effect against *Staphylococcus Lantus, Staphylococcus aureus, Escherichia coli, Routella Ornithonolytica, Trichophyton Rubrum, Candida Albican,and Aspergillus nigar* with a maximum zone of inhibition of 22 mm, 20 mm, 15 mm, 12 mm, 12 mm 12 mm, and 10 mm, respectively. *S. aureus* and *C. albicans* have been inculpated in causing skin infections including boils, thrush 54 and impetigo etc. The susceptibilities of these organisms to the soap indicate the therapeutic ability of the soap in the treatment of such diseases. Secondary metabolites phytochemicals present in the extract of the medicinal plants used in this study may play a role in defense through cytotoxicity towards pathogenic microorganisms (Briskin, 2000). The cell wall of *S. aureus* which is a gram-positive bacterium is made up of mainly peptidoglycan. Peptidoglycan is found to be distorted by long chain fatty acids that are found in vegetable oils of which is present in both *Olive* oil and pko oil an active ingredient in the soap (Ugbogu, 2006). The effect of long-chain fatty acid may be the destruction of the fungal membrane causing leakage of macromolecules such as nucleotide, inorganic acid or phosphorylated ammonium compound (Arora, 2004). This explains the inhibitory effect exerted by the soap against the fungus (*C. albicans*). *E. coli* being 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). The resistance of *E. coli* to antimicrobial agents is usually due to chromosomal mutation which lowers the permeability of the bacteria to the agents or acquisition of resistance (R) plasmids and transponsoms (Arora, 2004). Therefore, the immune showed by *E. coli* to the soap may be due to chromosomal mutation which may have resulted to lower permeability of the bacterial cell.

SUMMARY

This study describes the formulation of preparing antimicrobial soap using the medicinal plants, aloe vera and *ziziphus jujube* as antimicrobial agents and also evaluated the physicochemical properties and the antimicrobial property of the prepared soap. The physicochemical properties include the

pH, hardness, foamability and solubility while an antimicrobial susceptibility test was carried out to determine the antimicrobial property of the prepared soap. The preliminary qualitative phytochemical screening of the plant's extract revealed the presence of secondary metabolites such as alkaloids, flavonoids, saponin, tannins and anthraquinones most of which were reported by previous literatures to possess antimicrobial properties. The formulated soap was tested against 7 microbial strains (*S. lentus, S. aureus, E.coli, R. ornithonilytica, T. rubrum, C. albicans* and *A. nigar*) for their antimicrobial properties. Sensitivity assays were carried out on the soap sample formulated and other market soaps to determine the zone of inhibition. The antimicrobial property exhibited by the prepared soap sample in this study can be attributed to the presence of the phytochemical constituents in the plant's extracts, which signifies the potential of the soap as a typical therapeutic agent.

CONCLUSION

The secondary metabolites alkaloids, flavonoids, saponins, and tannins were found present in the Ziziphus jujube leaf extract and in the aloe vera gel with addition of anthraquinones in the aloe vera gel, and the physicochemical parameters evaluated were not higher than the requirement set by regulating bodies especially in Nigeria which makes the prepared soap skin-friendly and not harmful to skin. Also Gram-positive bacteria (*S. aureus,S. lentus*) were found to be more susceptible than the gram-negative bacteria (*E. coli, R. ornithonolytica*) and the antimicrobial effect exhibited by the soap in this study signifies the potential of the soap as a typical therapeutic agent.

RECOMMENDATION

The economic analysis of the produced soap samples should be done to check their viability against the commercial antimicrobial soaps, and further studies should be conducted on other non-utilized medicinal plants for their antimicrobial activity and also to determine the Minimum Inhibitory Concentration (MIC) to demonstrates the lowest level of antimicrobial agent that inhibits microbial growth and the minimum bactericidal concentration (MBC) to demonstrates the lowest level of antimicrobial agent that results in microbial death.

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