

Research Article

Sahel Journal of Life Sciences FUDMA (SAJOLS) December 2023 Vol. 1(1):290-296 ISSN: 3027-0456 (Print) ISSN: xxxx-xxxx (Online) DOI: <u>https://doi.org/10.33003/sajols-2023-0101-031</u>



Antibacterial Potential of African Black Soap Decoctions against Selected Clinical Isolates

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Received: 16th December, 2023 Accepted: 28th December, 2023 Published: 31st December, 2023

ABSTRACT

Africa black soap is widely use in Africa. This study aimed at examining antibacterial potential of African black soap and its different decoction preparations against selected bacterial agents. Soap sample was collected and prepared into three different decoctions (i.e. black soap only, black soap with aloe vera and black soap, cam wood and lime water). Afterwards, different concentrations of 200 mg/ml, 100 mg/ml, 50 mg/ml, and 25 mg/ml of each decoction preparation were prepared. Antibacterial activities of each decoction concentration against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Streptococcus pneumoniae, and Bacillus subtilis was carried out using agar well diffusion method. The result revealed that black soap with cam wood and lime water at 200mg/ml tested against Streptococcus pneumoniae had the highest (26.5 mm) zones of inhibition while at similar concentration black soap with aloe vera showed zone of inhibition of 24.5 mm, 23 mm, and 21.5 mm against Escherichia coli, Pseudomonas aeruginosa and Bacillus subtilis, respectively. Black soap only showed lowest inhibitory activities compared to other mixtures because at 200mg/ml, zone of inhibition of 18.5 mm, 17.5 mm and 12.5 mm against Pseudomonas aeruginosa, Streptococcus pneumonia and E. coli, respectively were observed. This study has shown that African black soap mixed with the tested mixtures could have a better synergetic effect in inhibiting different types of pathogenic bacteria compared to using the soap only, therefore, could have potential ability to increasing the antimicrobial properties of the soap during usage. Hence, addition could improve Africa black soap quality by indigenous companies.

Keywords: Antibacterial activity, African Black Soap, Clinical isolates, Decoctions concentration

Citation: Abdulmalik, U., Wata, I., Abdullahi, K. and Adesoji, T. A. (2023). Antibacterial Potential of African Black Soaps Decoction against Selected Clinical Isolates. *Sahel Journal of Life Sciences FUDMA*, 1(1):290-296. DOI: <u>https://doi.org/10.33003/sajols-2023-0101-031</u>

INTRODUCTION

Soap making is part of western world and Africa regions are not left out in the business of soap making as the African traditional soap often referred to as African black soap originated from the region. In Africa, traditional soap (black soap) is known with different names from various regions of the continent. For instance, in the western part of African, black soap is known as Anago soap or Alatasimena in Ghana, and in Nigeria, it is known by the Hausa as Sabulun-salo, the Yoruba's called it Osedudu while in Igbo land it called NchaNkota. Vegetable or animal derived oils, made into salts are Known as soaps (Al-Doori *et al.,* 2003). Modified detergents are added in their formulation to enhance their antibacterial activity. Such soaps have potential ability to remove 65 to 85% bacterial population, prevalent on human skin (Larson *et al.*, 2004).

Traditional medicine can be described as total combination of knowledge, practice and belief incorporating plant, animals and minerals based medicine whether explicable or not, used in diagnosing, preventing or eliminating a physical, mental or social diseases and which may rely exclusively on past experience handed down from generation to generation either verbally or in writing (David, 2005).

The traditional African black soap which has in combination, water, roasted plantain skin or cocoa pod, palm oil, palm kernel oil, or shea butter, when put together, are collectively referred to as "black soap" (Al-Doori *et al.*, 2003). Certain additive such as aloe vera, cam wood, etc. may be added to the soap to provide additional antibacterial activity (Larson *et al.*, 2004). Black soap made with shea butter offers protection against UV rays while black soap made with plantains contains a high concentration of iron along with vitamins A and E (Treehugger, 2008).

The African black soap has numerous benefits and importance. Black soap enjoys a reputation for improving or eliminating uneven skin tone, razor bumps caused by ingrown hairs and skin rashes (Ahmed et al., 2005). It is not scented and can be used by anyone who wishes to improve the quality of his/her skin. It is excellent for clearing up oily skin, acne price for its antiseptics properties. African people also use black soap to prevent the skin from rashes, ring worm, measles, and eczema and body odor (Larson et al., 2004). It is used as a natural shampoo to avoid dry itchy scalp (Aliyu et al., 2012). Black soap is used in the treatment of many infectious diseases caused by micro-organisms (Ajose et al., 2007). Black soap is also used in Africa for spiritual purification (Karen, 2004; Jones, 2001).

Vegetable or animal derived oils, made into salts are known as soaps (Al-Doori *et al.*, 2003). In south western part of Nigeria, indigenous black soap may be made from roasted plantain pills or dried waste cocoa pods and vegetable oil, palm oil or palm nut oil. In the Northern part of Nigeria, it is produced from a mixture of vegetable oil, palm kernel oil and sheabutter (Aliyu *et al.*, 2012).

Black soap is preferred for bathing due to its natural source of vitamins A and E (Getradeghana, 2000). Because of its phenolic contents, it is generally used

to cures skin rashes. Excellent for clearing up oily skin and priced for its antiseptics properties. African people also use black soap to prevent the skin from rashes, ring worm, measles, eczema, and body odour. In traditional medicine, black soaps are very common vehicles for application of medicinal plants especially for external use and also for the treatment of skin diseases (Ajaiyeoba *et al.,* 2003; Ahmed *et al.,* 2005; Ajose, 2007). Currently, the soap has been improved industrially into more presentable forms with different trade names such as 'Dudu Osun' 'Zee Black Soap' etc. in-order to be more attractive to the users.

Bacteria that attack human body are of great importance with regards to health. Fuls et al. (2008) reported the inhibitory potential of antimicrobial and non-antimicrobial soaps in clinical cases. Larson et al. (1987) and Toshima et al (2001) indicated that soaps containing antimicrobial active ingredients could be used to remove more bacteria as compared to those without antimicrobial agents. Osborne and Grube (1982) reported that antibacterial containing soaps can remove 65% to 85% bacteria inhabiting human skin. When used properly, washing with antimicrobial soap could remove and reduce propiobacterium acnes and prevent secondary infections in acne skin (Kuehl et al., 2003) and healthcare-associated transmission of contagious diseases more effectively (Arya et al., 2005). Moody et al., 2004 reported that locally manufactured soaps have some antimicrobial properties. Black soap has been employed to get rid and treat many infections caused by microorganisms as well as for exfoliating and deep cleansing (Underwood 2008). The use of soaps as vehicles for the application of medicinal plants for external use and in the treatment of skin diseases has been previously reported (Ahmed et al. 2005; Ajaiyeoba et al., 2003; Ajose 2007).

Many herbalist/traditionalists from Nigeria prepared black soap decoction mixed with different plants and other local medicinal products with the believe of curing many surface bacterial infections. However, the scientific evident of this traditional believe is very scanty globally. Therefore, this study aimed at determination of antimicrobial properties of different black soap decoction prepared in various part of southwestern part of Nigeria. Studies like this will suggest how local industries producing this soap can improve the antimicrobial qualities of the soap.

MATERIALS AND METHODS

Preparation of Different Concentration of African Black Soap Decoctions

Black soap sample was obtained during September, 2023 from Afijio Local Government area of Oyo state,

Nigeria. The sample was divided into three equal portions each weighing 12.50g. First portion contains the black soap only. The second portion constitutes black soap with aloe vera plant while the third portion contains black soap with cam wood as shown in (Table 1).

Table 1: Compositions of African	black soap decoctions	used in this study
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Serial Numbers	Decoctions	Compositions
1	Decoction A	Contains 12.50 g of the African black soap only
2	Decoction B	Contains 12.50 g of the African black soap, 5 g of aloe vera squeezed with hand
3	Decoction C	Contains and 12.50 g of the African black soap, 1g of cam wood 5 ml lime water

Collection and Biochemical Identification of Bacteria Isolates

Clinical bacterial isolates used as test isolates for this study were obtained from Microbiology Laboratory of Federal University Dutsin-Ma (ML-FUDMA) and Federal Medical Center (FMC), both are located in Katsina State, Nigeria. Isolates from ML-FUDMA include: *Staphylococcus aureus* and *Escherichia coli* while *Pseudomonas aeruginosa, Streptococcus pneumoniae* and *Bacillus subtilis* were obtained from FMC.

The collected isolates were sub-cultured (using streak method) on to another freshly prepared nutrient agar and incubated at 37°C for 24 hours (Cheesbrough, 2009). The identity of the isolates was confirmed according to established microbiological methods which include Gram-stain characteristics and biochemical test described by Cheesbrough (2009). Isolates were stored on a nutrient agar slant at 15°C.

Preparation of Different Concentrations of the Black Soap

The stock solution was prepared by measuring 5ml dimethyl sulphoxide into a sterile mortal and 1g of each decoction (A, B or C) differently was added and pounded until the soap dissolved completely. Two fold or doubling dilution technique was used to obtain different concentration of the prepared stock solution. The final concentrations prepared were 200 mg/ml, 100mg/ml, 50 mg/ml, 25 mg/ml. This procedure was carried out according to the method of Akinpelu and Kolawole. (2004).

Preparation of the Bacterial Inoculum

An overnight broth culture following 18 hrs of incubation at 37^oC of the test organisms was used by inoculating two colonies from the purified bacteria into 5ml of normal saline. The overnight broth culture was standardized using 0.5 McFarland standards.

Antibacterial Activities of Various Decoctions of African Black Soap

The antibacterial activities of African black soap decoctions were determined using agar well diffusion method described by Atata et al., 2003. Twenty-five milliliter of Mueller Hinton agar was prepared and was aseptically poured on each sterile petri dishes and allowed to solidify. Therefore, plates were seeded with 0.1 ml of the standardized inoculums of the test organisms using spread plate method. The inoculums were spread over the surface of the Mueller Hinton agar in each plate with a sterile glass spreader. The seeded plates were allowed to dry in an incubator at 37°C for 20 minutes. Afterwards, a standard sterilized cork borer of 6mm diameter was used to bore a uniform well on the surface of the agar in the plates and different concentration s of the black soap decoction were dispense into the wells. The inoculated plates were incubated at 37°C for 24 hours and the zone of inhibition was measured in millimeter (mm). These were carried out in triplicates for all the soap samples.

Determination of Minimum Inhibitory Concentration (Mic) of the Decoctions

The minimum inhibitory concentrations of the black soap decoction were determined using method described by Akinpelu and Kolawole. (2004). Two milliliters of different concentrations of the solution was added to 18ml of pre –sterilized molten nutrient agar. The media were poured into sterile petri dishes and allowed to set. The surfaces of the media were allowed to dry before streaking with 18 hours old of each test bacterium. The plates were incubated at 37°C for 24 to 48 hours after which they were examined. The minimum inhibitory concentration was taken as the lowest concentration that prevented the growth of the bacteria.

Determination of Minimum Bactericidal Concentration (MBC) of various Decoctions Preparation

The minimum bactericidal concentrations of the black soap decoctions were determined by the modification of the method of Spence and Spencer. (2004). Samples were taken from plates with no visible growth in the minimum inhibitory concentration assay and sub-cultured onto a freshly prepared nutrient agar plates and later incubated at 37° C for 48 hours. The lowest concentration of the extract that did not show any growth on the new set of plates was taken as the minimum bactericidal concentration of the extract.

RESULTS

Table 2 showed the results of the antibacterial activities of different soap decoctions against the test isolates. The soap decoctions were found to have antibacterial properties against the test bacteria. The

result showed that the highest zones of inhibition obtained were 26.5 mm, 26 mm and 24.5mm, against Streptococcus pneumoniae in the mixture of black soap prepared for decoction C (black soap with cam wood and lime water). While the result for decoction B (black soap with aloe vera) showed the zone of inhibition of 24.5 mm, 23 mm, and 21.5 mm against Escherichia coli, Pseudomonas aeruginosa and Bacillus subtilis respectively. And also, the results for the decoction A (containing black soap only) showed the zones of inhibition of 18.5 mm, 17.5 mm and 16 mm against Pseudomonas aeruginosa and Streptococcus pneumonia, respectively which was found to possesses the least activities

Table 3 and 4 showed the results of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the various Africa black soaps preparation against the test bacteria at different concentrations. Decoction A (black soap with no additive) showed MIC and MBC values of 25 mg/ml and 50 mg/ml, against *Escherichia coli* and *Staphylococcus aureus*, respectively while 25 mg/ml and 50ug/ml were noticed against *Streptococcus pneumoniae* and *Staphylococcus aureus* for decoction B (black soap with aloe vera). MIC and MBC of 25 mg/ml and 50 mg/ml value were observed for *Streptococcus pneumoniae* and *Pseudomonas aeruginosa*, respectively for decoction C (Black soap with cam wood and lime water).

					Con	centration (mg	g/ml)				
Test Bacteria		Soap decoction A (mg/ml)			Soap decoction B			-	Soap decoction C		
		1	2	3	4	(m	g/ml)		(r	ng/ml)	
						12	3	4	12	3	4
S. aureus	Zones of	14	12.5	23	12	16.5 20.5	5 11.5	11	22.5 2	1.5 18.	5 16
S. pneumoniae	inhibitions	17.5	00	17.5	15.5	13 20	10	00	26.5 26	õ 24.5	22.5
P. aeruginosa	(mm)	18.5	17	16	12.5	23 23	18.5	15	17.5 1	.8 16.5	13
E. coli		12.5	5 13	11	9.5	24.5 23	21.5	15	18.5 1	6.5 14	14.5
B. subtilis		14.5	5 13.	5 10.5	9.0	21 19.5	19	00	20.5 2	20 17.5	13
(EYS : 1=200mg/m	l, 2=100m	ıg/ml,	3=50	mg/m	l, 4=	-25mg/ml,					

 Table 2: Antibacterial activities of Black soap decoctions against some selected Bacteria Isolates

Soap portion A = Black soap with no additive, **Soap portion B** = Black soap with Aloe vera, **Soap portion C** = Black soap with cam wood and lime water

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Test Bacteria	Decoction A (mg/ml)	Decoction B (mg/ml)	Decoction C (mg/ml)	
S. aureus	50	50	50	
S. pneumoniae	50	25	25	
P. aeruginosa	50	50	25	
E. coli	25	25	25	
B. subtilis	25	50	25	

Table 3: Minimum Inhibitory	Concentration (MIC) of different	Decoctions against the tested bacteria
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KEYS: Decoction A = Black soap with no additive, **Decoction B** = Black soap with aloe vera, **Decoction C** = Black soap with cam wood and lime water

Test Bacteria	Decoction A (mg/ml)	Decoction B (mg/ml)	Decoction C (mg/ml)	
S. aureus	50	50	50	
S. pneumoniae	100	25	25	
P. aeruginosa	50	50	50	
E. coli	50	25	50	
B. subtilis	50	100	50	

KEYS:

Decoction A=Black soap without additive, **Decoction B=**Black soap with aloe vera, **Decoction C** =Black soap with Cam wood and lime water.

DISCUSSION

The bacterial isolates collected were identified as S. aureus, S. pneumonia, B. subtilis P. aeruginosa and E.coli. The decoction activities were found to be active base on increasing concentration of the decoction. The Decoction A (black soap only) were found to be active against gram negative bacterium Pseudomonas aeruginosa and gram positive S. pneumoniae. However, when aloe vera was added to the black soap it was more active against two gram negative bacteria i.e. Escherichia coli and Pseudomonas aeruginosa than the gram positive bacterial while black soap with cam wood and lime water was found to be better active against tested gram positive bacteria that includes S. pneumoniae, S. aureus and B. subtilis compared to gram negative bacteria (i.e. P. aeruginosa and E. coli) and consider as the most active soap preparation. Activities of some of the preparation against gram positive bacteria may be as a result of previous discovery by Aliyu et al. (2012) which attributed the antimicrobial action of the traditional medicated soap specifically against gram positive organisms like S. aureus to the ability of the soap's long chain fatty acid content to distort the Peptidoglycan present in the bacteria cell wall. The activity of the soap against S. aureus could be attributable to the palm kernel oil present in the soap (Ugbogu, 2006). Ugbogu (2006) opined that palm kernel oil has inhibitory effect on S. aureus and Streptococcus sp. The major fatty acids in palm kernel oil used for the production of black soap are lauric acid, myristic acid and oleic acid. This agreed with the work of (Bhattacharya *et al.*, 2007; Liao *et al.*, 1999) which also possesses the same antibacterial properties.

It was observed that, antibacterial activity of the soaps was enhanced by an increase in the concentration of the soaps, this correspond with the work of (Mann *et al.*, 2008) i.e. the higher the concentration of the plant extract the greater the zones of inhibition. This can only mean that the black soap believably induced antibacterial effects of both gram positive and gram negative bacterial through the leakage of intracellular materials which is demonstrated in its membrane damaging action. This is at equivalence with past findings of Aliyu *et al.* (2012) and Adebajo *et al.* (2004) that antimicrobial activities of the black soap on bacterial is due to its oil contents with disruptive actions on cell wall

The Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentration MBC showed that the black soaps and the various decoctions in this research are generally effective antibacterial soaps. All black soap decoctions had MIC and MBC that range between 25 mg/ml and 50 mg/ml for all the test pathogens. This reveals that the concentration used in this research was able to inhibit the organisms rather than killing them. i.e. Bacteriostatic at lower concentration and bactericidal at higher concentration, this correspond with the work of (Abalaka et al., 2008).

CONCLUSION

This study has revealed that African black soap decoctions prepared with different concentrations of cam wood, lime water and aloe vera could have a better synergetic effect in inhibiting different pathogenic bacteria compared to the use of the soap only, therefore, could have potential ability in improving the antimicrobial qualities of the soap during usage. Therefore, mixing with the soap during production by local industries can improve its antimicrobial quality.

Hence, government should improve the quality and acceptability of the soap and encourage it large scale production to help in creating job opportunities for youths, thereby, reducing poverty and improving standard of living in African countries. Further, detailed study on this African black soap is necessary to determine the toxicity, side effects, and stability and standard dosage and usage forms.

REFERENCES

Abalaka, M. E., Daniya, S. and Mann, A. (2010). Evaluation of the antimicrobial of two ziziphus species (*Z. mauritania* and *Z. spinachristii*) on some microbialpathoges. *African journal of pharmacology and pharmacy. Vol.* **4**(4) *pp* 135-139 *April 2010.*

Adebajo, M.,O., Akanni, M. S. and Frost, R. L.(2004). Effect of Palm Kernel Oil Extraction Method on the Electrical Conductance of Nigerian Traditional Soaps in Alcohols. *Journal of Surfactants and Detergents*, **7**(1), 81-85.

Adewuyi, G. O., Egbedo, O. and Babayemi, J. O. (2008). Evaluation of ten different African wood species for potash production. *International Journal of Physical Sciences* **3**; 63-68.

Ahmed, O. A., Odunukwe, N. N., Akinwale, O. P., Raheem, T. Y., Efienemokwu, C. E., Ogedengbe, O., and Salako, L. A. (2005) Knowledge and practices of traditional birth attendants in prenatal services in Lagos State, Nigeria. *African Journal of Medical Science*, **34** (1), 55-58.

Ahmed, O.A., Odunukwe, N.N., Akinwale, O.P., Raheem, T.Y., Efienemokwu, C.E. and Ogedengbe, O., (2005). 'Knowledge and practices of traditional birth attendants in prenatal services in Lagos State, Nigeria'. *African Journal of Medicine and Medical Sciences*, **34**(1), 55–58.

Ajaiyeoba, E. O., Oladepo, O., Fawole, O. I., Bolaji, O. M., Akinboye, D. O., Ogundahunsi, O. A., Falade, C. O., Gbotosho, G. O., Itiola, O. A., Happi, T. C., Ebong, O.

O., Ononi, I. M., Osowole, O. S., Oduola, O. O., Ashidi, J. S., and Oduola, A. M. (2003). Cultural categorization of febrile illnesses in correlation with herbal remedies used for treatment in south western Nigeria. *Journal of Ethnopharmacol*, **85** (2-3), 79-85.

Ajaiyeoba, E.O., Oladepo, O., Fawole, O.I., Bolaji, O.M., Akinboye, D.O. and Ogundahunsi, O. A. (2003). 'Cultural categorization of febrile illnesses in correlation with herbal remedies used for treatment in Southwestern Nigeria', *Journal of Ethnopharmacology*, **85**(2–3), 179–185.

Ajose, F. O. (2007) Some Nigerian plants of dermatologic importance. *International Journal of Dermatology*, **46**(11), 48-55.

Akinpelu, D. and Kolawole, D. O (2004) Determination of minimum inhibitory concentration. *African journal of Microbiology* **5**(10)59.

Al-Doori, Z., Morrison, D., Edwards, G. and Gemmell,C. (2003). Susceptibility of MRSA totriclosan.*Journal of Antimicrobial Chemotherapy* 51: 185-186.

Aliyu, M. S., Tijjani, M. B., Doko, M. H., Garba, I., Ibrahim, M. M., Abdulkadir, S. M., Abba, D. and Zango, U. U. (2012). Antimicrobial Activity of Sabulun Salo a Local Traditional Medicated Soap. *Nigerian Journal of Basic and Applied Science* **20**(1), 35-38.

Arya, M., Arya, P., Biswas, D. and Prasad, R. (1966). 'Antimicrobial susceptibility pattern of bacterial isolates from post-operative wound infections', *Indian Journal of Pathology and Microbiology* **48**(2), 266–269.

Atata, R., Sani, A., and Ajewole, S.M. (2003). Effect of stem bark extract of Enantia chloranta on clinical isolates. Biokemistri, **15** (2): 84-92

Barrow, G.I. and Felthan, R. K. (1993).*Cow and Steel's Manual Identification of Medical Bacteria*.(3rdEd), Cambridge University Press.

Bauer, W. M., Kirby, J. S. and Sherris, M. (1996) Agar well diffusion method. *American Journal of Clinical Pathology*. **45**: 439-496.

Bella, O. (2008). African Black Soap http: // www.bellaonline.com / article/art 26546.asp.

Bhattacharya, S., Mula, S., Gamre, S., Kamat, J.P., Bandyopadhyay, S.K. and Chattopadhyay, S. (2007). 'Inhibitory property of Piper betel extract against photosensitization-induced damages to lipids and proteins', *Food Chemistry*, **100**: 1474–1480. https://doi.org/10.1016/j.foodchem.2005.12.041 Cheesbrough, M. (2000-2009) District Laboratory Manual for Tropical Countries Part2, Cambridge University Press 62-78 & 178-194.

David, O. (2005). Brief History of black soap and ingredients (plantain skin), http: // www.Oakfordavid.com.

Fuls, J.L., Rodgers, N.D., Fischler, G.E., Howard, J.M., Patel, M., Weidner, P.L. et al., 2008, 'Alternative hand contamination technique to compare the activities of antimicrobial and non-antimicrobial soaps under different test conditions', *Journal of Applied & Environmental Microbiology*, 74(12): 3739–3744. https://doi. org/10.1128/AEM.02405-07

Getradeghana, B. T., (2000). Evalution of African traditional soap. *Global journal of pure and applied sciences* **6**, 174-179.

https://doi.org/10.1016/S0378-8741(02)00357-4 Ajose, F.O., 2007, 'Some Nigerian plants of dermatologic importance', *International Journal of Dermatology*, **46** (Supple 1), 48–55. https://doi.org/10.1111/j.1365-4632.2007.03466.

Kareen, A. (2004). Black Soap Story: Traditional soap making in Ghana http:// <u>www.yendordrums.com</u>.

Kuehl, B.L., Fyfe, K.S. & Shear, N.H. (2003). 'Cutaneous cleansers', in Skin Therapy Letter **8**(3), pp 1–4

Larson, E., Eke, P.I., Wilder, M.P. and Laughon, B.E (1987). Quantity of soap as a variable in hand washing, infection control **8**, 371-375.

Liao, Y.L., Chiang, Y.C., Tsai, T.F., Lee, R.F., Chan, Y.C. and Hsiao, C. H. (1999). 'Contact leukomelanosis induced by the leaves of Piper betle, L. (Piperaceae): A clinical and histopathologic survey', *Journal of The American Academy of Dermatology* **40**: 583–589. https://doi.org/10.1016/S0190-9622 (99)70441-X

Mann A. Yahaya Y. and Boson G. O (2008). Phytochemical and antibacterial screening of annogeissus leiocarpus against some microorganism associated with infectious wound. *African Journal of Microbiology Research*. **2**: 060-062.

Moody, J.O., Adebiyi, O.A. & Adeniyi, B.A., 2004, 'Do Aloe vera and Ageratum conyzoides enhance the antimicrobial activity of traditional medicinal soft soaps (Osedudu)?', *Journal of Ethnopharmacology*, **92**(1): 57–60. https://doi.org/ 10.1016/j.jep.2004.01.018.

Osborne, R.C. & Grube, J., 1982, 'Hand disinfection in dental practice', *Journal of Clinical Preventive Dentistry* **4** (6), 11–15.

Spence and Spencer (2004). Determination of Minimum Inhibitory Concentration and Minimum Bactericidal Concentration. http //www.qlaboratories.com Minimum Bactericidal Concentration.

Toshima, Y., Ojima, M., Yamada, H., Mori, H., Tonomura, M. and Hioki, Y. (2001). 'Observation of everyday hand-washing behavior of Japanese, and effect of antibacterial soap', *International Journal of Food Microbiology*, **68**(1–2): 83–91. https://doi.org/10.1016/S0168-1605(01)00481-0

Treehugger. (2008). what is Black soap? Http: //<u>www.treehugger.com/style/</u> what is black soap.html.

Ugbogu, O.C., (2006). Lauric acid content and inhibitory effect of palm kernel oil on two isolates and C. Albican, *African Journal of Biotechnology* **5**(11), 1045-1047.

Underwood, (2008). What is black soap? Viewed 15 January 2017, from https://www. treehugger.com/style/what-is-black-soap.html.