

Antimicrobial properties of prepared Neem oil extract based medicinal oily bar soap

Ameena Parveen^{1,2**}

¹Department of Physics, Government First Grade College, Gurumitkal, Karnataka, India

²Department of Physics, SC/ST Residential Government First Grade College, Mudnad, Yadgir – 585205, Karnataka, India

ABSTRACT

The standard bar soap and medicinal soap were prepared by neem oil continuous process. The prepared soap characterized by XRD for structural analysis of scribe and surface morphology was studied by TEM images. It is observed from XRD that the orthorhombic structure of montmorillonite clay and hexagonal wurtzite with cubic zincblende structure of incorporated ZnO in medicinal soap. TEM images shows the nanostructured ZnO and MMT clay embedded in the medicinal soap homogeneously and its size is around ~7 nm of ZnO and around ~22 nm of MMT clay respectively. The standard bar soap was also prepared by blending 1:4 ratio of coconut oil and vegetable ghee. These blends of standard bar soap and medicinal soap with small amount of MMT clay, carboxy methyl cellulose (CMC), and ethylene diamine tetra-acetic acid (EDTA) have shown high foam length of 41 ml for standard bar soap, 45 ml for medicinal soap and 42 ml for commercial soap respectively. The oil interaction indicates the formation of emulsion and forms precipitate with salts such as $MgCl_2$ and $FeCl_3$. The antimicrobial activity of the prepared soap was studied against bacteria especially *E. coli*. The soap with medicinal value was highly effective against *E. coli* and has high area of inhibition of 24 mm vertical diameter and 23 mm horizontal diameter compared with Lifebuoy soap.

Keywords: Coconut oil, Carboxymethyl cellulose, Antibacterial agent, *E. coli*

INTRODUCTION

Soap is sodium or potassium salts of fatty acids are composed of a long chain of hydrocarbon with carboxylic acid group at one end has ionic bond with metal ions such as sodium or potassium [1]. There are several factors which affect the soap making process such as quality of oil, amount of sodium hydroxide and essential oils used in the fabrication of soap. In continuous process the fabrication of soap is influenced free fatty acid content of the oils, heating temperature, starting time and speed [2]. The most common oils and fats used to prepare soap are coconut oil because of its easy hardening even though it contains high volume of moisture contents compare to other vegetables oils. The vegetable fats mixed with oils in proper ratio can reduce the water retain capacity of coconut oil during saponification and soap quality can be improved significantly [3, 4].

Soap is used every day and it plays vital role in maintaining the health, skin nourishment and freshness of our body. Hence, it is important to manufacture multifunctional soap that can have not only cleaning properties but also should contains cosmetic and medicinal values. It is well know that the many natural ingredients have medicinal and cosmetic values which is low cost, easy to extract, good aroma and does not have any side effects. Many natural ingredients such as Neema oil which contains various essential fatty acids, triglycerides and vitamin E which can be penetrate into the skin and can be heal the micro cracks and wounds on the skin [5 - 7]. We have used other oils such as Moringa oil aand black cumin oils to prevent and curing of disease such as pyoderma and constipation [8]. Aloe vera and Eucalyptus oil extracted oils have multi-benefits that relive skin irritation, rejuvenate, promoting skin circulation, healing wounds, and easing muscle tension in harsh environment [9]. Some of the oils such as Rosemary oil and Olive oil have large cosmetic values that including its ability to reduce inflammation, improve skin tone and clarify skin, improve blood circulation [9, 10]. Therefore, authors have prepared the various standards oily bar soap and medicinal soap by continuous process using different essential and fixed oils. The prepared soaps were characterized by Fourier transmission infrared spectroscopy (FTIR) and X-ray diffraction (XRD) for structural analysis. Further, different test were conducted in comparison with commercial Lifebuoy (medicinal soap) soap.

MATERIALS AND METHODS

All the chemicals are used for preparation of soaps are Analytical Research (AR) grade. Sodium hydroxide, Magnesium chloride, Ferric chloride, Carboxy methyl cellulose (CMC), Ethylene di-amine tetra-acetic acid (EDTA), Ethanol, Zinc oxide, Sulphuric acid, Phenolphthalein, Saturated sugar solution, montmorillonite clay (MMT clay) and perfume were procured from Sigma Aldrich, India. The MMT clay was active by sulphate process before using for soap preparation [11]. Coconut oil (parachute double filtered oil was procured from Parachute oil. Pvt. Ltd, India), Neem oil (99.99 %), Olive oil (99 %), Aloe vera (99.99 %), Black cumin (98 %) and Eucalyptus oil (95 %) were procured from Bioresearch and agro innovation, India, Sheno (high quality vegetable ghee), Natural massage oil (99.99 %, Bioresearch and agro innovation, India) with rosemary oil (99.99 %, Bioresearch and agro innovation, India), Skin care with thyme lavender and frankincense oil (98 % Bioresearch and agro innovation, India), Moringa sesame oil, Argo for arthritis and gout (95 % pure) from locally available market.

Preparation of standard bar soap

25g of high quality vegetable ghee (sheno) was weighed in to 100ml beaker and allowed to melt on hot plate. This was allowed to cool to room temperature and mixed with 98.8g coconut oil in 1litre beaker with stirring to ensure the complete blending of the components. The sodium hydroxide solution was prepared by dissolving 12g NaOH pellet in 37 ml distilled water in 250 ml volumetric flask with continuous shaking to ensure complete dissolution and allowed to cool to room temperature prior to introduce in to the oil mixture. The mixture in 1000 ml beaker was put on hot plate and mixed with previously prepared sodium hydroxide solution and then heated at 60 °C for 45 minute with continuous stirring using mechanical stirrer. Further to the above solution, 0.25g EDTA, 0.625g clay, 0.625g of ZnO and 0.3125g carboxy methyl cellulose (CMC) were slowly added and allowed for the saponification process. When thick homogenous mixture formed, it was removed from heater and allowed to cool to room temperature [12]. Later, the prepared soap solution was casted (molded) in to small polyethylene container and kept in dark place for 3 days to ensure complete saponification and hardening of soap as shown in figure 1 (a, b). Finally, the harden bar soap was removed from the mold and transferred to polyethylene bag as shown in figure 2 and cured for 15 days in order to ensure complete consumption of sodium hydroxide solution by fatty acid [13].

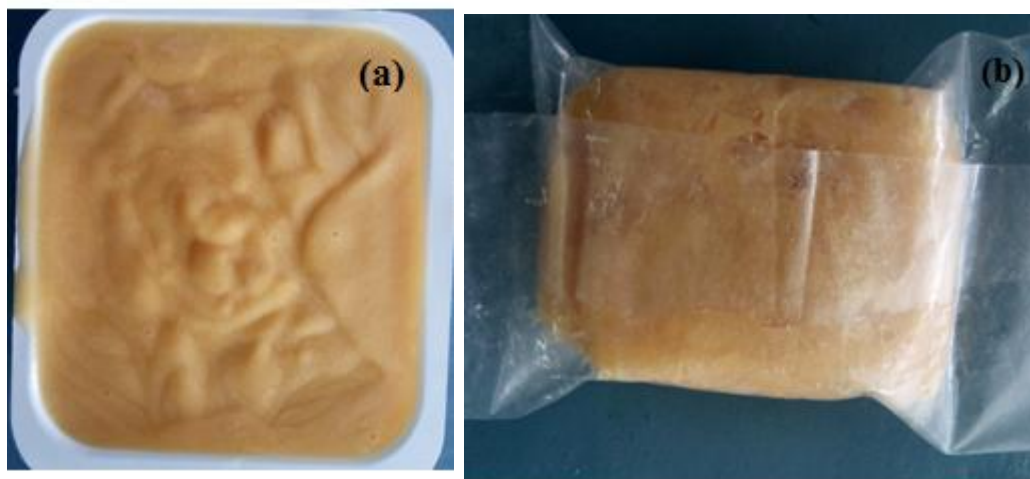


Figure 1 (a) show the soap solution molded (casted) in polyethylene container and (b) shows the soap packed in polyethylene bag for curing

The same procedures were followed to prepare the different standard samples in various compositions designed using soap making calculator software (SMC software) as indicated in table 1.

Table 1 the proportion of ingredients used in production of standard bar soap

Soap type	Name of ingredients					
	Composition	Vegetable ghee	Coconut oil	clay	CMC	EDTA
bar soap	In percentage (%)	20	79.05	0.5	0.25	0.2

Sample 1	In gram (g)	12.5	43.5	0.3	0.16	0.13
Sample 2	In gram (g)	16.7	65.91	0.42	0.21	0.17
Sample 3	In gram (g)	25	98.8	0.625	0.3125	0.25

Preparation of medicinal soap

The same procedure was followed to prepare medicinal soap. In this case however, 87.5g coconut oil was used to be mixed with 25g vegetable ghee. The soap was made medicinal by adding different composition of fixed and essential oils according to the proportion listed in table 2. Essential and fixed oils were added to the soap solution at the point where the temperature of the mixture was reduced from 60 °C to 35 °C during saponification process because some of the essential and fixed oils are volatile in nature. A 97% ethanol was used as solvent and perfume was added to enhance the odor of the soap. Molding and curing process is also similar to that of the standard bar soap.

Table 2: the proportion of ingredients used in production of medicinal soap with cosmetic value

Name of ingredients	Medicinal soap			
	Composition in Percent (%)	Composition in gram (g)		
		Sample 1	Sample 2	Sample 3
Vegetable ghee	20	12.5	16.70	25
Coconut oil	70	43.75	58.36	87.5
Neem oil	6	3.75	5.00	7.5
Rosemary oil	1	0.63	0.83	1.25
Eucalyptus oil	0.3	0.20	0.25	0.38
Aloe vera	0.125	0.10	0.12	0.16
Olive oil	0.125	0.10	0.12	0.16
Thyme, lavender & frankincense oil	0.25	0.16	0.21	0.31
Moringa sesame oil	0.25	0.16	0.21	0.31
Argo for arthritis and gout	0.25	0.16	0.21	0.31
Black cumin	0.25	0.16	0.21	0.31
Zinc oxide	0.5	0.3	0.42	0.63
Clay	0.5	0.3	0.42	0.63
EDTA	0.2	0.13	0.17	0.25
CMC	0.25	0.16	0.21	0.31

Characterizations of prepared soap

The characterization of prepared soaps has been carried out to continuous process were analyzed by Rigaku Miniflex X-Ray Diffractometer (RM-XRD) with Cu K α as source of radiation at 30 kV and mA is used for montmorillonite (MMT) clay and ZnO structure in the soap analyzed. The morphology of the pure MMT clay, ZnO and medicinal soap (sample 3) was investigated using transmission electron microscopy (TEM) (JEOL-2010).

Three separate soap solution were prepared to perform tests on soap in comparison with commercial soaps as follows: (a) 1g of standard bar soap was taken in to 100ml beaker and dissolved with 50ml warm deionized water. This solution was swirled well to mix it and properly labeled. (b) 1g of commercial soap was dissolved with 50ml deionized water in 100ml beaker and swirled well until all it completely dissolved. The content of the beaker was labeled and (c) 1g of medicinal soap solution was mixed with 50ml warm deionized water and swirled and labeled properly [14].

The two commercial soaps were used to compare their properties with the prepared soaps. These are Kris beauty soap and Lifebuoy medicinal soap. Kris Beauty soap is high quality coconut oil based soap with following ingredients Salicylic acid, Lactic acid, Tranexamic acid and Kojic acid, Glucono-delta-lactone, Sodium Lactate, Tetrasodium EDTA, Tocophenyl acetate and Butylated hydroxytoluene. Since Lifebuoy soap has high medicinal value because it has following ingredients such as Sodium palmate, Sodium palm kernelate, Aqua, NaCl, Tetrasodium EDTA, Tetrasodium etidronate, Limonene and Linalool. It was used only for antimicrobial effectiveness tests. The following tests were performed to compare prepared soap properties with commercial soap and detergent.

pH test

Three separate measuring cylinders were taken and labeled properly. 10ml of standard bar soap solution, 10ml of commercial soap solution and 10ml of deionized water were taken in to 1st, 2nd and 3rd measuring cylinder respectively. One by one each solution was stirred with stirring rod and then the stirring rod was touched to pH paper and the pH of the solution was recorded. The pH value of the soap solution was also measured by immersing electrode of pH meter in to the solution. This solution was saved for the next part [15].

Foam test

The solutions taken in measuring cylinder for pH test was properly supported from top and bottom and shaken continuously for 20 seconds. Then the amount of suds (foam) each soap solution produces was observed and recorded. This solution was saved for the oil interaction test.

Oil interaction test

The interaction of soap solution and oil was tested by placing 5ml of oil into each of measuring cylinder containing soap solution and deionized water in part II. Then each cylinder was properly supported from the top and bottom and shaken continuously for 20seconds. The amount of foam formed in each cylinder was measured and compared. Finally the result was recorded and the solution was discarded in to sink.

Hard water test

As it was done for oil interaction test, three separate measuring cylinders were taken and labeled properly. 5ml of standard bar soap solution, 5ml of commercial soap solution and 5ml of medicinal solution were taken in the 1st, 2nd and 3rd measuring cylinder respectively. 20 drops of 1% MgCl₂ solution was added to each test tube and each test tube shaken continuously for 20 seconds. Then the result was compared by observing whether the soap form precipitate with Mg²⁺ and the amount of suds formed was recorded. The same procedure was repeated with 20 drops of 1% FeCl₂ solution and the result was then recorded and compared [16].

Basicity test

Soap with free alkali can be very damaging to skin, silk, or wool. Small piece (0.5g) of prepared soap was dissolve in 15 mL of ethanol and then two drops of phenolphthalein indicator was added. The presence of free alkali was noted by observing whether the soap solution form pink color with indicator [17].

Determination of the number of moles of NaOH reacted with fatty acid by titration

50ml burette was prepared and rinsed twice with 0.2M NaOH solution. Then the rinsed burette was filled with 0.2M NaOH solution. 2g of prepared soap was accurately weighed in to 250ml Erlenmeyer flask. To this, 50ml of ethanol was added and heated to boil on hot plate set at medium temperature. By carefully holding the flask with tongs the mixture was cautiously swirled until the soap dissolves. The hot fatty acid-ethanol solution was then titrated with standard 0.2M NaOH solution by adding 5 drops of phenolphthalein indicator.

The solution was continuously swirled as the titrant added and the titration was terminated when faint pink color appeared. The reading of the volume of burette was recorded at the end of titration and the number of moles of NaOH needed to react with the same number of moles of fatty acid was determined [18].

Antimicrobial effectiveness test

0.072g of Mueller-Hinton Agar (MHA) was weighed in to 250 ml conical flask and dissolved with 72ml distilled water. This was covered with aluminum foil and sterilized in autoclave for 15minute at 121^oC together with wire loop, forceps, measuring cylinder and three petri-dish. Then 24ml of the sterilized MHA solution was transferred to each of petri-dish and kept for 5 minute until it become solid. One colon of pure E.Coli culture was taken with sterilized wire loop and inoculated in to the prepared agar media. Then the colon was dispersed with the sterilized glass spreaders in each petri-dish and each of petri-dish was pierced by pressing with cork and bore [19].

Three soap samples (Standard bar soap, Medicinal neem soap and Lifebuoy commercial soaps) were taken and soap solutions were prepared by taking 2.8g of each of soaps. The soap was dissolved in 9 ml of distilled water and each was stirred until it completely gets dissolved using stirring rod. 0.5 micro liter of each of soap solution was dropped in to the petri-dish containing E.Coli through the three holes. Finally the inoculum was kept for 24hrs in fume hood and the zone of inhibition was measured.

RESULTS AND DISCUSSIONS

X-ray diffraction (XRD) pattern

Figure 2 shows that the X-ray diffraction (XRD) pattern of Lifebuoy soap and medicinal soap (sample 3). It is observed that the Lifebuoy soap is amorphous in nature and does not contain any particles in it which can be acts as scribe for the body or with antimicrobial agents of inorganic oxides as shown in figure 2 (a). Figure 2 (b) shows the XRD pattern of medicinal soap (sample 3) prepared by continuous process. The characteristics peaks observed at 7.6° and 54.6° corresponds to (001) and (102) for the orthorhombic structure of montmorillonite clay (MMT- clay) and 38.29° , 39.6° , 42.1° , 63.4° and 69.2° which are corresponds to (100), (002), (101), (110) and (103) planes indicates the hexagonal wurtzite and cubic zinc blend structure of incorporated ZnO in medicinal soap [20].

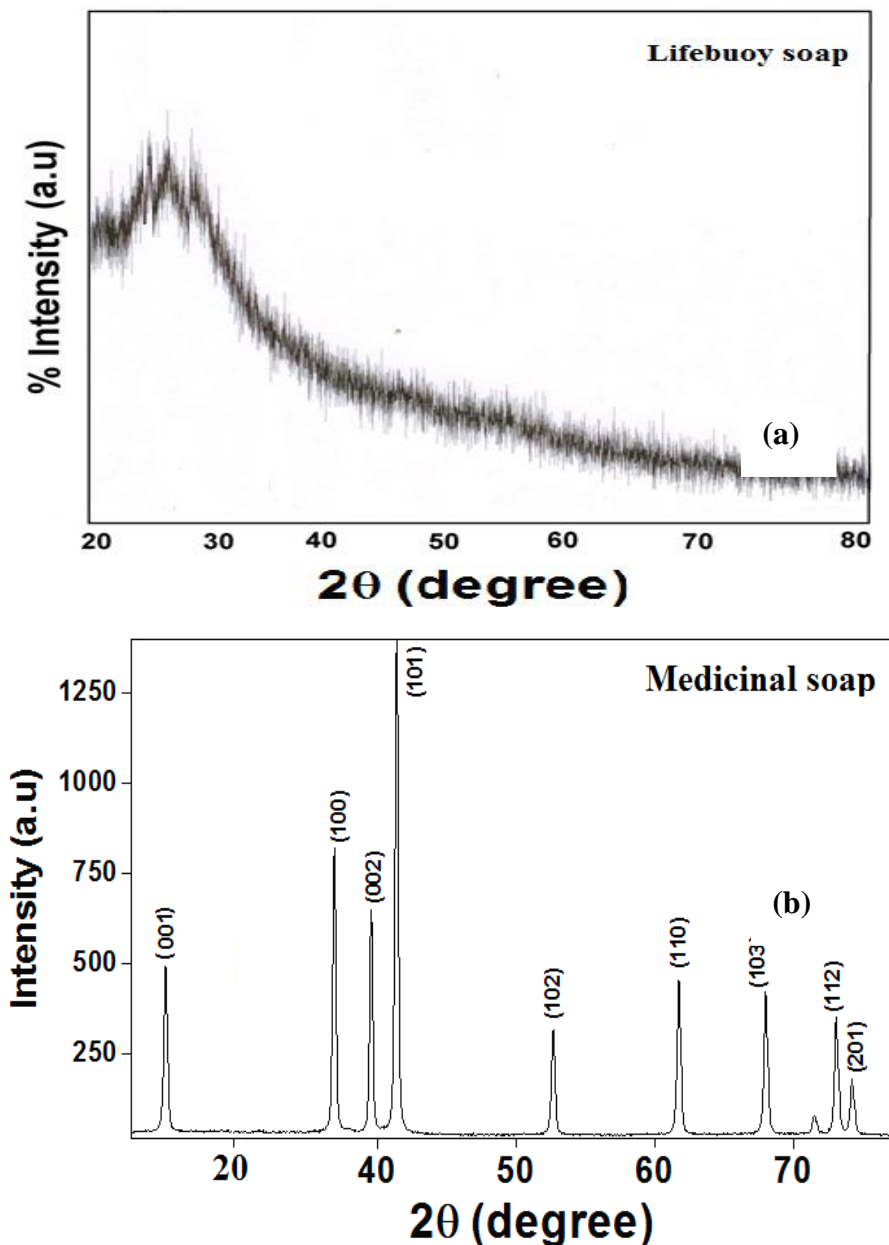


Figure 2: shows the XRD pattern of (a) Lifebuoy soap and (b) medicinal soap prepared in lab (sample 3).

Transmission electron microscopic (TEM)

Figure 3 shows that the Transmission electron microscopic (TEM) images of (a) zinc oxide, (b) MMT clay and (c) Medicinal soap (sample 3). It is observed that the ZnO is spherical in shape formed very indusial particles having particles size around ~ 7 nm which help as vehicle to carry the herbs extract to the bacteria surface to disintegrate its epidermal surface as shown in figure (a). It is also interesting to note that ZnO itself has good antimicrobial activity as reported in our earlier work [21]. The MMT clay shows higher particle size of ~ 22 nm and which are well connected each other due to the

SO⁴⁻ ions on its surface as shown in figure (b). In figure (c) it is observed that the ZnO surface connected with the MMT clay may be due to the electrostatics charges and it is important to note that the dense fixed and essential oils are surrounding to the nanoparticles that could helps in the antimicrobial activity of the medicinal soap.

Soap is sodium salts of fatty acid produced by saponification process. In this project both standard bar soaps and medicinal soaps were prepared by incorporating different types of essential and fixed oils in various proportions. The prepared soaps were characterized by comparing its properties with commercial Kris beauty soap and Lifebuoy soap. The major soap characterization tests include pH, foaming, oil interaction, interaction with hard water, basicity (alkalinity) and antimicrobial activity tests. The following table 3 summarizes the result obtained from pH tests of the soap solutions [22].

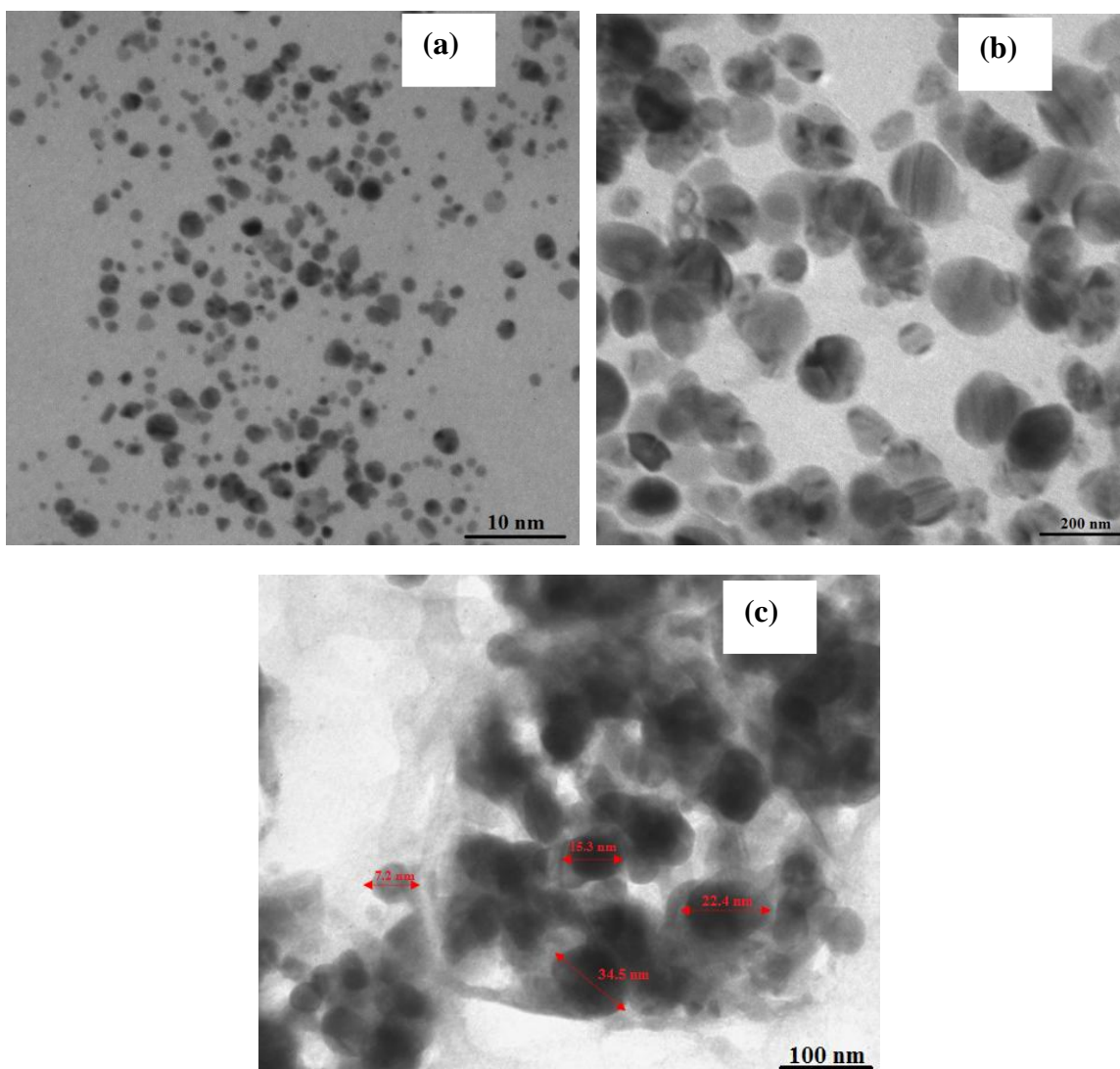


Figure 3: shows the TEM images of (a) ZnO and (b) MMT clay and (c) medicinal soap (sample 3).

pH value

The pH value of the soap show that the prepared soap was relatively basic when compared to the commercial Kris beauty soap, this is may be due to the excess unreacted NaOH present in the saponificated fatty acid as shown in the table 3. Since saponification is very slow process, it requires longer time for the fatty acid to completely react with NaOH solution in the soap mixtures.

Table 3 the pH value of different soap solutions and their interaction with pH papers

Soap types	Interaction with red litmus			pH value		
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3

Prepared lab soap solution	Blue color	Blue color	Blue color	10.9	11.3	10.4
Medicinal soap solution	Blue color	Blue color	Blue color	10.3	9.9	10.1
Lifebuoy soap solution	Blue color	Blue color	Blue color	10.2	10.2	10.2
Kris beauty soap solution	Blue color	Blue color	Blue color	10.2	10.2	10.2

Foaming /leathering

The result obtained from foaming, oil and hard water interaction and basicity (alkalinity) test was shown in figure 4. The foam length of water with prepared standard soap, medicinal soap in comparison with Lifebuoy commercial soap was measured. It is observed that the foam length of the medicinal is 32.5 ml and the prepared standard bar soap shows moderate foam length of 31 ml in compare with commercial Lifebuoy soap as shown in figure 6. This is due to the presence of carboxy methyl cellulose which acts as eye lubricants keep the eye moist, help to protect the eye from injury and infection, and decrease symptoms of dry eyes such as burning and itching. However, the foam length of the standard bar soap and medicinal soap shows lower foam length then detergent as normally desired.

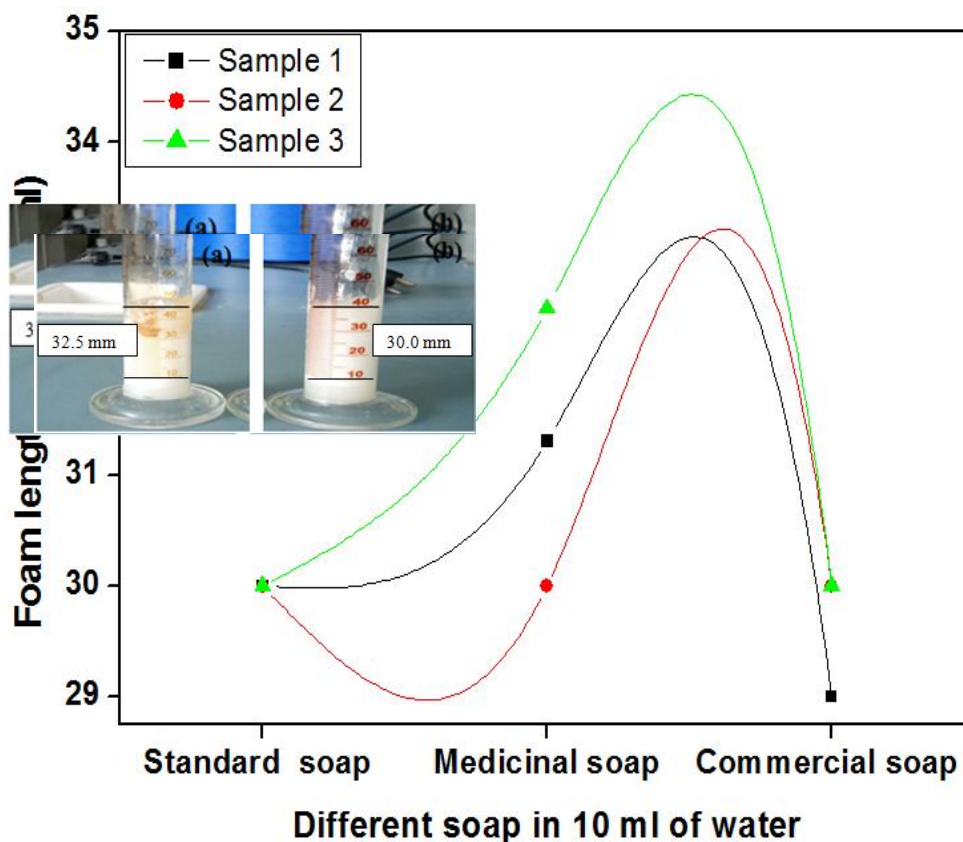


Figure 4: shows that the foam length of standard bar soap (sample 3), medicinal soap (sample 3) prepared in laboratory in compare to Kris beauty soap

Interaction with oil

From table 4, it is observed that the soap made from coconut oil has good interaction with oil to form foam (leather) compared to other commercial kris beauty soaps. It is observed that the foam length of the prepared medicinal soap (sample 3) was 32 ml which is higher than the commercial Kris beauty soap whose foam length is 30 ml after shacking for 20 s. The highest foam length of the prepared soap is mainly due to the presence of Carboxymethyl cellulose (CMC) which has high foaming capacity when used in soap solution [23]. When 5ml of oil was added and shaken for 20 seconds, both prepared and commercial soap solution emulsified the oil as summarized in the table 5. Soap has both non-polar hydrocarbon end and polar cationic end. As result, soap has ability to emulsify oil and it can also cable to react with polar substances.

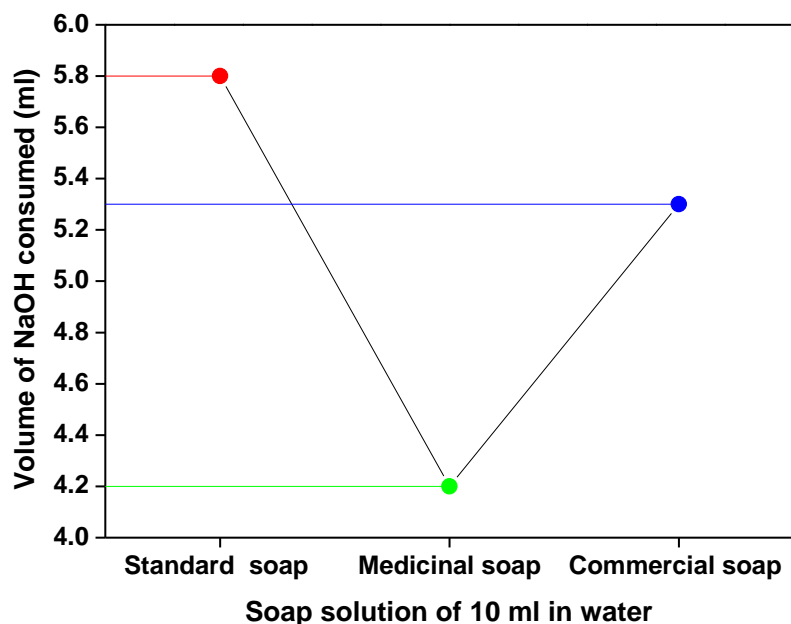


Figure 5: shows that the comparison of volume of NaOH consumed by standard bar soap, medicinal soap (sample 3) and Kris beauty soap

Table 4: Shows the oil interaction with various prepared soaps and commercial soap

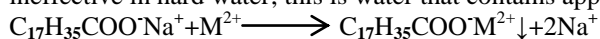
Soap types	Interaction with oil		
	Sample 1	Sample 2	Sample 3
Standard bar soap	Emulsified the oil	Emulsified the oil	Emulsified the oil
Medicinal soap	Emulsified the oil	Emulsified the oil	Emulsified the oil
Lifebuoy soap	Emulsified the oil	Emulsified the oil	Emulsified the oil
Kris beauty soap	Emulsified the oil	Emulsified the oil	Emulsified the oil

Table 5: Show the interaction of hard water with prepared soap and commercial soap

Soap types	Interaction with 1% MgCl ₂			Interaction with 1% FeCl ₃		
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
Standard bar soap	Very little ppt formed	Very little ppt formed	Very little ppt formed	No ppt formed	No ppt formed	No ppt formed
Medicinal soap	Very little ppt formed	Very little ppt formed	Very little ppt formed	No ppt formed	No ppt formed	No ppt formed
Lifebuoy soap	No ppt formed	No ppt formed	No ppt formed	No ppt formed	No ppt formed	No ppt formed
Kris beauty soap	No ppt formed	No ppt formed	No ppt formed	No ppt formed	No ppt formed	No ppt formed

Salt test

Soap has been largely replaced by synthetic detergents, because soap has two serious drawbacks. One is that soap becomes ineffective in hard water; this is water that contains appreciable amounts of Ca²⁺ and Mg²⁺ salts.



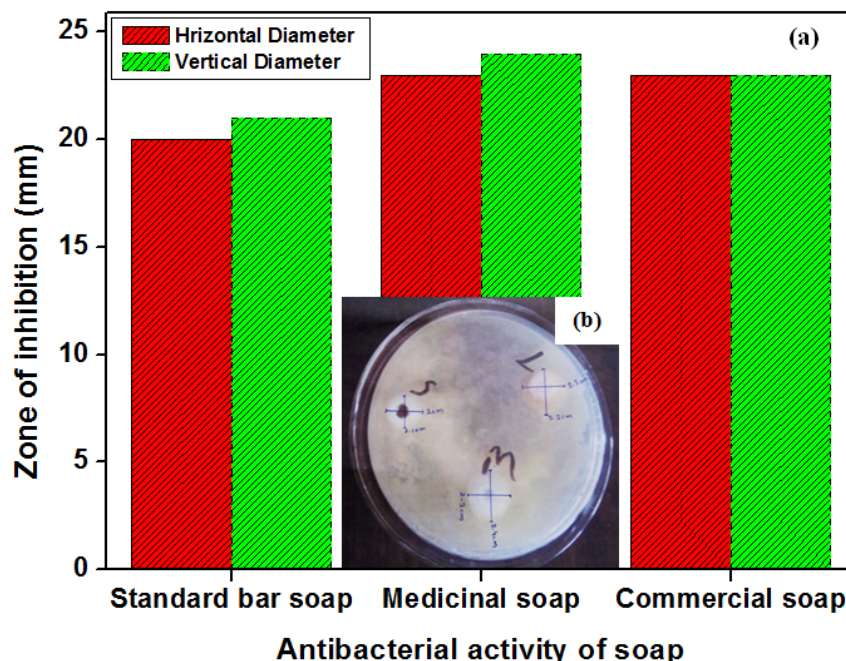


Figure 6: Shows the zone of inhibition of standard bar soap (sample 3), medicinal soap (sample 3) prepared in laboratory in compare to commercial Lifebuoy soap

CONCLUSION

Soap was prepared using all varieties of oils including that of all the various blends of oils. The soap was tested for various properties and compared with commercial soap and detergents. The prepared soap characterized by XRD for structural analysis of scribe and surface morphology was studied by TEM images. It is observed from XRD that the orthorhombic structure of montmorillonite clay and hexagonal wurtzite with cubic zincblende structure of incorporated ZnO in medicinal soap. TEM images shows the nanostructured ZnO and MMT clay embedded in the medicinal soap homogeneously and its size is around ~7 nm of ZnO and around ~22 nm of MMT clay respectively. The standard bar soap made with 1:4 ratio of coconut oil and vegetable ghee has high foam length. Its foam length reading was 32.5 ml in measuring cylinder which is higher than that of commercial Kris beauty soap with 30 ml height. All standard bar soap, medicinal and cosmetic soaps were fully emulsified the oil and had good interaction with salts that can causes hardness in water. Since soap is sodium salts of fatty acids, it is basic and turns red litmus paper to blue. The pH value of prepared standard bar soap, prepared medicinal soap and commercial soap were 10.9, 10.4 and 10.2 respectively which satisfies the requirement of most commercial soaps with pH value ranges from 9 to 11. The prepared medicinal soap was also effective against bacteria such E. coli. It has highest area of inhibition when compared with commercial and standard bar soaps. Their areas of inhibition were 23 mm, 23 mm, 20 mm horizontally and 24 mm, 23 mm, 21 mm vertically for medicinal, Lifebuoy soap and standard bar soap respectively. Therefore, the soap made from coconut oil is hard enough, has high cleaning power and incorporating different proportion of essential and fixed oil give the soap medicinal value.

REFERENCES

- [1] Bassett, I.B., Pannowitz, D.L., Barnetsm, R.S, Med. J. Aust, 153 (1990) 455-8
- [2] Biswas K, Ishita C, Ranajit K B, Uday B, Current Science, 82 (2002)1336-1345
- [3] Buck D.S, Nidorf D M, Addino J G, J. Fam. Pract., 38 (1994) 60-605
- [4] Gata-Goncalves L, Nogueira J M F, Bruno de Sousa O M R, J Photochem. Photobiol. B: Biol. 70 (2003) 51-54
- [5] Holetz F B, Pessini G L, Sanches N R, Cortez D A G, Nakamura C V, Filho B P D, Rio de Janeiro, 97 (2002) 1027-1031.
- [6] Karen W M, Edzard E, J. Antimicrob. Chemother. 51 (2003) 241-246
- [7] Moses, N. N., James, A. M., Pierre T., Vincent P.K. T., Afri.JTrad. Compl. Alt. Med. 3 (2006): 84-93.
- [8] Pulok K M, Kakali M, Rajesh K M, Pali M, Phytother. Res., 17 (2003) 265-268
- [9] Ravikumar P H S, Makari H K, Gurumurthy H, J. Environ. Agri. Food Chem. 6 (2007) 2318-2332
- [10] Kareru, P.G., Gachanja, A.N., Keriko, J.M. and Kenji, G.M., Afri. J Trad. Compl. Alt. Med. 5 (2008) 51-55

- [11] Syed Abusale Mhamad Nabirqudri, Aashis.S.Roy, M.V.N.Ambika Prasad, Journal of Materials Research, 29, (2014) 2957-2964
- [12] Mathabe M C, Nikolova R V, Lall N, Nyazema N Z, J Ethnopharmacol. 105 (2006) 286-293.
- [13] Meléndez P A, Capriles V A, Phytomed. 13 (2006) 272-276.
- [14] Millogo-Kone, Guissou I P, Nacoulma O, Traore A S, Afri.JTrad. Compl. Alt. Med.3 (2006) 74-78
- [15] Abdulkarim, S. M, Lai, O. M, Muhammad, S. K. S, Long, K and Ghazali, H. M Food Chem. 93, (2005). 253-263.
- [16] Simon W.J. Gould, Mark D. Fielder, Alison F. Kelly, Declan P. Naughton, Comp. Alt. Med. 9 (2009) 23-27
- [17] M. H.Gordon, I.A.Rahman, 68 (1991) 574–576
- [18] WarraAa, Hassan Lg, GunuSy, Jega Sa. Nigerian Journal of Basic and Applied Science. 18 (2010) 315–321.
- [19] Williams D F, Schmitt W H, Chemistry and Technology of Cosmetics and Toiletries Industry, 1st edition, Blackie Academic Press, Glasgow, (1992)123-125.
- [20] Aashis. S. Roy, A. Parveen, Raghunandan Deshpande, Ravishankar Bhat and K. R. Anilkumar, Journal of Nanoparticle Research, 15(2013) 14:1337
- [21] Wongthongdee N, Inprakhon P, Science Asia; 39 (2013) 477–485.
- [22] Wijetunge W.M.A.N.K, Perera B.G.K, International Journal of Pharmacy and Biological Sciences, 6 (2016)7-16
- [23] A. O Ameh, J.A Muhammad and H. G Audu, African Journal of Biotechnology, 12 (2013) 4656-4662
- [24] E. E. Mak-Mensah and C. K. Firempong, Asian Journal of Plant Science and Research, 1 (2011) 1-7
- [25] VioricaPopescu, AlinaSoceanu, Simona Dobrinasi, Gabriela Stanciu, DanutTiberiuEpure, Food Industry, 12 (2011) 257 – 261
- [26] Venubabu Thati, Aashis. S. Roy, M.V.N. Ambika Prasad, C. T. Shivannavar and S.M. Gaddad, J Biosci Tech, 1 (2010), 64-69.
- [27] Aashis. S. Roy, Ameena Parveen, K. R. Anilkumar and M.V.N.Ambika Prasad, J. Biomaterials and Nanobiotech, 1, (2010), 37-41