Optimisation of Antiseptic Soap Production from a Blend of Neem Seed Oil and Eucalyptus Oil

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***Abstract*: - In this work, Neem oil and eucalyptus oil were mixed in various proportions and used in preparing soaps which were subsequently characterized. The combination of neem oil to eucalyptus oil considered were 100:0, 90:10, 80:20,70:30, 60:40, 50:50, 40:60, 30:70 20:80 10:90 and 0:100 (wt:wt). The physical properties of the prepared soap including hardness, foamability and pH were analyzed. The antibacterial properties of the prepared soaps in terms of sensitivity, minimum inhibitory concentration and minimum bacterial concentration were analyzed. The antimicrobial properties of the prepared soap in terms of sensitivity, minimum inhibitory concentration (with respect to staphyylococcus aureus and Staphylococcus epidermidis) indicated that the properties observed from the use of the blending ratio 20:80 correlates with that gotten from the commercial soap sample and clearly agrees with world health organization standard for antiseptic soap.**

I. INTRODUCTION

Soap is a product formed from saponification reaction, where esters are split into alcohol and salts. Saponification is more widely used in general terms as alkaline hydrolysis of ester. Soap is sodium or potassium salt of fatty acid produced by saponification reaction using sodium or potassium hydroxide. Based on its chemical properties as an anionic surface active agent (surfactant), soap is used to clean and wash skin and clothing. The fatty acids, stearic, palmatic, myristic, lauric and oleic acids, contribute to lathering and washing properties of the soaps (Alander, 2004: Maranz et al., 2004), other oils such as lard and tallow from animal sources, coconut, palm oil and olive oil are the commonest plant oil used in soap production (Okumura and Nishikawa, 1996).

Palm oil has been widely used as fatty raw material in the manufacture of soap. The chemical characteristics of soap depend on several factors: the strength and purity of alkali, the kind of oil used, completeness of saponification and age of the soap. Such chemical characteristics include moisture content, total fatty acids (TFA), pH, free alkali, and percent chloride. Soap is a substance of ancient origin, the manufacture of which according to the literature (Akhter et al, 2008) has evolved from primitive beginning into a sophisticated chemical process. There are two main processes of soap production they include cold process of soap production, and hot process of soap production. Cold process soap (commonly referred to as "CP" soap) is made by combining fatty acid and sodium hydroxide together. Fatty acid can be almost any oil. Cold process soap making is a combination of an art and science. The condensed version of this type of soap making is that there is a certain proportion of lye (sodium hydroxide) and water to fatty acid that form a chemical reaction called "saponification. During saponification, the oil and lye (sodium hydroxide) mixed and form soap while in the hot process of soap making there is variation on the cold process method. Hot process soap is an interesting take on the cold process method. The simple explanation is that we take the entire ingredient and add it all together into a pot or a container that can be place over a fire source e.g. stove and stir frequently until the soap goes through various stages. The excess water is evaporated off and the soap is ready for use once cooled. The use of conventional sources of oil for the production of soaps example PKO, Coconut etc has begun to take its negative effect on the economy Nigeria as a case study to be precise, and this is because this conventional oil is eatable oil so when the demand for consumption for this oil rises there eventually will a drop in production of soap generally, and the effect of this on the economy will surely lead to a drop in the country's GDP (gross domestic income). Furthermore, these oils have been used for long period of time so the tendency that new microorganisms that won't be affected by the oils that is for antiseptic soap production would have been formed making humans susceptible to these microorganisms. The questions that always come to the mind of a is the way forward, and that is what has fueled the desire for the use of non-conventional forms of oil for the production of not just antiseptic soap, but all forms of soap generally.

Neem oil, extracted from the seed of neem plant (Azadirachta indica) is reported to contain natural organic antimicrobial agents, largely used in the Indian sub-continent in traditional/folk medicine. Upadhyay et al. (2010) reported neem oil to be highly bactericidal. Neem oil has been used in the treatment of inflammation, pain and swelling that occur in arthritis (Subapriya et al., 2005). When properly used, the oil combats vaginal infection and sexually transmitted diseases and kills lice (Abdel-Ghaffar and Semmler, 2007). It is used

in the treatment of skin diseases such as scabies, ringworm and athlete's foot and also in the treatment of phyto-phthora infestans, (Mirza et al., 2000) etc. Mak-Mensah and Firempong (2011) prepared toilet soap using neem oil and suggested that due to the phytoconstituents in neem oil and the favorable chemical characteristics of the soap, it can be used as medical and cosmetic toilet soap as also reported by Warra (2012).

Eucalyptus is a tall, evergreen tree, native to Australia and Tasmania, successfully introduced worldwide, now extensively cultivated in many countries. Eucalyptus oil has antibacterial effects on pathogenic bacteria in the respiratory tract. Inhaled eucalyptus oil vapor is a decongestant and treatment for bronchitis. Cineole controls airway mucus hyper secretion and asthma via anti-inflammatory cytokine inhibition.Pre-clinical results also show that eucalyptus oil stimulates innate cell-mediated immune response by effects on the phagocytic ability of human monocyte derived macrophages. Eucalyptus oil also has anti-inflammatory and analgesic qualities as a topically applied liniment ingredient.

With the uncontrollable increase in the population of the world and the use of an eatable source of oil (PKO) for the production of antiseptic soap and an increase in human activities such as indiscriminate dumping of refuse, release of untreated waste in the environment by industries. the increase in harmful organisms have become more rampant there by leading to an increase in skin infection and competition for the oil by the soap industries and food industries. So there is need to produce a more effective antiseptic soap whose oil is not consumable so as to control these skin infections.

The aim of this research is to produce an antiseptic soap using a blend of eucalyptus oil and Neem seed oil.

With the production of a cheaper and effective antiseptic soap, germs will be controlled and humans will have an opportunity to live a safer and tranquil life. Also eucalyptus oil and Neem seed oil when blended serves in preventing the inflammation of skin cells and due to their high level of water content they help in keeping the skin well moisture.

The project is aimed at varying the blending ratio of both oils to find the best set of blend that satisfies the world health organization standard for antiseptic soap production and the oil in question are the Neem oil and eucalyptus oil.

II. EXPERIMENTAL

2.1 Materials and Reagents

The Neem seed oil was bought from National Research Institute for Chemical Technology Zaria (Kaduna state) and the eucalyptus oil was bought from quality skills limited mararaba Nasarawa state. The reagents and materials used in the production and characterizations of the antiseptic soap includes; Chloroxylenol, Sodium Silicate, Sodium Tripolium Phosphate, Soda Ash, Sodium Sulphate, Calcium Carbonate, Methylparaben, Perfume, Color, Glycerin, Sodium Hydroxide.Others includes; Measuring Cylinder, Stop Watch, Weighing Balance, Stirrer, Bunsen Burner, Filter Paper, pH Meter, mould, Aluminum Foil.

2.2 Soap preparation

100ml of the blended oil was measured into a plastic container that is 100:0 of neem to eucalyptus oil. 50ml of the fermented NaOH solution was weighed. The caustic soda was stirred well using a stirring rod. The caustic soda was then poured gradually into the blended oil the mixture was stirred gently in one direction (laminar flow) to enhance thorough mixing of the solution. Then 10ml of each (Chloroxylenol, perfume, glycerin) were added respectively to the mixture and stirring was carried out until a homogeneous mixture was obtained. The plastic container which was insulated with pieces of cloths to prevent the fat from hardening before the soap mixes properly. Small amount of sodium sulphate (25g) and sodium silicate (20ml) were added into the soap, the mixture was stirred properly until it blended with every other chemical. Sodium sulphate helps in the binding of the soap chemicals and it induces the foaming ability of the soap. It is equally a binder and an extender. Sodium silicate hardens the soap: it eases removal of dirt and prevents deposition of dirt particles. 25g of Sodium tripolium phosphate which is a foaming agent is added, 25g of Calcium carbonate is added to increase the soap hardness and finally 5g of methylparaben is added to serve as a preservative all this are stirred and 2ml of color is applied to the soap for beautification. After this, the soap was molded into a proper shape and kept in a filter paper. This soap was then taken to an air oven to dry up the moisture. Before taken to the oven the weight of soap was taken.

The above method is followed to prepare soap using the two different blends of oils. The same procedure was followed for all the blended samples. The combination of Neem oil to eucalyptus oil considered were 100:0, 90:10, 80:20,70:30, 60:40, 50:50, 40:60, 30:70 20:80 10:90 and 0:100 (wt:wt). According to the method reported by Warra, (2009).

Neem oil and eucalyptus oil were mixed in various proportions and used in preparing soaps which were subsequently characterized.

2.3 Chemical and physical characteristics of the soap produced

2.3.1 Hardness test

To determine the hardness of the soap, a needle (6.4 cm in length: 1 mm in diameter) to which a lead fishing weight (130 g) was attached was lowered unto the soap, the distance into which the needle penetrates the soap, after 30 s, was recorded as a measure of its hardness. All this was done for the twelve samples.

2.3.2 Foamability test

2.00 g of the soap was dissolved in 50 ml of distilled water in a 100 ml measuring cylinder and shaken vigorously for 2 min.As reported by Isah, (2006) for synthetic detergent. It was allowed to stand for 10 min after which the height of the foam was determined. This was done for all twelve samples.

2.3.3 pH analysis

The pH values of the soaps produced were analyzed using a pH meter (Kent EIL 7055). 5.0 g of the produced soaps were dissolved in 100 ml of deionized water and the pH determined using the meter. This was done for all twelve samples as reported in literature (Dalen and Mamza, 2009).

2.3.4 Determination of soap sensitivity, minimum inhibitory concentration

The antibacterial properties of the soap samples were studied using two clinical isolates of bacteria, Staphylococcus aureus and staphylococcus epidermidis obtained from the micro biology laboratory federal polytechnic Nasarawa. From the slant culture of the test organisms, a colony was obtained and suspended into sterile distilled water. 10 g of each soap sample was weighed and dissolved in a sterile bottle containing 10 ml of distilled water, and stock solution (1000 mg/ml). The stock solution of the soap was used to prepare various concentrations of each soap sample (30, 20 and 10 mg/ml).

2.3.5 Sensitivity of the soap samples

The agar well diffusion method was used. 20 ml of freshly prepared Muller-Hinton agar was poured into sterile Petri-dishes and the agar was allowed to solidify. Using sterile syringe, 0.2 ml of each of the standardized organisms was inoculated into three Petri-dishes. Three (3) wells each of diameter 6 mm were made into each agar plate using a cork borer and the plate were labeled. Unto each plate, 0.5ml of appropriate soap dilution was placed in appropriate wells, that is, 100, 50 and 25 mg/ml, respectively. The plates were left for about 1 h for the soap to diffuse into the agar and then were incubated at 37^{o}C for 24 h. After incubation, the plates were observed for evidence of inhibition which will appear as a clear zone completely devoid of growth around the well (zone of inhibition). The diameters of the wells were measured using a calibrated ruler in millimeters. The experiment was performed in duplicates and the mean of the zone of inhibition computed for all twelve sample

III. RESULTS AND DISCUSSION

Table 3.1 Nomenclature of Neem oil (N) to Eucalyptus Oil (E) Samples

Parameter	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
Ratio(N:E)%	100:0	90:10	80:20	70:30	60:40	50:50	40:60	30:70	20:80	10:90	0:100

3.1.1 Properties of soap produced

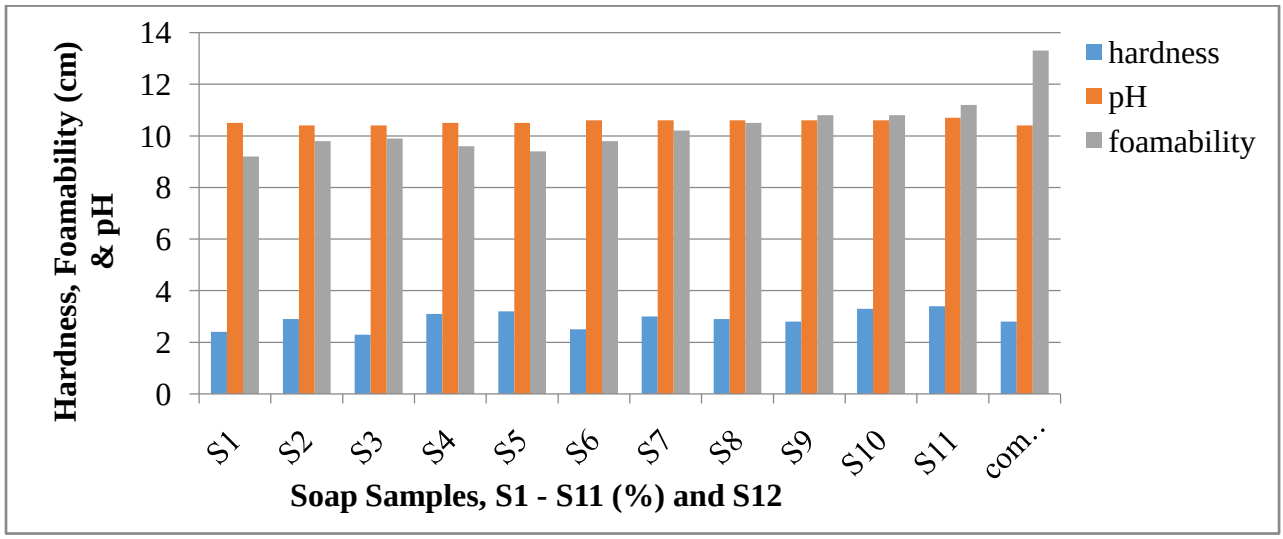

Figure 3.1: Chart of Hardness, pH and Foamability Analysis of Soap Samples

Figure 3.1 displays the result of Hardness, pH and formability tests of the soap produced (S1 to S11) as compared to the commercial one (S12) used as control. The measure of the hardness of each soap sample is dependent on the amount of free alkali present in it (Hassan et al, 2005). The effect of the alkali can be nullified by adding at least 10% excess fat which will help stabilize the hardness of the mixture that is when the soap is excessively hard, for the case of the soap been too soft it is recommended that an additional calculated amount of the alkali be added.

From the result obtained, S2, S8 and S9 had penetration value of 2.9cm, 2.9cm and 2.8cm which clearly agreed with penetration value of 2.8cm for the commercial sample. These values obtained were found to be within the range of acceptable standard for antiseptic soap hardness as given by NAFDAC. The rest of the samples have penetration deep outside the standard range given. From the analysis, it was observed that the characteristic of the oil used played a major role in the hardness of the soap produced. The ratio of oil used exhibited various degrees of hardness but the most preferable blend when compared to others was sample eight which had a penetration of 2.8cm, same as that of commercial one. Other soap samples which had penetrations of less than 2.5cm which included sample one and three were neither characterized as soft or hard which made their result with respect to hardness inconclusive. Other blend that had penetrations of more than 2.9 was characterized as hard soap which may be hard on the skin as directed by the World Health Organization.

Soap being salt of strong base and weak acids should be weakly alkaline in aqueous solution. But soap with free alkaline (pH 11 -14) can cause damage to skin. Hence pH of 9-11 is considered as low level by NAFDAC, a regulatory Agency in Nigeria (Umar, 2002). The commercial sample had a pH of 10.4 which is in line with the WHO standard. The first five samples have pH ranging between 10.4 and 10.5 which were found to be within the acceptable range as given by NAFDAC (Umar, 2002). As the ratio of the Eucalyptus oil increases, there is an increase in pH of the soaps as observed in samples 6 to 11 which clearly indicates that the eucalyptus oil is more basic than neem oil and hence controls the pH of the soap. Furthermore, the pH of all the soap samples produced where in agreement with the standard as given by NAFDAC and also in agreement with the commercial sample used as control. This makes the soap safe for use.

The foamability height produced by different soap samples can be explained based on the fatty acids composition of the oil used in the eleven soap sample formulation, it was reported that, lauric acid and myristic acid, which are all saturated fatty acids produces soap with fluffy and high foamability height (Phansteil et al 1998). However, the observed difference in the height of foam formed in each soap sample formulation may be due to the method use in the soap preparation in addition with the nature of fatty acid composition of oil. The height of the foam obtained from the control sample was 13.3cm. Sample one had a height of 9.2 which was a low height when viewed in line with the control sample and this could be as a result of the fatty acid composition of the oil. Sample 2 had a foamability height of 9.8cm which was better than that obtained from the first sample but never the less it was low when compared to that obtained from the control sample, a slight increase was recorded from sample three which had a had a height of 9.9cm, Sample four on the other hand had a slight variation in trend from the others with a foamability height of 9.4 so also sample five which had a height of 9.4cm this slight variation could be as a result of negligence. Sample six had a height of 9.8 which was an increase in height, sample seven had a height 10.2, which made us believe that the fatty acid composition for the eucalyptus oil was convenient for a good foamability. Sample 8 had a height of 10.5 which made our assumption a hypothesis: Sample 9 and 10 both had a height of 10.8cm finally sample 11 had a height of 11.2 which was the nearest to that obtained from the control. The fatty acid composition of the eucalyptus oil was observed to be better than that of the neem oil with respect to their formability levels.

3.1.2 Sensitivity analysis of soap with s. aureus

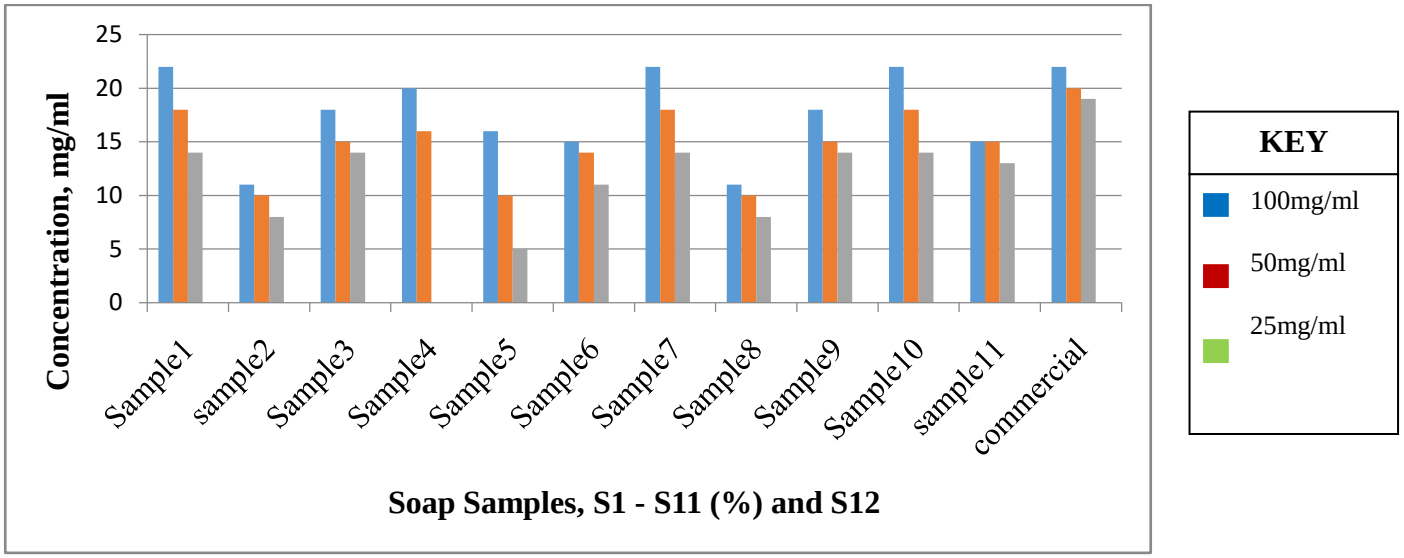

Figure 3.2 Sensitivity of Various Soap Samples to S. Aureus at Different Concentrations

3.1.3 Sensitivity analysis of soap with s. epidermidis

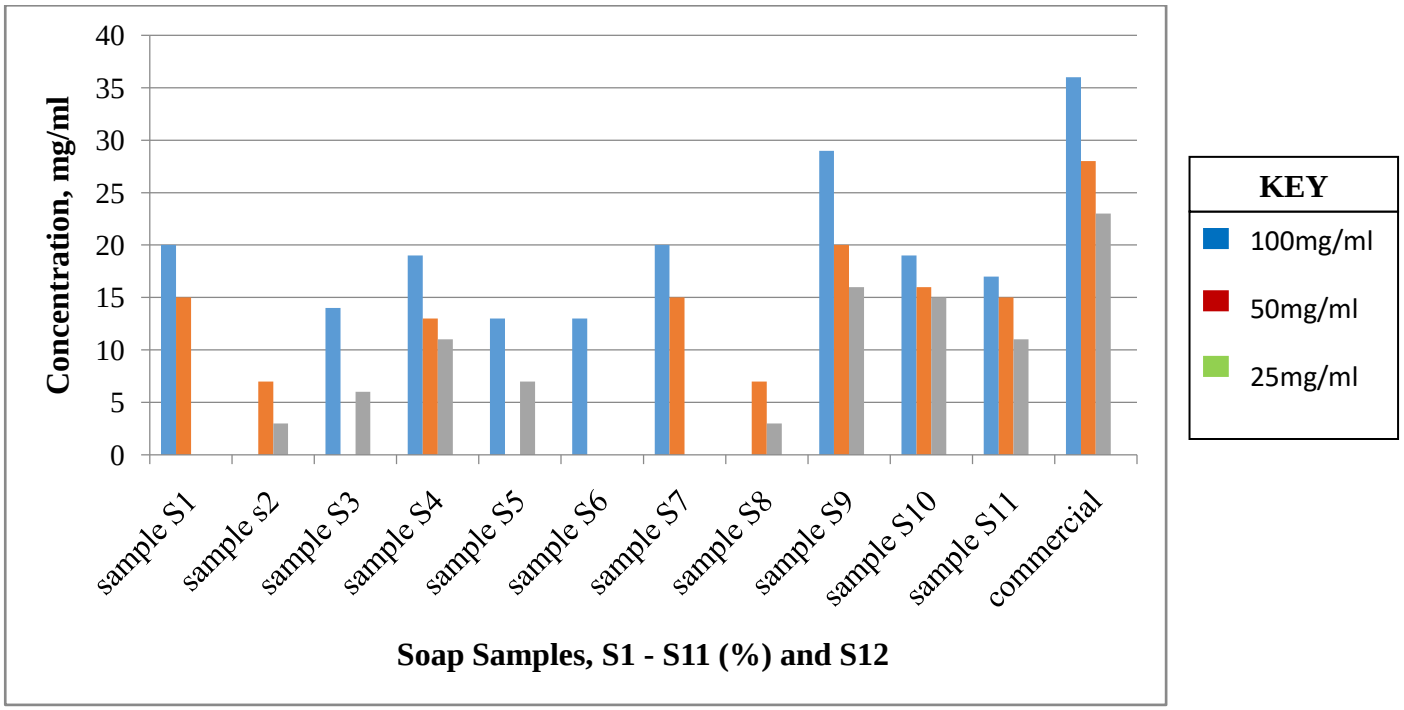

Figure 3.3 Sensitivity of Various Soap Samples to S. Epidermidis at Different Concentrations

Figure 3.2 and 3.3 shows the sensitivity of the two microorganisms (Staphylococcus aureus and Staphylococcus epidermidis) to various concentrations of the soaps. The commercial antiseptic soap (S12) recorded the highest zone of inhibition for all soap concentrations considered when used against S. aureus and S. epidermidis. The microorganisms (S. aureus and S. epidermidis) were similarly susceptible to all prepared neem/eucalyptus oil soaps (S1 to S11) but the zones of inhibition were generally lower than those for S9 and S12. It may also be observed that the zone of inhibition decreased with decreasing soap concentration. It may be inferred from the figures that the soap samples produced exhibited the greatest inhibitory effect on S. aureus and S.epidermidis even though not as high as that recorded for the commercial one and that the extent of inhibition was for all soap solution and was concentration dependent. The table also shows that the inhibitory effect on S.epidermidis improved with increasing eucalyptus oil concentration as S4 and S9 showed inhibition against the organism for all soap concentration considered.

A high value means that the micro organism is susceptible, that is, they can't grow if the soap sample is present. This means that the soap sample at that concentration is effective against the bacteria at that concentration.

A low value means that a higher dose of the soap sample is needed to prevent growth at that concentration.

R means that the organism is resistant to the soap sample present at that concentration.

3.1.4 Analysis of inhibitory concentration

Table 3.2:Minimum inhibitory concentration

Test organism	Concentration of S1			Concentration of S2			Concentration of S3		
	30	20	10	30	20	10	30	20	10
S.Aureus	-	MIC	-	-	+	+	MIC	+	-
S.Epidermidis	+	+	+	+	MIC	+	MIC	+	+

Test organism	Concentration of S4			Concentration of S5			Concentration of S6		
	30	20	10	30	20	10	30	20	10
S.Aureus	-	MIC	+	MIC	-	MIC	-	+	+
S.Epidermidis	+	+	-	-	+	+	+	+	-

Test organism	Concentration of S7			Concentration of S8			Concentration of S9		
	30	20	10	30	20	10	30	20	10
S.Aureus	MIC	MIC	-	-	-	MIC	-	-	+
S.Epidermidis	-	+	-	+	+	+	-	-	+

Test organism	Concentration of S10			Concentration of S11			Concentration of S12		
	30	20	10	30	20	10	30	20	10
S.Aureus	-	+	+	-	MI	MIC	-	-	+
S.Epidermidis	MIC	+	-	+	+	+	-	-	MIC

Table 3.2 shows the minimum inhibitory concentration test analysis of soap samples and it was observed that samples S9 had the highest effect on the two microorganisms and this agrees with that recorded for the commercial sample (S12).

IV. CONCLUSION

Soap from a blend of Neem seed oil and Eucalyptus oil was produced and characterized. All the soap samples produced exhibited various degree of satisfaction from the analysis carried out. Neem seed oil and Eucalyptus oil are a good blend for any good antiseptic soap production. The properties of the soap samples produced showed that most of the characteristics like pH, Hardness and formability agreed to what was recorded for the commercial sample used as control and the acceptable standard as given by NAFDAC.

The alkaline nature of the eucalyptus oil used help stabilize the pH of the soap produced which makes it suitable for use on human skin without fear of skin irritation or damage. Also, the medicinal properties of Eucalyptus and Neem oil greatly improved the quality of the soap produced as evident in the antibacterial test of the soap.

The antibacterial test carried out on the soap showed that the soap had high inhibitory effect on S. Aureus and S. Epidermidis and higher concentration of Eucalyptus oil favored the inhibition rate.

The blending ratio of Neem seed oil to Eucalyptus oil of 20:80 was found to be the best blending ratio for the antiseptic soap production as the characteristic of the soap at these ratios showed better result than the rest when compared to the properties of the control sample and the standard given by NAFDAC.

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