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FORMULATION AND ASSESSMENT OF THE ANTIMICROBIAL PROPERTIES OF SOAP FORMULATED FROM THE OILS OF *HARUNGANA MADAGASCARIENSIS* AND *CYPERUS ESCULENTUS* SEEDS

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ABSTRACT

Soap is a source of fatty acid useful in variety of forms as surfactants, thickeners, components of some lubricants and precursors to catalysts. The study is aimed at the formulation and evaluation of soap made from extracted seed oils of *H. madagascariensis* and *C. esculentus* plant. Four batches of formulations were made and identified as: batch A soap (*C. esculentus* oil alone), batch B soap from (*H. madagascariensis* oil), batch C soap from oils of (*C. esculentus/ H. madagascariensis* (3:1) and batch D (*C. esculentus/ H. madagascariensis* (1:3). The products of the various batches were compared for efficacy and antimicrobial activity with standard (Dettol soap). Formulation of the soap involved simple mixture of fats and oils with a base (NaOH) by process of continuous stirring of the mixture, transferring to a mold for curing and allowing for completion of the saponification process. Using the agar diffusion assay, zones of inhibition of some strains of micro-organisms including bacteria and fungi were determined. The soap formulations were evaluated for various physicochemical properties such as appearance, pH, percentage free alkali content, foam height, moisture content, total fatty matter and alcohol insoluble content. The pH of the product from the various batches ranged between 10.3 to 11.5 and this is comparable to dettol soap. The results of the antimicrobial analysis of batch C showed clear zones of inhibition ranging between 12mm and 13mm for *E.coli* and *S.aureus* and that for batch D showed clear zone of inhibition between 10mm and 15mm respectively for *E.coli* and *P.aerugnosa* while the results found with other formulations (batches A and B) showed low activity. Results of the physicochemical properties of all the batches had % free alkali content ranging between 0.26 – 0.29, moisture content 4.65- 5.51%, total fatty matter 0.25-0.28g, alcohol insoluble content 18-21g and foam height of 7.5- 9.5cm. From the study, product of batch D, seems promising by showing appreciable physicochemical characteristics with better emollient property and antibacterial activities comparable to that obtained with commercial products than the other formulations.

KEYWORDS: *Harungana madagascariensis*, *Cyperus esculentus*, Formulation, Assessment, oil extracts, Soap.

INTRODUCTION

Plant derived lipids are important in nutrition, health, cosmetology and as biofuels. Oil is one of the oldest form of natural herbal medicine, with majority being those sourced from plants. These oils are either used solely or in combination with other forms of medicinal components example powders, for the treatment of illness and diseases. In the pharmaceutical industry oil is used in the manufacture of emulsions, suppositories, ointment, creams and so on, while in cosmetic it is used to formulate hair and body cream, lotion and soaps in other to improve appearance of the skin.[1]

Oils are major constituents of natural origin such as plants, animals and algae. They could be obtained from the cotyledons of *H. madagascariensis* which appears as broadly spathulate, with margins marked with dark 'oil' glands which looks dark but visible in transmitted light.

The seeds of *C. esculentus* has also been found to contain about 20-36% oil and this is suggested as potential oil crop for the production of biodiesel. A study found that chufa as it is often called, produces about 1.5 metric tonnes of oil per hectare based on a tuber yield of 5.67 t/ha and an oil content of 26.4%. The oil of the tuber was found from studies to contain 18% saturated (palmitic acid and stearic acid) and 82% unsaturated (oleic acid and linoleic acid) fatty acids.[2]

PHARMACEUTICAL FORMULATION

Pharmaceutical formulation is a combination of several chemical substances including the active ingredients to

produce a finished dosage form. It is a mixture of pharmaceutically active with inactive ingredients (excipients).

Formulation types is dependent on the type of active ingredient, including its physicochemical properties, compatibility and route of administration hence Pharmaceutical formulations can be divided into enteral, parenteral and topical based on the route of administration.[3]

The topical Formulation involve those intended for application on the body surface (skin) and mucous membrane. They include powders (finely divided solid), ointments (mixture of oil and water with oil being in higher proportion), lotions, pastes (mixture of oil, water and powder), foam, gel (this in contact with skin liquefies in presence of alcohol as solvent) and creams (emulsion of oil and water in almost equal proportion). In essence, a formulation can only be termed a product when the different ingredients have been mixed together in the right proportions to give a homogenous and elegant finished product.

SOAP

Soap is a source of fatty acid used in a variety of cleaning and lubricating products. In the domestic setting, soaps are surfactants usually used for washing, bathing, and other types of housekeeping but in industries, they are often used as thickeners, components of some lubricants and precursors to catalysts. When used for cleaning, soap solubilizes particles and dirts, which can then be separated from the material being cleaned. In handwashing, soap is used as a surfactant which when lathered with a little water, kills microorganisms by disorganizing their membrane lipid bilayer and denaturing their proteins.[5] It also emulsifies oils, enabling them to be carried away by running water. Soap is formulated by mixture of fats and oils with a base and it has excellent cleansing and good biodegradable property whereas some special metallic soaps made from heavier metals can be used as additives in polishes, inks, paints and lubricating oils.

Skin Care

Skin care is a range of practices that support skin integrity, enhance its appearance and relieves skin conditions. They include nutrition, avoidance of excessive sun exposure and use of appropriate emollients. Practices that enhance appearance includes the use of soaps, cosmetics, botulinum, exfoliation, fillers, laser resurfacing, microdermabrasion, peels, retinol therapy and ultrasonic skin treatment. Skin care is a routine daily procedure in many settings such as skin that is either too dry or too moist, and for prevention of dermatitis and skin injuries.[6]

Skin Infections

Skin infections occur when harmful germs (usually bacteria, fungi or virus) find their way into the body through a cut or break in the skin and are able to grow. Many skin infections respond well to simple, non – prescription remedies such as use of soaps but sometimes, a course of antibiotics and medical application is needed to treat them. Bacteria, fungi and virus are usually implicated for these skin infections. Like all harmful germs, they can be spread from person to person by skin to skin contact; and around the home on our hands, clothes, pets, food, and household objects.

Different types of such skin infections as: boils, cellulitis, ringworm and athletes foot (Tinea pedis) exists and the type, depends on the microorganism causing it.

Bacterial infection

Bacteria are living things which have one cell and are often seen with the aid of a microscope. They are ubiquitous and occur in several places including the skin surface as part of the composition of normal flora. Most bacteria flora in less than 1% would not cause any harm to the individual.[7] The majority of bacterial skin infections could be caused by Gram – positive bacteria example; Staphylococcus and Streptococcus species.

In countries like Nigeria where irrational drug use is common and bacteria have found several means to adapt gaining resistance, antibiotics are used empirically with consideration for resistance but some have proved futile in the fight against bacterial infections and hence more sources for commercial production of new moiety especially from natural sources is encouraged.

Also some bacteria like *Salmonella spp*, *Shigella*, *Staphylococcus aureus* and *Escherichia coli* amongst others have been found to have multi drug resistant strains hence the need for new remedies such as use of soap and new antibiotic evolving.

This search for novel moiety has led to the acceptance of plant and animal products which can combat issues like multi-drug resistant strains, leading to the discovery of new lead compounds or antimicrobial compound itself. Some methods involved in the detection/diagnosis of bacteria/bacterial infections includes Gram staining, Acid fast bacillus smear and culture, bacteria culture test, blood culture test, pro calcitonin test, protein electrophoresis, immuno-fixation, electrophoresis, immunoglobulin blood test and white blood cell in stool test. To carry out the assay, a couple of methods have been employed to assess the activity of new antimicrobial agents and these include; well diffusion, disk diffusion, broth and agar diffusion techniques.[8]

Fungi infection

Fungi are quite distinct from bacteria in size, cellular structure and chemical composition. They may contain one or more nuclei surrounded by a cell wall. Their life cycle varies from simple to complex and they may reproduce by both asexual and sexual method. Fungi can be divided into yeasts and moulds based on their physical

appearance.[9] Approximately 1-2% of the world's population is affected by dermatophytoses (superficial fungal infections of the skin) with skin fungi infection being the most common yet. Examples of fungi includes *Candida spp, Aspergillus, Histoplasma spp,* etc; and proliferation of these can be combated by topical medicinal components.

The study is aimed at the formulation and evaluation of soap from the oil extracts of *H. madagascariensis* and *C. esculentus* using varied ratios and in comparison for efficacy and antimicrobial activity with standard or known soap.

MATERIALS

Dettol soap, 1% ethanolic KOH, 0.5M hydrochloric acid, 0.1diethylether Hydroxide, carbon tetrachloride, potassium iodide solution, sodium thiosulphite solution, starch, glacial acid (SIGMAALORICH 2.5, LOT NO.83280, Germany), Standard glucose and boric acid, steric acid, (loba chemie, India), sodium hydroxide, sodium sulphate, sodium silicate, borax, sodium lauryl sulphate, kaolin, Cetostearyl alcohol, extracted oil, nutrient sugar(Titian biotech, India), pH Meter (Helmreasinn, PHS 25), Homogenizer (Master chef), Brookfield viscometer (DV2T, Thermostat hot water Bath (HH-6, Techmel and Techmel, USA), oven (scanfrost), refractometer (ABBE, Germany), conductometer (DDS-22c, Hanna instrument), and UV-spectrophotometer (Jenway, England), Muffle furnace, Cathode lamp autoclave, incubator, universal bottle, photo colorimeter, centrifuge (PEC Medicals, USA).

METHOD

Table 1: Formulae for Soap formulation.

Ingredients	Percentage composition			
	Batch A: (200g) (%w/w)	**Batch B**: (200g) (%w/w)	**Batch C**: (200g) (%w/w)	**Batch D**: (200g) (%w/w)
C.esculentus oil	44.4		33.19	11.08
H. madagascariensis oil		44.95	11.27	33.69
Sodium hydroxide	12.5	12.5	12.5	12.5
Sodium sulphate	6.25	6.25	6.25	6.25
Sodium silicate	2.4	2.4	2.4	2.4
Borax	1.25	1.25	1.25	1.25
Sodium lauryl sulphate	6.25	6.25	6.25	6.25
Kaolin	6.25	6.25	6.25	6.25
color	0.5	0.5	0.5	0.5
Fragrance	0.95	1.05	0.95	0.95
Water	19.25	18.60	19.25	19.25

Batch A: Soap from C. esculentus (200g) (%w/w), **Batch B**: Soap from *H. madagascariensis* (200g) (%w/w), **Batch C**: *C.esculentus*/Harungana soap 3:1 (200g) (%w/w) and **Batch D**: *C.esculentus*/Harungana/soap 1:3 (200g) (%w/w)

Formulation of soap

A 25g of sodium hydroxide was weighed and dissolved in a little quantity of water to achieve a specific gravity of 1.275g/ml (specific gravity of toilet bar soap). An 88.8 g of *C.esculentus* oil was weighed and introduced into a container using a small amount of it to dissolve the 1.0g of colorant. The NaOH solution was added to the oil with continuous stirring while 12.5g, of kaolin, sodium lauryl sulphate and sodium sulphate were added to the mixture with stirring and 1.904g of lavender fragrance was added with continuous stirring. The mixture was then transferred into a mold and allowed for 24 hours to solidify after which it was brought out of the mold. The soap was then kept undisturbed for 6 weeks to cure (allowed for full drying and completion of the saponification process).

This process was repeated for the soaps made with *H.madagascariensis*, *C.esculentus*/*H. madagascariensis* (3:1), and *H.madagascariensis*/ *C.esculentus* (3:1) imputing the proper proportion and composition of the ingredients appropriately.

Evaluation of formed soap

Organoleptic

The following physicochemical parameters were evaluated to confirm the quality of prepared formulations. Determination of clarity, texture, roughness, color and odor.

Clarity and color was checked by visual observation against white background, the odor was smelled and the texture and roughness checked by feeling with the index fingers.

pH Determination: 1.0g of the soaps were separately dispersed in 10ml of water and stored for two hours and pH determined using previously calibrated digital pH meter.

Determination of Percentage free Alkali content: About 10g of dry soap was weighed and transferred to a beaker containing 150 ml of distilled water. It was boiled under reflux on a water bath for 30 to 40 minutes to dissolve the soap. This solution was cooled and transferred along with the washings to the 250 ml conical flask and made

up to the volume with distilled water. 10 ml of the soap solution was taken in the titration flask and two drops of phenolphthalein indicator added. It was then titrated against 0.1M HCl until the solution turn colorless.[10]

Determination of Foam height: 0.1g of the soap made was weighed into a 50ml measuring cylinder and dispersed with 10 ml of distilled water. The mixture was vigorously shaken for 10 seconds and allowed to stand and the foam height was measured and recorded. This same procedure was repeated with soaps from all batches.[11]

Moisture content: 10g of soap sample was weighed immediately and recorded as "wet weight of sample". This wet sample was dried to a constant weight, at a temperature not exceeding $115^{O}C$ using the oven. The sample was cooled, weighed again and recorded as the "dry weight of sample". The moisture content of the sample was calculated using the equation.[12]

$$\% M = 100 \frac{(W - D)}{D} \times 100$$

% M = Percentage of moisture in the sample, W= Weight of wet sample (grams), and D = Weight of dry sample (grams).

Total fatty matter: 5.0 g of soap was accurately weighed and transferred into 250 ml beaker. 100 ml of hot water was added to completely dissolve the soap. 40 ml of 0.5 N Nitric acid was added until contents were slightly acidic. The mixture was heated in a water bath until the fatty acids were floating as a layer above the solution. The fatty acids were cooled in ice and separated while, 50 ml of chloroform was added to the remaining solution and transferred to a separating funnel. The solution was shaken and allowed to separate into two layers. The bottom layer was drained while 50 ml of chloroform was added to the remaining solution in the separating funnel. The Separated fatty acid dissolved in chloroform again as in the previous case and transferred to the collected fatty matter. The fatty matter was weighed in a pre-weighed dish. The content was allowed to evaporate and the residue weighed. From the difference in weight, the percentage of fatty matter in the given soap sample was calculated.[13]

Alcohol insoluble content: 5.0g of soap sample was dissolved in 50ml hot alcohol. The solution was filtered through a tarred filter paper with 20 ml warm ethanol and dried at $105^{O}C$ for 1 hour. The weight of dried filter paper was taken and percent alcohol insoluble matter calculated as:

$$\% \text{ alcohol insoluble matter} = \frac{\text{Wt. of residue}}{\text{Wt. of soap}} \times 100$$

Anti- microbial analysis

Preparation of Soap solutions: 1.0g of the soap was weighed and aseptically dissolved in 10ml of sterile water to prepare a 10% w/v soap solution. The mixture was used to carry out the antimicrobial analysis.

Preparation of Test organism solutions

5ml of sterile water was aseptically transferred into MacConkey bottle. Using a wire loop, a colony of *Pseudomonas aerugnosa* organism from culture medium was inoculated into the water in the bottle and shaken. The same method was used to prepare solutions of *Klebsiella pneumonia, Escherichia coli, Staphlococcus aureus* and *Candida albican.*

Agar diffusion assay: 0.1ml culture of test organisms was separately added to the nutrient agar, poured into the universal bottle prepared and mixed properly. The contents of the mixture was poured into a sterile petri dish and allowed to solidify. Using a sterile cork borer, 4 discs were removed from the agar layer in other to produce 4 wells in the agar plate. Using a sterile pipette, drops of the soap solution were added into the corresponding wells and the fourth well filled with control solution (Dimethyl sulphoxide). The plates were allowed to stand on the table top at room temperature for 15 minutes for proper diffusion of the soaps, then incubated at $37^{O}C$ for 24 hours. The diameter of zones of inhibition were measured through the base of the plate in millimeters.

Determination of percentage (%) chloride. 10 grams of soap was weighed and made up to 100 mL with distilled water and heated to dissolve the sample. The resulting solution was transferred into a 250 mL volumetric flask and 20 mL of 15% ($Ca(NO_3)_2$) added to it and shaken to dissolve the soap completely. Distilled water was added to the solution to make up to 250 mL mark. The solution was filtered and methyl red added to 100 mL of the filtrate. The solution was titrated against 10 N $H_2SO_4(aq)$ until a pink color was obtained. The resulting solution was again titrated against 0.1 $AgNO_3$ using $K_2Cr_2O_7$ as indicator till a brick red color was obtained. The % Chloride used was calculated as[15]:

$$\% \text{ Chloride} = \frac{\text{Titer volume}}{\text{Weight of soap}} \text{ X } 0.585$$

Emolliency test: This evaluates the occlusiveness of soap formulations. A 2.0 g portion of each soap formulation was smeared onto the surface of white sheets of paper over approximately 5 cm^2 surface area and left to stand on the laboratory shelf for 24 h after which the degree of translucency was graded into a three-level ranking: mild, moderate, or strong translucency.[16]

RESULTS

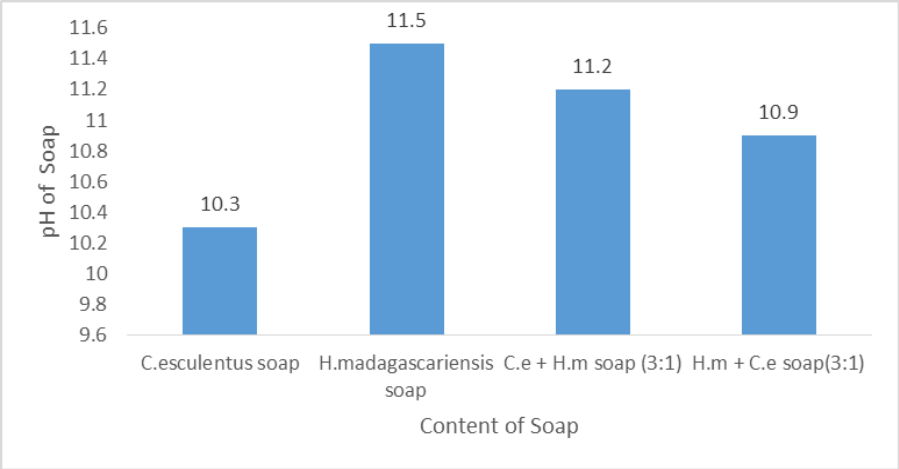

Figure 1: pH of Formulated Soap.

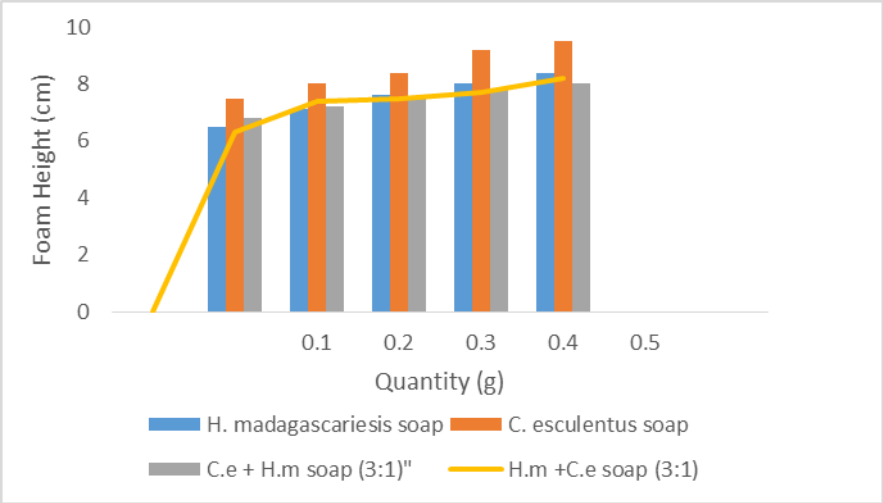

Figure 2: Foam Heights formed from the oils and the combination.

Organoleptic properties of soaps

Table 2: Physical characterization.

Soaps	Physical Characteristics			
	Colour	**Odor**	**Texture**	**Shape**
C.esculentus	milky	pleasant	rough	square
H. madagascariensis	Deep red	pleasant	rough	square
C.e + H.m (3:1)	Light brown	pleasant	smooth	rectangular
H.m +C.e (3:1)	Brownish red	pleasant	smooth	rectangular

Note: C.e = *Cyperus esculentus* and H.m = *Harungana madagascariensis*

Antimicrobial analysis of the soaps

Table 3: Zone of Inhibition of The Soaps.

Soaps	Zone of inhibition (mm)				
	E.coli	***P. aurugnosa***	***S. aureus***	***K. pneumonia***	***C. albican***
C. esculetus soap	8	-	7	3	-
H.madagascariensis soap	3	8	5	-	7
C,e + H.m (3:1) soap	12	5	13	9	3
H.m +C.e (3:1) soap	10	15	9	7	12

Table 4: Physicochemical analysis of formulated soaps.

Oil content	% Free alkali content	Foam Height (cm)	Moisture content (%)	Total fatty matter (g)	Alcoholic insoluble content
C.esulentus oil	0.26	9.5	5.51	0.25	18
H.madagascariensis oil	0.28	8.2	4.65	0.27	21
C. e + H.m 3:1 oil	0.27	7.8	6.21	0.26	18.5
H.m + C.e 3:1 oil	0.29	7.3	4.95	0.28	19

Emolliency Test

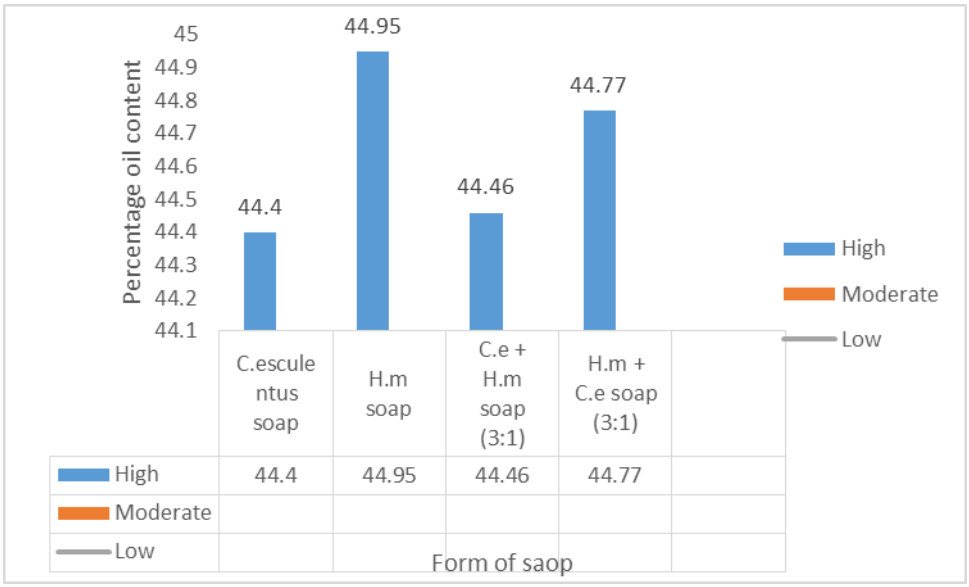

Fig. 3: Emolliency ranking of soap base formulation relative translucency produced on white paper.

DISCUSSION

Soap formulations were evaluated for various physicochemical properties such as appearance, pH, percentage free alkali content, foam height, moisture content, total fatty matter and alcohol insoluble content in which they exhibited satisfactory characters even as total fatty matter is the most important characteristics describing the quality of soap.

Emolliency test usually evaluate occlusiveness of the soap formulations. Occlusive agents such as the residual oils in soap formulation produce translucency on white paper. Therefore, the extent of translucency shows the relative amount of residual oils present in the soap sample after the saponification process. The result in Figure 3, illustrates this where the highest emolliency was observed with soaps containing high concentrations of *H. madagascariensis* oil, when used singly or applied in higher proportion at combined ratio. Emollients are occlusive, humectant and/or restorative in their mechanism of action. Occlusive agents build a thin skin surface film that prevents moisture loss, primarily due to the presence of natural oils and residual fatty matters. Topical products containing emollients can correct problems in skin scaling disorders and may also have suppressive effects on epidermal thickening, in addition to anti-inflammatory activity and transient relief from irritation.[17] Due to the possible benefit of the soaps contributing their moisturizing quality to the user's skin, probable function of glycerol (end-product of saponification reactions) in soap formulations was not separated from the formulated product.

The soaps from uncombined oil extracts appeared to have a brown colour suggesting the dominant colour of the plant sources. Overall, soaps containing the combined extracts of the plants show a better foam formation and stability. The skin has a pH range of 4 to 6. Skin products are expected to have pH as close to this range as possible in order to reduce irritation although the pH of the oil extracts (pH 5.4 and 5.6 for *H. madagacariensis* and *C. esculentus* respectively) was closer in value to the pH range for the skin.

Moisture content is a parameter that measures the shelf life of a product. High moisture content in soap would lead to reaction of excess water with un-saponified fat to give free fatty acid and glycerol in a process called hydrolysis of soap on storage. All the formulated soaps fall within the limits of 5-10% and the Free caustic alkali measures the abrasiveness of any given soap. This mostly results from improper or incomplete saponification. From the current analysis, the % alkalinities of the formulated soaps were similar and within 0.26 -0.29%. This shows that there are no free NaOH in the formulated soaps (table 4). The determination of percentage chloride levels in soap is important as excess amount causes soaps to crack. From

this study, the % NaCl for the formulated soaps remain within the limit of ≤0.75 set by official standards. The reason for a high chloride content of any soap may be caused by the use of chlorinated water used to dissolve NaOH pellets.[18]

The foams produced by the soaps was observed in overall to have stayed longer (indicating higher foaming capacity and stability) when left in distilled water than in tap water. This could be as result that tap water is often prone to containing divalent and trivalent metals which may reduce foaming and foam stability of the monovalent sodium soaps leading to the formation of water immiscible divalent soaps.[19]

Soap base containing only *H.madagascariensis* oil retain their foam much longer than that with only *C. esculentus* oil and that containing combination of the oils. As expected all the soap base formulations produced alkaline pH solutions as seen in figure 1, values of which decreased gradually over about 4 weeks of observation. Relatively high concentrations of NaOH used with low oil concentrations could result in higher pH of the soap base solutions

The emolliency results rank of soap base formulations showed a trend. It revealed that the relative translucency produced by the formulations bear a significant general correlation with overall concentrations of oil present in the soap base formulations (Figure 3). Consequently, most of the soap base formulations that produced strong translucency contained very high total *H.madagascariensis* oil concentrations.

The skin has a pH range of 4 to 6 and skin products are expected to have pH as close to this range as much as possible in order to reduce irritation but apart from the pH of the oil extracts, those of the formulated soap product does not bear closer value to the pH range for the skin rather to that of the control (dettol) which is not within close range. However, the control soap is popularly used with no reported adverse effect on the skin due to pH, hence the formulated soap product can also follow suit.[20]

Upon antimicrobial analysis, the results showed that soaps formulated from combined oil extracts of the plants exhibited maximum antimicrobial activity as seen in table 3. Clear zones of inhibition was found to be 12mm and 13mm for *E.coli* and *S.aureus* with formulation of C.e + H.m (3:1) while for formulations of H.m+C.e (3:1) clear zone of inhibition was in the range between 10mm and 15mm respectively for *E.coli* and *P.aerugnosa* and these were better than that observed with soaps formulated with plant seed extracts used alone.

From overall result the formulation with H.m+C.e (3:1) seems to be more promising as it has shown better physicochemical characteristics with better emollient property and antibacterial activities compared to other formulations and comparable to commercial products.

Hence the soaps can further be processed and used as biopharmaceutical product in the fight against bacterial skin infection as well as its usage as bathing soap.

With regard to the antifungal activity of the formulated soaps, the results reveal that the formulation containing only one extract show less significant antifungal activity than that with two or more extracts combined especially formulation containing H.m +C.e (3:1) as in table 3 for *C.albican*. This indicates a possible potent synergism for the constituents present in this extract which could together be responsible for the characteristic antifungal and antibacterial activities recorded in the course of the study.

CONCLUSION

Soaps can easily be formulated by processes involving simple mixture of fats and natural oils with a base (NaOH) by process of continuous stirring of the mixture, curing and allowing for completion of the saponification process.

Although the soaps made from the oil extracts of *C.esculentus* and *H.madagascariensis* showed appreciable physicochemical and anti-microbial properties that made using the combined oil extracts showed better activities especially with batch D, consisting of *H. madagascariensis* and *C.esculentus* in a ratio (3:1). This batch formulation depicts a more promising activity with better physicochemical, emollient and anti-bacterial properties comparable to standard formulations.

Hence formulations of such oil extracts and the combination should be more elaborated so as to incorporate such into wide varieties of topical formulation and to help cushion the economic implication of expensive chemical/synthetic derivatives currently in use.

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