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Biochemical Assay of Medicated Soap from Shea Butter

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Shea butter is a fatty raw material derived from shea nut and is associated with skin softening as well as inflammatory and healing properties. This study aimed to explore the potential of the Shea butter for soap production and assayed of its medicated usage. Oil (65.8g) was extracted from the Shea fruit using soxhlet extraction with n-hexane as the extracting solvent. The physicochemical properties of the extracted shea butter oil have saponification value (63.76mg/KOH), acid value (1.45mg/KOH), iodine value (85.26I_2/100g) and specific gravity (0.91). The FT-IR spectra of the oil showed absorption peaks at 2918.5cm^{-1}; 1738.8cm^{-1} and 1463.0cm^{-1} which is ascribed for alkane, carbonyl and cyclic alkene groups respectively. Furthermore, physicochemical properties of the formulated soap showed foaming value (2.6cm) and pH value (9.63). Formulated shea butter soap exhibited stronger antimicrobial effects against *Staphylococcus aureus* (44 mm) and *Candida albicans* (24 mm) than DuduOsun soap (14 mm and 12 mm), especially at 500 mg/cm^3 concentration. The physicochemical properties and antimicrobial activity of shea butter oil infer it viability as a potent raw material for production of medicated toilet soap.

Keywords: Anti-microbial activity; physicochemical properties; shea butter oil; medicated.

1. Introduction

Shea butter is an organic fat extracted from the nuts of the Shea tree, scientifically known as *Vitellaria paradoxa* (previously *Butyrospermum paradoxa*), which is indigenous to West Africa (Elias and Carney, 2007). The Shear tree, from which butter is derived, is widespread across a vast expanse of savannah that spans 5000km in East and West Africa and stretches across 20 countries, from Senegal in the West to Kenya and Uganda in the East. The East African subspecies, *nilotica*, produces a more fluid shea fat, predominantly due to its higher oleic acid content in kernel (ranging from 50% > 60% in Uganda), (Carette *et al*., 2009). The sub-species paradoxa is more prevalent in the West African region.

The primary product derived from the Shea tree is the Shea fruit, which contains an edible fat content varying from 20% to 50% (Carette *et al*., 2009). The tree, an important aspect of the natural ecological system, is deciduous and thrives in the wild savannah regions. It can manage to survive on minimal rainfall of about 400-500mm annually, temperatures that range between 30 to 45 degrees Celsius, and relative humidity beneath 10%. A Shea tree's average height is approximately 13 meters (Hall *et al.,* 1996).

The Shea butter is famous for its nourishing and moisturizing qualities, and for centuries, African communities have been exploiting it for hair care and skincare treatments (Hetta, 2016). There has been an escalation in demand for natural and organic skincare products recently, as many individuals are anxious regarding the probable health risks of synthetic components present in various commercial cosmetics. Consequently, there has been a rise in the use and production of skincare products that contain Shea butter in the industry.

Incorporating medicinal ingredients like active pharmaceutical agents, essential oils, or herbal extracts into the soap enables it to provide augmented benefits beyond conventional cleansing.

Medicated soap made from Shea butter offers numerous potential benefits. Shea butter inherently contains vitamins A, E, and F that help nourish and shield the skin, promoting healthy skin (Goreja, 2004). Furthermore, based on the specific medicinal ingredients incorporated, the soap can provide additional curative effects like anti-aging, antibacterial, anti-inflammatory, or antifungal properties (Arora *et al.,* 2019)

Shea butter oil has been utilized to create various products, such as toothpaste, natural cosmetics, skin and hair care products, ointments, medicinal cosmetics, emulsions, and liquors (Coulibaly *et al.,* 2009). The oil can be obtained either mechanically (hot or cold process) or chemically (solvent extraction) from the edible nut of the fruit sourced from the Karite tree (*Butyrospermum parkii*) grown in regions such as Ghana, Mali, Burkina Faso, and other Savannah Grasslands located within West Africa

The superior quality Shea butter oil that retains a majority of its phytoconstituents is acquired through the cold process. During this process, the oil obtains a lighter colour and retains almost all of its natural qualities, resulting in a milder scent (Shahinuzzaman *et al.,* 2016; Warra *et al.,* 2010). Additionally, potential health hazards to consumers from residual solvents present in chemically extracted oil are mitigated, as solvents are not utilized in cold processing techniques.

A study revealed the presence of some phenolic compounds in Shea butter, which mostly belong to the catechin family known for their antioxidant properties. The catechin content of Shea butter oil is notably higher than the total phenolic content of ripe olives (Honfo *et al.,* 2014). The catechin is known to reduce inflammation, pain, and swelling associated with arthritis (Badoussi *et al*., 2015; Honfo *et al.,* 2014). Notably, Shea butter oil is effective for treating vaginal infections (Shahinuzzaman *et al.,* 2016), and sexually transmitted diseases, eliminating lice, scabies, ringworm, Athlete's foot fungus, and *Phytophthora infestans* (George and Raymond, 2015). Additionally, applying it to the skin repels mosquitoes, fleas, and houseflies and addresses hair problems like dandruff, baldness, and graying of hair. Shea butter oil can cure leprosy, rheumatism, chronic syphilitic sores, and indolent ulcers, clear herpes simplex, *E. coli*, *Staphylococcus aureus*, chickenpox, cholera, pneumonia, tuberculosis, peptic ulcers, dry psoriasis, diabetic foot, heal wounds, and resolve other skin disorders (Israel, 2014).

The popularity of natural and organic skincare products has risen due to increasing concerns over the possible health risks linked to synthetic additives present in many commercial cosmetics. This has resulted in an increasing production and use of skincare products using Shea butter as an integral component.

Medicated soap is one such product that utilizes the moisturizing properties of Shea butter and combines it with therapeutic benefits from different medicinal ingredients, including herbal extracts, essential oils, or active pharmaceutical agents. These additional medicinal ingredients allow the soap to offer considerable benefits beyond basic cleansing.

The aim of this research is to study shea butter oil as an alternative source of fatty material in medicinal soap formulation and to determine the physicochemical properties of oil extracted from shea butter seed and the produced soap which may help to impart its medicinal characteristics.

2. Materials and Methods

2.1 Materials

All the solvents and reagents used were of Analytical grade which includes ethanol, glycerol, Sulphuric Acid, Ethyl acetate, Potassium hydroxide, Sodium hydroxide, Sodium Sulphate, and dilute hydrochloric acid. pH meter, rotary evaporator, water bath was all used, also all glassware was washed, cleaned and dried in an oven at 105^{o}C.

Table 1. List of equipment, their Model and Manufacturer.

Equipment	Model	Manufacturer
FT-IR Spectrometer	Cary 630	Agilent Tech, USA
GC-MS	Intuvα9000	Agilent Tech, USA
pH Meter	Tenway 3105	
Incubator	----------	----------

The organisms used are *Staphylococcus Aureus* and *Candida albicans*, both were cultured from growth on potato dextrose agar (fungi) and nutrient agar (bacteria) plates.

2.2 Collection of plant material

The seeds of butyrospernum parkii were collected from Zuru Local Government Area, Kebbi state. The seeds were dried at ambient temperature, de-shelled and pulverised. The *Aloe vera* and turmeric were obtained from Badariya area, Birnin Kebbi. The *Aloe vera* leaf was peeled to extract a gel. The turmeric was also peeled to remove the thin papery skin. The *Aloe vera* gel and turmeric were blended using a blender and sieved.

2.3 Extraction of Shea Oil

The pulverised *Vitellaria paradoxa* seed (235.9 g) was subjected to soxhlet extraction using n-hexane as the extracting solvent at 60°C for six hours and oil yield of 65.80g was obtained at the end of the extraction.

2.4 Physicochemical properties of shea oil

Physicochemical parameter determination for the oils were determined according to the standard protocols for oil analyses with minor modifications where necessary (Gerpen, 2005; Ibeto *et al*., 2012). Through sensory inspection, the physical state, colour, and smell were determined.

(a). Determination of Acid value

In this technique, 250cm³ container was filled with 5cm³ of diethyl ether and 5 cm³ of ethanol. The liquid that was obtained was then mixed with 2g of the extracted oil and a few drops of phenolphthalein indicator. The volume of 0.1M KOH (V_o) was recorded after the combination was titrated against 0.1M KOH to the endpoint (dark pink colour) (Ogunlade *et al.,* 2008). The acid value was calculated using equation (i):

$$\text{Acid Value} = \frac{M \times C \times Tv}{W} \quad \text{.....(i)}$$

Where, M= molar mass of KOH (56.1g/mol); C = concentration of KOH (0.1M); TV= titre value; = weight of oil sample.

(b). Determination of Saponification value

The saponification value was calculated using the method outlined by (Warra, 2017). Two gram of the oil was placed into 250cm^3 conical flask, then 10cm^3 of 0.1M ethanolic potassium hydroxide was added into the oil. The mixture was continuously stirred gently and gently warmed for thirty minutes (Warra, 2017). In order to ensure complete dissolution of the sample a reflux condenser was set up on top of the flask holding the liquid. After adding the phenolphthalein indicator (1cm^3), the sample was titrated to a pink endpoint against 0.5 M HCl. The oil's saponification value was determined using equation ii:

$$\text{SV(mgKOH/g)} = \frac{mL\ of(Tv-B) \times M \times 56.1 g/mol}{Weight\ of\ oil(g)} \quad \text{.....(ii)}$$

Where, Tv=sample titre value (ml); B=blank titre value (ml); M=Molarity of KOH;

56.1 g/mol= Molecular weight of KOH.

(c). Determination of Iodine value

The method described by Onwaku (2005) was used for determination of the butter iodine value. A conical flask containing 2.0g of oil was mixed with 25cm^3 of carbon tetrachloride (CCl_4) to dissolve the oil. In the fume hood, a safety pipette was used to add 25 cm^3 of Wiji's reagent to the mixture. After inserting the cork, the flask's contents were swirled and left in the dark for two and half hours. 20cm^3 of 10% aqueous potassium iodide and 125cm^3 of distilled water were added to the mixture. Using a sodium thiosulphate (0.1M) solution, the solution was titrated until the yellow tint nearly vanished. Two drops of the 1% starch solution indicator was added and the titration continued by adding sodium thiosulphate dropwise until, the blue colouration vanished. Equation iii was used to determine the iodine value:

$$\text{Iodine Value} = \frac{126.9 \times M \times (V_o - V_1)}{W} \quad \text{.....(iii)}$$

Where, M= Concentration of sodium thiosulphate; V_0= Volume of NaS_2O_3 used for blank;

V_1= Volume of NaS_2O_3 used for determination; W= Mass of oil sample (g)

(d). Determination of Specific gravity

The technique employed by Adam *et al*., (2015) was employed for the specific gravity (S. G.) measurement. An empty specific gravity bottle was weighed and was recorded as M_1. After being filled with water, the bottle was weighed and marked as M_2. Shea butter oil was then added to the bottle once it had been emptied and dried. The weight that was attained was noted as M_3. After that, the specific gravity was determined using the equation iv

$$\text{S.G} = \frac{M_3 - M_1}{M_2 - M_1} \quad \text{..... Equation (iv)}$$

2.5 Fourier-Transformed Infrared Spectral Analysis of Shea butter oil

The FTIR (4000-650cm^{-1}) analysis of the shea butter oil was performed using an FT-IR spectrometer and the analysis was conducted under ambient laboratory conditions. Small quantity of the oil sample was placed on the FT-IR Spectrometer and the transmittance method was used with resolution 8 to identify the type of functional groups present.

2.6 Soap formulation

The soap was prepared using a method outlined by Warra (2017). In a mixing container, 200cm^3 of shea butter oil and 100cm^3 of caustic soda were added, the mixture was stirred continuously until a paste was formed. Then 200cm^3 of phenol was added into the mixture, while stirring. 50cm^3 of 2M sodium sulphate was added to the mixture and stirred. Then, sieved mixture of turmeric and aloe Vera were added to the mixture, it was vigorously stirred until a homogenous paste was obtained. The soap mixture was then poured into moulds and allowed to cool and harden, resulting in soap tablets. The soap tablets were left in a well-ventilated area to cure. (drying and hardening).

2.7 Physiochemical properties of the soap

(a). Determination of Texture and Stability

A sample of the soap tablet was immersed in suitable vessel containing distilled water at 27±2^{o}C for an hour. The sample soap was withdrawn from the water and allowed to dry at room temperature. It is then examined for defects after 24hours (Warra, 2017).

(b). Determination of Foaming Ability

Exactly 10g of the sample soap was immersed in 500cm^3 beaker containing 200cm^3 of distilled water. It was shaken vigorously for 2minutes and allowed to stand for 20minutes. The height of the foam in the solution was measured and recorded.

(c). Solubility tests

A soap sample 2.0g of each soap was added to a 100mL measuring cylinder containing 10 mL of distilled water. The duration of the dissolution of the soap after continuous shaking was recorded**.**

(d). Determination of pH

A 10% soap solution was prepared and the solution pH was determined using a pH meter (Tenway 3150) according to method of Mendes *et al.,* (2016).

2.8 Anti-Microbial Test of the Soap

Using the agar-well diffusion method, the test organisms' susceptibilities to the shea butter soap formulation were evaluated (Ndukwe *et al.,* 2005; Aliyu *et al.,* 2009). The test organisms from the growth on potato dextrose agar and nutrient plates were suspended in saline solution (0.85% NaCl) and adjusted to match a turbidity of 0.5 McFarland standard. The bacteria and fungus were incubated at 37^{o}C at room temperature for 18 hours, respectively. Using sterile cotton swabs, the standardised suspension was used to inoculate the surfaces of Mueller Hinton agar plates (90mm in diameter). By combining 5g of the soap with 10mL of sterile water in a universal bottle and heating it, a stock solution containing 500 mg/ml of the soap was created to dissolve the soap into solution in a water bath at 60^{o}C. Using the stock solution, various concentrations of the soap; 250, 125, and 100 mg/ml were obtained. For the purposes of the bacteria and fungus, the plates were incubated for 24hours at 37^{0}C and 48hours at room temperature, respectively. The transparent metre rule was used to measure and record the zones of inhibition that were found on the plates around the wells. The full examination was carried out twice.

3. Results and Discussion

An acid value is the measure of the amount milligram of potassium hydroxide to neutralize the free acids in 1g of fat or oil (Ikaya *et al.,* 2013; Samuel *et al.,* 2017). The acid value of 1.45mg was observed in this study which is lower than the FAO/WHO standard of 4mgKOH/g. However, 0.84mgKOH/g was obtained by Maurice *et al.,* (2022), the acid value of shea butter oil which is much lower than the value obtained in this study. Jatropha seed oil, was reported to have a much low acid value of 1.20mgKOH/g (Warra *et al.,* 2012). A low acid value is an indication that the oil sample contains minimal amount of free acids, which reduces its rancidity and ensuring its suitability for use in food and other industrial applications (Samuel *et al.,* 2017). Saponification value of oil is an index of the average molecular mass of fatty acid in the oil sample, and is an important parameter in indicating the suitability of the oil for soap production. The saponification value of the shea seed oil was analysed in this study and found to be 196.89mgKOH/g and this value is within the FAO/WHO range of 195-205mg KOH/g for edible oils.

Iodine value (IV), is the degree of unsaturation of a fat or oil, is defined as the amount in grams of iodine absorbed by 100g of fat (Hassan *et al.,* 2015). The iodine value of 85.26gI_2/100g obtained in this research means that shea butter oil belongs to the class of non-drying oil. A non-drying oil (iodine value less than 100gI_2/100g) is an oil which does not harden when it is exposed to air, while a drying oil hardens completely through polymerization and a semi-drying oil partially hardens (Datti *et al.,* 2021). It was reported by Maurice *et al.,* (2022), shea butter has Iodine Value of 51.01 I_2/100g, lower than the obtained value in this study.

Table 2: Physicochemical Properties of shea Butter oil

Parameter	Value
Yield(g)	65.3
Acid value (mg/KOH/g)	1.45
Saponification value (mg/KOH/g)	63.76
Iodine value (I_2/100g)	85.26
Specific gravity	0.91

From Table 2; the specific gravity obtained for the shea butter oil was 0.91. This value was close to the one published by Boadu *et al.,* (2017) which was between 0.87, the same value obtained by Adam *et al.,* (2015) was 0.91. This property means that shea butter floats on the surface of water when dropped on it. It was based on this property that makes it easier to collect shea butter on the surface of water

during shea butter extraction from the shea nut (Coulibaly *et al.,* 2009).

The FT-IR spectra of shea butter oil (Figure 1) revealed the presence of C-H stretching at 2918.5 cm^{-1} and 2851.4 cm^{-1} stretching for carbonyl while 1738.8 cm^{-1} is due to the C=O present and benzene –C=C- at 1463.0 cm^{-1}

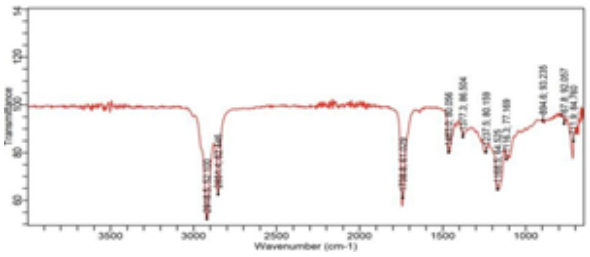

Figure 1: FT-IR Spectra of Shea Butter oil

The presence of peak at 2918.5cm^{-1} in the FT-IR spectra, was due to C-H of alkane which confirms the presence of saturated hydrocarbon chains in the sample, which is consistent with the composition of shea butter. Peak at 2851.4cm^{-1} was due to C-H of carbonyl, which indicates the presence of ester functional groups in the sample. This suggests that the soap is made from a saponification reaction of shea butter with a strong base, resulting in the formation of carboxylate ions and glycerol. The C=O stretching vibrations of carbonyl groups observed at 1738.8cm^{-1}, further confirmed the presence of ester functional groups in the sample. The presence of peak at 1463.0cm^{-1} was due to C=C stretching vibrations, indicating the presence of cyclic alkene, which suggests the presence of unsaturated hydrocarbon chains in the shea butter, which is consistent with the natural composition of shea butter. The data in Table 2 are in good agreement with the published report of Mehul *et al*., (2011) on IR spectroscopic data.

Table 2: FT-IR Absorption bands for Shea Oil

Bands	Frequency (cm^{-1})	Inference
C-H	2918.5.	Stretching, C-H of alkane present
C-H	2851.4	Stretching, C-H of carbonyl group
C=O	1738.8	Stretching, carbonyl group C=O
C=C	1463.0	Stretching, cyclic alkene

Physicochemical properties of the oil (Table 3) revealed that the extracted oil is pale yellow. The colour may be due to carotene, a pigment found in butter of the shea seed. The soap have smooth texture and creamy, and it maintain its stability throughout the testing period. This indicate that the product is well-formulated and will not degrade or change in texture over time, providing a consistent user experience.
The soap showed a good solubility in water, indicating that it can be easily rinsed off during use. This is a positive attribute as it ensures the soap can be effectively removed from the skin without leaving a residue. The foaming ability of a soap (or the measure of the foam height) is an important factor that attracts the interest of the consumer, even though it has little contribution to the cleansing ability of the soap (Mak-Mensah and Firempong, 2011). Evaluating the foaming ability of a soap is thus important to the producer rather than the consumer. In this study, the foam height of the produced shea butter oil was found to be 2.60cm^3, and this result is close to 2.73 cm^3 (Hassan *et al.,* 2015), 2.36cm^3 (Datti *et al.,* 2021) and 2.0cm^3 (Warra *et al.,* 2011) for some samples of Neem seed oil soaps. The result from other studies shea nut oil soap (4.2cm^3), cotton oil soap (4.5cm^3) Jatropha oil soap (5.4cm^3), Sesame oil soap (4.8 cm^3) all reported by Warra *et al.,* (2011).However, Warra *et al.,* (2011) have, on the other hand reported a much lower foam height for Castor oil soap (1.6cm^3).

Table 3: Physical Properties of Shear Butter soap

Property	Value
Colour	Pale yellow
Texture and stability	Smooth
Solubility	Soluble
Foaming value	2.6cm^3
pH value	9.63

The pH of the prepared Shea butter oil soap was found to be 9.63. The result obtained is comparable with pH of 9.0 for shea butter oil soap prepared and reported by Boadu *et al.,* (2017), and pH of 9.38 for cotton seed oil soap prepared and reported by Warra *et al.,* (2011). pH of 10.22 was reported for soap prepared from milk bush (*Thevetia peruviana*) seed oil (Alabi 2014), and pH of 10.46 for neem seed oil was reported by Datti *et al.,* (2021). The result from the present study is however within the normal pH range for soap 7-11 (Rafaela *et al.,* 2016). High pH value makes cleasing soap harsh and corrosive to the skin, and is mainly contributed to an incomplete alkali hydrolysis during the saponification process, but is usually treated by adding extra oil to lessen the harshness of the soap (Latifat *et al*., 2012; Alabi 2014), Even though alkalinity increases the soap's detergency (Kaoru 1998). On another hand, pH range of 3-5 is considered very low and highly acidic (Oyedele 2002; Alabi 2014). The pH value of the shea butter oil indicated that the soap is gentle and non-irritating to the skin. This is an important factor for a soap as it ensures it will not disrupt the natural pH balance of the skin.

All the microorganism tested were susceptible to formulated shea butter soap and commercial soap

(DuduOsun). It was observed that the zone of inhibition decreased with decreasing soap concentration of which we can affirmatively claim that the sensitivity of microorganisms was concentration-dependent. On the other hand, 125mg/ml soaps concentration showed higher colony growth of *Candida albicans* and *Staphylococcus aureus*, whereas 500mg/ml soap concentration inhibited the growth of all the microorganisms of both formulated shea butter soap and DuduOsun soap.

Table 4: Zone of inhibition of microbial organisms against formulated Soap of Shea butter oil

Test organisms	Zone of inhibition against fractions (mm)		
	500mg/ml	250mg/ml	125mg/ml
Staphylococcus aureus	44	39	14
Candida albicans	24	14	7

The results obtained from this study(Table 4) showed that Shea butter formulated soap have higher significant antimicrobial activities against *Staphylococcus aureus*, and *Candida albicans*, with a maximum zone of inhibition of 44mm and 24mm respectively, though to a specific identifiable extent as shown by the inhibition of growth pattern of the isolates (Table 5), while Commercial soap (DuduOsun) have least significant antimicrobial activities with maximum inhibition zone diameter (IZD) of 14mm and 12mm against *Staphylococcus aureus*, and *Candida albicans* (Table 5). *Staphylococcus aureus* and *Candida albicans* have been inculpated in causing skin infections including boils, thrush 54 and impetigo etc., the susceptibilities of these organisms to the soap indicate the therapeutic ability of the soap in the treatment of such diseases (Shehu *et al.,* 2020)

Table 5: Zone of inhibition of microbial organisms against DuduOsun soap

Test organisms	Zone of inhibition against fractions (mm)		
	500mg/ml	250mg/ml	125mg/ml
Staphylococcus aureus	14	9	7
Candida albicans	12	9	6

The study comparatively evaluated the antimicrobial activity of formulated Shea butter Soap to enhance awareness of the economic viability of the skin care products

4. Conclusion

In this study, medicated soap was formed from shea butter. The physicochemical properties of the shea butter oil such as acid value, saponification value, iodine value, and specific gravity were evaluated, which shows the shea butter is of good quality. Also, the physicochemical properties of the soap such as solubility, foaming ability, texture and stability, colour, and pH value were evaluated, and shows that the soap provides a consistent user experience. Fourier Transform-Infrared spectroscopy of the oil was carried out to identify the major functional groups present in the oil, which are consistent with the natural composition of shea butter. The study comparatively evaluated the antimicrobial activity of formulated Shea butter Soap to enhance awareness of the economic viability of the skin care products. Thus, the findings of this research study validate the potential of shea butter oil as active ingredient in cosmetic industry.

Conflict of Interest

The authors declare no conflict of interest.

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