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Formulation of Cosmetics Using Selected Fruits of Medicinal Plants: *Persea americana* (Avocado pear) and *Azadirachtha indica* (Neem)

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Abstract
The use of herbal cosmetics has been practised by ancient people and cultures because of their perceived minimal to zero negative effects. This study was aimed at producing soap from *Persea americana* and *Azadirachtha indica* determining the percentage yield of their extract and identifying bacterial strains in them using standard methods. The result showed that avocados generated a higher yield extract (28.20%) while neem had 19.40%. The result of the zone of inhibition of soap formulated from neem at 100mg/ml concentration revealed a significant difference ($p<0.05$) between the test organisms ranging from 5.65 to 8.40 mm. The result of minimum inhibitory concentration (MIC) of neem-formulated soap showed activity at a concentration of 0.50 mg/dl against all the test organisms, while MIC of avocado soap showed activity against all the test organisms at 0.50 mg/dl concentration. However, when the concentration was increased to 1.00 mg/dl, the activity of the soap sample was active against *Staphylococcus aureus, Listera monocytogenes, Escherichia coli and Streptococcus pneumonia*, but the soap sample showed no action at increased concentrations of 1.50 and 2.00 mg/dl respectively. The inhibitory properties of soap samples against gram-positive and negative bacteria are attributed to the concentration of the bioactive compounds present in the plant materials used to formulate the soap. Therefore, it is recommended that people should be using herbal cosmetics since it has bioactive compounds that inhibit the activities of bacteria.
Keywords: Cosmetics, Medicinal plants, Persea Americana, Azadirachtha indica

Introduction

The term "cosmeceuticals" was first used in 1961 by Raymond Reed, a founding member of the United States Society of Cosmetic Chemists. Dr. Albert Kligman later used it in 1984 to describe substances that have both cosmetic and therapeutic benefits (Saha, 2012). Cosmeceuticals are cosmetic-pharmaceutical hybrids intended to enhance health and beauty through ingredients that influence the skin's biological texture and function (Sharma and Paramesh, 2010). The effects of plant-based chemical compounds on the human system are mediated by mechanisms that are similar to those that are widely known to occur with chemical substances in conventional medications (Lai and Roy, 2004). Thus, herbal medicines do not differ greatly from conventional drugs in terms of how they work. The idea of cosmetics and beauty dates to early human history. Natural cosmetics and herbal cosmetics are used interchangeably. Herbal cosmetics are items that contain herbs in their raw or extracted form. (Sahu *et al*., 2011). Herbal cosmetics are created by mixing numerous cosmetic materials to create a base, then adding some plant-based substances to handle a range of skin conditions, alleviate irritation, improve appearance, or make the skin more beautiful (Devi *et al*., 2018). Plants are extensively utilized in the creation of novel medicinal and cosmetic goods (Joshi, 2012).

Herbal cosmetics, sometimes referred to as products, are prepared by combining some legally allowed cosmetic chemicals as a base and then adding some plant-based elements to exclusively produce specific cosmetics (Devi *et al*., 2018). Though herbs do not generate quick cures, they offer a way to rebalance the body's relationship with nature (Pandey *et al*., 2010). A variety of cosmetic and toiletry formulas have lately been created and developed using Indian herbs. In addition to their historically recorded uses, Indian herbs have also been included in certain recent studies of personal care products (Pal *et al*., 2022).
The natural ingredients in herbs provide the body with nutrition and other beneficial minerals with minimal to

no negative effects on humans (Gediya *et al*., 2011). Likewise, pure herbal cosmetics manufactured from shrubs and herbs have no negative side effects. There is a growing demand for herbal medicines because they are gentle to the skin and have little to no negative effects. Though there are several plants used for herbal medicines, however this study aimed at producing soap from *Persea americana* and *Azadiractha indica*.

Persea americana is an evergreen grey-trunked small tree belonging to the Lauraceae family. It is indigenous to South and Central America and is highly utilised for its nutritional value and in traditional medicine to cure a range of illnesses (Shruti and Padma, 2015). *P. americana* has various common names depending on the country in which it is cultivated; it is commonly called Alligator in Florida, Xiene in Mexico, Palta in Colombia and Ecuador, Abacoteiro in Brazil, Avocado pear (Ube Beke – Igbo) in Nigeria and pear in Cameroon (Omodamiro *et al*., 2016). The avocado is a leafy tree up to 60 feet high with a trunk diameter which can reach 100 cm. It possesses alternative and elliptical leaves. The flowers are small and unisexual (Akpuaka *et al*., 2003). The fruit is a drupe (fleshy fruit with seed inside) with greenish and thin skin whose taste is reminiscent of walnut and has a very oily pulp, commonly used as food. Avocados are a good source of B vitamins, which help our organisms fight against diseases and infections (Omodamiro *et al*., 2016).

Azadiractha indica commonly called Neem tree is mainly grown in southern Asia and some parts of Africa, where it is known to have traditional medicinal uses through the ages. Worthy of note is that virtually all parts of the Neem tree (leaves, bark, fruit, flowers, oil, and gum) are associated with the medical folklore for the treatment of certain medical conditions such as cancer, hypertension, heart diseases, and diabetes. The potential effects that are seen when using these extracts can certainly be attributed to cellular and molecular mechanisms. These mechanisms include free radical scavenging, detoxification, DNA repair, cell cycle alteration, programmed cell death mitigation and autophagy, immune surveillance, anti-inflammatory, anti-angiogenic, and anti-metastatic activities and the ability to modulate various signalling pathways (Arumugam *et al*., 2014; Omobowale *et al*., 2016; Patel *et al*., 2016).

Synthetic cosmetics have the potential to irritate skin, resulting in pimples and other skin conditions. They could clog skin pores and cause excessively dry or oily skin. With natural cosmetics, one need not worry about these problems caused by synthetic beauty products. The natural ingredients used ensure no side effects; one can apply them anytime, anywhere. Natural cosmetics are safer to use than conventional beauty products. Dermatologists have tested and confirmed that they are hypo-allergenic and safe to use at any time or location. People do not have to be concerned about developing skin rashes or feeling itchy on their skin because they are comprised of natural substances. For example - BHA (Butylated Hydroxyanisole) and BHT (Butylated Hydroxytoluene) are closely related synthetic antioxidants and are used as preservatives in lipsticks and moisturizers. BHA and BHT can induce allergic reactions in the skin.

Materials and methods

Materials

Azadirachtha indica (Neem) fruits were collected from Michael Okpara University of Agriculture, Umudike, Nigeria while *Persea americana* (Avocado pear) fruits were obtained from Afor Oru market in Mbaise, Imo State, Nigeria. The samples were transferred into properly labelled polyethene bags and then taken to the laboratory for identification and preparation. The identification and authentication of fruits were carried out at the Department of Forestry and Environmental Management, in the College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike Abia State. Voucher specimens were deposited at the FOREM herbarium for both *Persea americana* and *Azadirachtha indica*.

Methods

Oil extraction

Ripe fruits of *Persea americana* and *Azadirachtha indica* were washed with water and put in polyethene bags for 24 hours for the fruit to soften. The pulps were removed and mashed using porcelain mortar and pestle to have a smooth paste. Unripe fruits of *Persea americana* and *Azadirachtha indica* were kept for about 48 hours at room temperature until they were ripened. The fruits were cut open with a stainless-steel knife to remove the seeds from the pulps, and the skins were removed from the pulps. Each of the mashed samples was spread on stainless-steel trays and sun-dried for 6 hours. The samples were scraped from the trays into clean white cotton cloths, wrapped and squeezed. The oil yield was calculated following the method of Otaigbe *et al*. (2016) as expressed in Equation 1 below. The oils collected were stored in airtight bottles in a functional refrigerator for further use.

$$\text{Yield (\%)} = \frac{A1}{A2} X \frac{100}{1} \quad \ldots\ldots (1)$$

Where: A_1 = weight of extracted oil in grams (g)

A_2 = weight of dry sample in grams (g).

Soap production

The method of Mak-Mensah and Firempong (2011) was adopted for soap production. The individual oils (100 g) of *Persea americana* and *Azadirachtha indica* respectively were weighed into separate 500 cm^3 beakers, and heated to about 100°C. Saponification was initiated by adding 20 cm^3 of 23.5% sodium hydroxide (NaOH) solution to the resulting solution. 60 g of NaOH pellets dissolved in 100 cm^3 of deionised water was gradually added while stirring until the completion of saponification. NaCl (8g) dissolved in 30 cm^3 of deionised water was then added to grain soap. The salt was added to separate the spent lye in the bottom, while the saponified mass floated on the surface to reduce the soap viscosity and to separate the glycerol water in the bottom. The glycerol water was removed by siphoning.

The soap paste was washed with 10 cm^3 of hot water (90°C) to reduce excess sodium hydroxide and sodium chloride and any impurities found in the soap paste. The soap obtained was washed again with 10 cm^3 of distilled water; filtered using a linen cloth, and then a small amount of water was added to soften it whilst heating. The soap was placed in a mould and allowed to dry.

Anti-bacterial test

Four reference bacterial strains and three laboratory strains from our laboratory stock culture-confirmed to be multi-drug resistant bacteria (Iweriebor *et al*., 2015; Iwu *et al*., 2016) were used for the antibacterial assay. The reference and laboratory strains bacteria are four Gram-positive: S. aureus (NCIB 50080), M. smegmatis (ATCC 700084), L. ivanovii, (ATCC 19119), S. uberis (ATCC700407) and three Gram-negative bacteria: E. cloacae (ATCC 13047), E. coli 180 and V. paraheamolyticues reported to be resistant to sulphamethoxazole, ampicillin, streptomycin, cefuroxime, cephalexin, tetracycline and nalidixic (Adefisoye and Okoh, 2016) was examined in comparison to the neem and avocado cosmetics, following CLSI (Clinical and Laboratory standards Institute (2014) procedures. The bacterial suspensions were made by inoculating a fresh stock culture of the test bacteria strains into tubes containing 5 ml of sterile Luria Bertani broth and incubated for 24 h at 37 °C. Thereafter, active cultures were grown for 24 hours in sterile Luria- Bertani broth inoculated into Mueller-Hinton Agar (MHA) incubated for 24 hours at 37 °C. After incubation, single colonies were transferred from MHA plates into 4 ml of normal saline solution determined spectrophotometrically at 580 nm as previously reported by Omoruyi *et al*. (2014) and adapted by Igwaran *et al*. (2017) and the dilutions matching with 0.5 Mc-Farland standards were used for the assay.

The modified method of Gullon *et al*. (2016) was used for the determination of MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) of the neem and avocado cosmetics. Under the aseptic condition, two-fold serial dilutions were carried in sterile microcentrifuge tubes in a total volume of 100 µl of Muller Hinton (MH) broth mixed with the NC of various concentrations ranging from 0.0125–0.800 mg/mL. The positive and negative controls were ciprofloxacin and water respectively; thereafter they were incubated at 37°C for 24 hours. The assay was carried out in triplicates and tubes with the lowest concentration without visible growth were reported as the minimum inhibitory concentration (MIC). The minimum bactericidal concentration (MBC) for each neem product and the control was determined by streaking out all tubes without visible growth in the MIC technique into fresh nutrient agar plates. Then, at 37°C, the culture was incubated for 24 hours. The lowest concentration of the cosmetics and ciprofloxacin that didn't on the solid medium yield any growth after 24 hours (Ramalivhana *et al*., 2014) was recorded as the MBC.

Statistical Analysis

The significant difference between the products and controls was carried out using SPSS 23.0 for Windows (Institution registration No for IBM-SPSS/OLRAC SPS 2012/1786646/07). All protocol results were expressed as means ± S.D of duplicate in cytotoxicity assay while the antibacterial, antioxidant and physicochemical tests were performed in triplicates. Significance at $P < 0.05$ was considered the confidence level.

Results and Discussion

The result of the percentage yield showed that avocado had the highest value of 28.20 % compared to that of neem which had a value of 19.40 % (Table 1). The increase in the percentage yield of avocados could be a result of high essential oil content. Omodamiro *et al*. (2016) reported that the result for the phytochemical constituents in *Persea americana* seeds showed the presence of the following compounds with their respective concentration: Alkaloids (2.92±0.028), Flavonoids (4.76±0.053), Saponins (3.22±0.055), Steroids (1.58±0.05), Tanins (0.18±0.00), Phenol (2.47±0.03). The decrease in the percentage yield of neem could be a result of a decrease in phenolic compounds present. According to Mariana *et al*. (2017), the presence of metabolites and their antimicrobial action can be affected by several factors, both qualitatively and quantitatively. One such element is seasonality, which can be thought of as the time of leaf harvest. Gram-positive bacteria are defined in bacteriology as those that respond positively to Gram stain, which is a commonly used method of rapidly classifying bacteria into two main groups considering the type of cell wall they possess (Madigan and Martinko, 2006). When observed under an optical microscope, gram-positive bacteria absorb the test's crystal violet stain and take on a purple hue. This occurs during the decolourization step of the test, when the remaining portion of the sample is cleaned of the stain due to the thick peptidoglycan coating in the bacterial cell wall (Madigan and Martinko, 2006). Gram-positive bacteria lack an outer membrane; this makes them more susceptible to some antibiotics that target their cell walls than gram-negative bacteria, even though they have a thicker peptidoglycan layer (Madigan and Martinko, 2006).

The result of the zone of inhibition of soap formulated from neem at 100mg/ml concentration revealed a significant difference ($p<0.05$) between the test organisms ranging from 5.65 to 8.40 mm, with *Streptococcus pneumoniae* having the highest value of 8.40 mm compared to *Listera monocytogenes* which had the lowest value of 5.65 mm (Table 2). Compared to the control (ciprofloxacin) which had a value of zone of inhibition ranging from 10.50 to 12.40 mm was higher than that of the neem-formulated soap. On the other hand, the zone of inhibition of the soap formulated from avocado showed that at 200 mg/ml concentration showed that neem formulated soap had a significant difference ($p<0.05$) ranging from 8.00 to 10.20 mm. For the avocado-formulated soap, the zone of inhibition had

a value between 6.5 to 8.4 mm. The control (ciprofloxacin) had a higher zone of inhibition ranging from 15.00 to 16.40 mm. The result obtained is comparable to that of Ukaoma *et al*. (2019) who observed the inhibitory effects of neem increased as the extract's concentration increased. Also, the finding of this study was different from the reports of Paz *et al*., (2015) who reported that the antibacterial activity of avocado was greater against gram-positive bacteria, primarily *S. aureus*, than gram-negative bacteria, this might be explained by the membrane structure.

Gram-negative bacteria are bacteria that do not retain the crystal violet stain used in the Gram-staining method of bacterial differentiation (Table 3). They are characterized by their cell envelopes, which are composed of a thin peptidoglycan cell wall, sandwiched between an inner cytoplasmic cell membrane and a bacterial outer membrane (Pelletier, 2011). Gram-negative bacteria are found in virtually all environments on Earth that support life. The gram-negative bacteria include the model organism *Escherichia coli*, as well as many pathogenic bacteria, such as *Pseudomonas aeruginosa*, *Chlamydia trachomatis*, and *Yersinia pestis*. They are an important medical challenge, as their outer membrane protects them from many antibiotics (including penicillin), detergents that would normally damage the inner cell membrane, and lysozyme, an antimicrobial enzyme produced by animals that forms part of the innate immune system. Additionally, the outer leaflet of this membrane comprises a complex lipopolysaccharide (LPS) whose lipid A component can cause a toxic reaction when bacteria are lysed by immune cells. This toxic reaction leads to low blood pressure, respiratory failure, reduced oxygen delivery, and lactic acidosis life-threatening condition known as septic shock (Pelletier, 2011). The result of the zone of inhibition of soap formulated from neem against gram-positive bacterial at 100mg/ml concentration revealed a significant difference ($p<0.05$), with *Escherichia coli* having the highest value of 7.30 mm compared to that of *Proteus mirabilis* which had the lowest value of 6.5 mm. Compared to the control (ciprofloxacin) which had values ranging from 10.30 to 11.40 mm was higher than that of the neem-formulated soap. On the other hand, the avocado-formulated soap sample had values of the zone of inhibition at a concentration of 100mg/ml ranging from 5.20 to 6.50 mm, which is lower than that of the control which ranges from 10.30 to 11.40 mm. At the concentration of 200mg/ml of the avocado-formulated soap the zone of inhibition ranged from 6.50 to 8.00 mm which is lower than that of the control (ciprofloxacin) which ranged from 18.40 to 21.50mm. The findings of this work agreed with that of Daieni *et al*., (2019) who reported that the Quintal peel extract of avocado (which had the highest levels of phenolic compounds and flavonoids) demonstrated the best results for antioxidant and antibacterial activities, followed by the Hass variety, which also showed low concentrations in the tests against the selected bacteria.

The Minimum Inhibitory Concentration (MIC) is defined as the lowest concentration of an antimicrobial ingredient or agent that is bacteriostatic (prevents the visible growth of bacteria) (Table 4). MICs are used to measure the impact of reducing antibiotic/antiseptic concentrations over a predetermined time frame in terms of preventing the growth of the microbial population. This allows researchers to measure the antimicrobial activity of different ingredients. The result of the minimum inhibitory concentration of avocado soap showed activity against all the test organisms at 0.50 mg/dl concentration, at 1.00 mg/dl activity of the soap sample was active against *Staphylococcus aureus, Listera monocytogenes, Escherichia coli and Streptococcus pneumoniae*. At concentrations of 1.50 and 2.00 mg/dl the soap sample showed no activity.

The result of the Minimum Inhibitory Concentration (MIC) of neem-formulated soap showed activity at a concentration of 0.50 mg/dl against all the test organisms (Table 5). At 1.00 mg/dl, it showed activity against *Listera monocytogenes*. It was non-active at a concentration of 1.5 mg/dl but showed activity against *Escherichia coli* at 2.00 mg/dl.

Conclusion

Yield also known as reaction yield, is a measure of the amount of product formed about the reactant used. It is derived from a chemical reaction typically stated as a percentage (Vogel, 2020). One of the key considerations for scientists is yield, which is crucial in both organic and inorganic chemical synthesis processes (Cornforth, 2013). The concentration of the bioactive compounds present in the plant materials used to formulate the soap was responsible for the inhibitory properties of soap samples against both gram-positive and negative bacteria. From the result of the percentage yield, avocado had a higher yield than neem. However, the result of the inhibition of the soap sample against the test organisms showed that at higher concentrations of the soap sample, the test organisms were susceptible, especially *Streptococcus pneumoniae.* It was also observed that the control antibiotics had higher inhibitory properties than the cosmetics (soap) that were prepared. This research work has shown that at the concentrations of the soap samples under study, the soaps have anti-bacterial activity against the test organisms. Therefore, it is important to note that more research be conducted on higher concentrations of the soap samples to ascertain the antibacterial properties and the toxicity levels.

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Table 1: Percentage yield of *Persea americana* and *Azadirachtha indica*

Samples	Weight of oil (g)	Weight of sample (g)	Percentage yield (%)
Azadirachtha indica (Neem)	80.78	416.40	19.40
Persea americana (Avocado pear)	108.73	385.60	28.20

Table 2: Zone of the cosmetics (soap) against selected gram-positive bacteria

Samples	*Staphylococcus aureus* (mm)	*Streptococcus pneumoniae* (mm)	*Listera monocytogenes* (mm)	*Baccilus anthracis* (mm)
100mg/ml concentration				
Azadirachtha indica (Neem)	6.50^{b}	8.40^{b}	5.65^{c}	7.60^{b}
Persea americana (Avocado pear)	5.40^{c}	6.20^{c}	4.80^{b}	5.30^{c}
Ciprofloxacin (Control)	12.40^{a}	11.80^{a}	10.50^{a}	11.60^{a}
200 mg/ml concentration				
Azadirachtha indica (Neem)	8.70^{b}	10.60^{b}	8.00^{c}	10.20^{b}
Persea americana (Avocado pear)	7.30^{c}	8.50^{c}	6.50^{b}	8.40^{c}
Ciprofloxacin (Control)	15.80^{a}	16.40^{a}	15.00^{a}	16.20^{a}

Values are the mean of two replicates. Means along the column with varying superscripts differed significantly at p<0.05

Table 3: Zone of inhibition of the cosmetics against gram-negative bacteria

Samples	*Pseudomonas aeruginosa* (mm)	*Proteus mirabilis* (mm)	*Escherichia coli* (mm)
100mg/ml concentration			
Azadirachtha indica (Neem)	7.00^{c}	6.50^{b}	7.30^{c}
Persea americana (Avocado pear)	6.50^{b}	5.80^{c}	5.20^{b}
Ciprofloxacin (Control)	10.80^{a}	11.40^{a}	10.30^{a}
200 mg/ml			
Azadirachtha indica (Neem)	9.40^{c}	8.25^{b}	9.00^{c}
Persea americana (Avocado pear)	8.00^{b}	7.30^{c}	6.50^{b}
Ciprofloxacin (Control)	19.40^{a}	21.50^{a}	18.40^{a}

Values are the mean of two replicates. Means along the column with varying superscripts differed significantly at p<0.05

Table 4: shows the minimum inhibitory concentration (MIC) for avocado cosmetics (soap)

Organisms	0.50 mg/ml	1.00 mg/ml	1.50 mg/ml	2.00 mg/ml
Staphylococcus aureus	+	+	-	-
Listera monocytogenes	+	+	-	-
Bacillus anthracis	+	-	-	-
Pseudomonas aeruginosa	+	-	-	
Proteus mirabilis	+	-	-	
Escherichia coli	+	+	-	
Streptococcus pneumoniae	+	+	-	-

Table 5: Minimum inhibitory concentration (MIC) for neem cosmetic (soap)

Organisms	0.50 mg/ml	1.00 mg/ml	1.50 mg/ml	2.00 mg/ml
Staphylococcus aureus	+	-	-	-
Listera monocytogenes	+	+	-	-
Bacillus anthracis	+	-	-	-
Pseudomonas aeruginosa	+	-	-	-
Escherichia coli	+	+	-	+