

Evaluation of Pulp Oil from *Persea Americana* (Avocado Fruit) in Pharmaceutical Cream Formulation

Ordu J I¹ and Jaja G O²

Department of Pharmaceutics and Pharmaceutical Technology,

Faculty of Pharmaceutical sciences

University of Port Harcourt

Nigeria.

ABSTRACT

The cosmetic properties of pulp oil extracted from Persea americana was compared with olive oil after the extraction of oil from Persea americana pulp by cold pressed chemical method using acetone. The percentage oil content from the pulp was 12%w/w. Physiochemical, proximate and elemental analyses were carried out on the extracted oil. Which was characterized based on pH, conductivity, specific gravity and refractive index and both oils analyzed for anti- microbial activities adopting the well diffusion method and using staphylococcus aureus and pseudomonas aeruginosa as micro -organisms. The avocado pulp oil showed zone of inhibition of 1.3-1.7mm against staphylococcus aureus but no activity against gram negative pseudomonas aeruginosa while olive oil did not show any activity against both organisms. Creams formulations were carried out using the extracted avocado pulp oil, mixture of extracted avocado oil and olive oil and evaluated for pH, density, viscosity, sunscreen activity, conductivity, centrifugation and alkali test.

Key words: Cosmetics, Creams, *Persea Americana*, Pulp oil, Olive oil.

1. INTRODUCTION

The secondary metabolites of natural products have been the most successful source of potential drug leads ^[1]. However, their recent implementation in drug discovery and development efforts have somewhat demonstrated a decline in interest ^[2]. Nevertheless, natural products continue to provide unique structural diversity in comparison to standard combinatorial chemistry, which presents opportunities for discovering mainly novel low molecular weight lead compounds. The biosynthesis of secondary metabolites is derived from the fundamental processes of photosynthesis, glycolysis and the Krebs cycle to afford biosynthetic intermediates which, ultimately, results in the formation of secondary metabolites also known as natural products ^[3].

Aged skin exhibits disturbed lipid barrier, angiogenesis and production of sweat, immune functions, and calcitriol synthesis as well as the tendency towards development of certain benign or malignant diseases. These complex biological processes comprise endogenous and exogenous factors. The development of advanced glycation end-products and the declining hormonal levels are major factors influencing intrinsic aging. Chronic photo damage of the skin is the prime factor leading to extrinsic skin aging. The deterioration of important skin functions, due to intrinsic and extrinsic aging, leads to clinical manifestations, which leads to several internal age associated diseases such as diabetes, arterial hypertension and malignancies ^[4].

1.1 Avocado Pear

The avocado is a dense, evergreen tree, shedding many leaves in early spring. It is fast growing and can reach 24m. It is commonly called avocado, alligator pear (English), aguacate, palta (Spanish), avocado (Afrikaans)^[5]. The fruit of the cultivated species vary greatly in size, shape, colour, texture and flavor. The edible part of the fruit, the flesh between the seed and the skin

varies in colour from cream to yellowish green. When ripe, the flesh should have a consistency of soft butter. The fruit has one seed^[6]. Avocado tree is an evergreen tree that attains heights of 40 to 80 feet with many branches. Flowers are small, greenish, and perfect (has both male and female parts)^[7].

1.1.1 Avocado (*Persea americana mill.*)



Fig 1: Avocado Tree



Fig 2: Avocado Fruit

1.2 Planting

Avocado will grow in many soil types, but for best growth and production the soil should be medium textured to provide good aeration. Due to the climatic variability between growing regions, most of the major cultivars are available over an extended period during the season^[8].

1.3 Harvesting

Fruit must be harvested without mechanical damage (cuts, scratches and abrasions), which can affect the cosmetic appearance of the fruit and act as an entry point for postharvest pathogens causing decay and localized softening during storage and transportation^[9]. The usual method of harvesting involves placing the fruit either into a soft picking bag attached to a harvesting pole or directly into a plastic crate to prevent damage of the fruit. For tall trees, hand picking poles or ladder is used for fruit that cannot be reached easily from the ground.

The avocado oil can be extracted in different ways. It is contained in a finely dispersed emulsion in the cells of the fruit pulp. Hence, the extraction process requires rupturing not only the cell walls, but also the structure of the emulsion^[10]. Traditionally, this oil used to be obtained by mashing the pulp in water, then heating and skinning off the supernatant oil. Recently for economic reasons, producers now extract oil from dried fruits by means of solvents^[11]. Two major methods are employed to extract avocado oils for industrial production. The first method involves drying and pressing of the fruit at high temperature and subsequently oil is extracted by means of organic solvents. In the second method, oil is separated from fruits by centrifugal or pressing forces, then oil cells are subjected to mechanical and enzymatic destruction^[12] reason being to cut energy costs and minimize air pollution

caused by organic solvents. Nevertheless, in both cases, the crude avocado oil still needs to be refined before final consumption and use in the cosmetic industry, where it is particularly appreciated for its high vitamin E content and emollient properties ^[13].

2. APPLICATIONS OF AVOCADO OIL

Avocado has many beneficial properties, including a high vitamin E content, which has been linked to a lower risk of coronary heart disease, a high amount of mono-unsaturated fatty acids, which help to reduce LDL cholesterol, a low amount of saturated fat, and high levels of sit sterol, which is believed to depress cholesterol levels ^[14].

2.1 Dosage

Given by mouth the un-saponified fraction of Avocado oil/soya oil has been used for osteoarthritis relief and treatment of the knee at 300 to 600 mg daily dosage ^[15].

2.2 Interactions

Warfarin (coumarin) has been known to slow down blood clotting and avocado oil has been reported to decrease the effectiveness of warfarin (coumarin). Decreasing the effectiveness of warfarin (Coumadin) might increase the risk of clotting. Based on the international normalized ratio (INR), patients may experience a fall in the INR during consumption of avocado (100 and 200 g daily) and when the avocado was eliminated from their diets, the INR increased and adequate anticoagulation was restored ^[16].

The fats in avocados helps the body absorb the carotenoids from other vegetables and researchers has discovered that adding avocado (75-150g) to salads helps in the absorption of these colorful pigments from carrot, lettuce, spinach and other vegetables hence the more avocado added, the more carotenoids were absorbed^[17].

Plants sterols help reduce cholesterol reabsorption in the intestine, thereby increasing the amount of cholesterol excreted from the body. Avocados contain small quantities of plant sterols especially beta-sitosterol around 100mg in a half an avocado ^[18]

A diet rich in fruits and vegetables such as avocado can help to reduce the risk of heart disease, maintain a healthy bowel function, fill one up and control appetite for a long period since avocado is a good source of fibre with around 5g per 120g serving up to 17% of the recommended dietary intake (RDI) for adults ^[19]

Cosmetic application of avocado oil involves its use in shampoos for babies and for damaged hairs, skin and body care, nourishing creams for damaged, dry and sensitive skins, suitable for baby skin, effective in sun products because of its protective action and it could also be found useful in massage oils and products for around the eyes.

Because of its richness in vitamin A, E and D in un-saponifiable compound, the it contains lecithin an essential ingredient for the skin,hence avocado oil has hydrating and regenerating properties and therefore useful as an anti-ageing and anti-wrinkle products, repairs dry skin and renders elasticity to the skin. It also protects the face against external aggressions such as wind, cold or sunlight and relieves itching and burns. When applied to dry hair it revitalizes it and makes it shiny; it plays a role by stimulating hair growth. Its content in un-saponifiable compounds gives its toning, softening and restructuring properties for the epidermis. Its rich content in phyto sterols eases skin penetration and therefore leaves no oily sensation. Avocado oil is effective against parasites and eczema and cares for some forms of rheumatism. Avocado oil can be formulated into a semisolid dosage form like cream containing one or more drug substances dissolved or dispersed in a suitable base ^[20].

3. MATERIALS USED

Glycerin, cetostearyl alcohol, tween 80, sodium lauryl sulphate , methyl paraben, emulsifying ointment, emulsifying wax, white soft paraffin, liquid paraffin, sodium carboxymethyl cellulose , purified water, beaker 250ml (pyrex), stirring rod, water bath homogenizer(eurosonic), Petri dish[disposable plates], Nutrient agar, Peptone water Olive oil, Aluminum made pot], hot plate acetone, muslin bag, syringes (1 and 5ml), Cork borer(6mm) Autoclave Incubator, extracted avocado oil from avocado fruit (*Perea americana*) purchased from Sangana market, Diobu, Port Harcourt, Rivers State , Nigeria.

3.1 Method

3.1.1 Sample Collection

The fruits were bought unripe, left undisturbed for 4 days to ripe, peeled and seed removed while the pulp was hand meshed into paste.

3.1.2 Extraction of Oil

The avocado paste was transferred into a big aluminum pot, allowed to heat using a hot plate for 3 days to evaporate the water content. After evaporation, acetone was added and the mixture was pressed using a muslin bag. The resultant oil was then heated to remove any residual aqueous and non-aqueous solvents present. The crude oil was then weighed to determine the amount of oil extracted.

4. PHYSICOCHEMICAL PROPERTIES

4.1 Determination of Fatty Acid

25ml of diethyl ether was mixed with 25ml alcohol and 1ml phenolphthalein (1%) and carefully neutralize with 0.1M NaOH, then 1.0g of oil or melted fat was dissolved in the mixed neutral solvent and titrated with aqueous 0.1M NaOH shaking constantly until pink colour which persisted for 15 seconds was obtained.

The Acid value was calculated as
$$\frac{\text{Titre volume (ml)} \times 5.61}{\text{Weight of sample used}} \quad \text{----- (1)}$$

The free fatty acid (FFA) is usually calculated as oleic acid (1ml/ 0.1M sodium hydroxide = 0.0282g oleic acid), in which case the acid value = 2 x FFA ----- (2)

For most oils, acidity begins to be noticeable in the plate when the FFA calculated as oleic acid is about 0.5- 1.5% and for palm oil as palmitic (1ml 0.1M NaOH = 0.0256g) while for palm kernel, coconut and similar lauric acid (1ml 0.1M NaOH = 0.0200g).

4.2 Determination of iodine value

1.5 ml of potassium iodine solution (10%) was added with 100ml water, properly mixed and titrated with 0.1M thio-sulphate solution using starch as indicator. Volume at the titration end -point was designated as a ml). A blank titration was also carried out at the same time commencing with 10ml of carbon tetrachloride and titre volume designated as b ml.

Iodine value =
$$\frac{(b-a) \times 1.269}{\text{Wt (g) of sample}} \quad \text{----- (3)}$$

If (b-a) is greater than b/2 the test must be repeated using a smaller amount of the sample. Note that the less unsaturated fats with low iodine values are solid at room temperature and conversely, oils that are more highly unsaturated are liquid (showing there is a relationship between melting points and the iodine value.

4.3 Peroxide Value

1g of oil or fat was weighed and introduced into a clean dry boiling tube and while still liquid 1g of powdered potassium iodide and 20ml of solvent mixture (2ml of glacial acetic acid 1ml volume chloroform) was added

The tube was placed in boiling water and the liquid allowed to boil vigorously for not more than 30 seconds after which the content was transferred immediately into a flask containing 20ml of potassium iodide solution (5%), the tube washed twice with 25ml water and titrated with 0.002M sodium thiosulphate solution using starch.

The peroxide value was obtained by multiplying the Titre value by 2 ----- (3)

4.4 Saponification Value

2g of oil or fat was weighed and introduced into a conical flask and added with 25ml of the alcoholic potassium hydroxide solution.

A reflux condenser was attached and the flask heated in boiling water for 1hr, with intermittent agitation then about 1ml of phenolphthalein (1%) solution added and the hot excess alkali titrated with 0.5M hydrochloric acid giving titre value as = a ml

A blank titration was carried out at the same time with titre value = b ml

$$\text{Saponification value} = \frac{(b-a) \times 28.05}{\text{Wt (g) of sample}} \quad (4)$$

4.5 Proximate Analysis

The proximate analysis (moisture, fiber, ash, lipid and carbohydrates) percentage composition of okra leaves gum was determined.

The moisture and ash were determined using weight difference; fibre content was estimated from the loss in weight of the crucible and its contents on ignition. Carbohydrate was determined when the sum of the percentage of moisture, ash, protein, lipid and fiber were subtracted from 100.

The nitrogen value which serves as precursor for protein content of a substance was determined by micro Kjeldalhi method described by Pearson (1976) ^[21] involving digestion, distillation and titration of the sample. The nitrogen value was converted to protein by multiplying with a factor of 6.25. Carbohydrate was determined by method of difference and all proximate values reported in percentage ^{[22][23]}

4.6 Metal Analysis

The following metals; Fe (248.3nm), Zn, pb (283.3nm) Ca, Mg (285.2nm), Na (589nm) and K (766nm) content of the okra leave gum were analyzed following standard method involving the use of atomic absorption spectrophotometer and in conformity with the absorbance wavelength of the designated metals ions.

5. CREAM EVALUATION

5.1 Density of cream

An empty beaker of a known volume was weighed then a known volume of cream was introduced into the beaker and weighed. The procedure was repeated for the other cream formulations

The density of the cream was determined following the relation:

$$\text{Mass of cream} = \text{weight of beaker and cream} - \text{weight of empty beaker} \quad (5)$$

$$\text{Density} = \text{mass/volume} \quad (6)$$

This procedure was repeated for the density determination of the oil.

5.2 pH Determination of Oil and Cream

A solution of the sample in a beaker was made and an electrode inserted into the beaker. The test was conducted in triplicates and the readings were recorded at each stable deflection

5.3 Conductivity Test of Oils and Creams

The electrode was inserted into a beaker containing the sample .and the tests conducted in triplicates while readings were taken after each stable deflection

5.4 Microbial Analysis Using Well Diffusion Method

19ml of molten nutrient agar was inoculated with 0.5ml of overnight *Staphylococcus aureus* culture and *Pseudomonas aereginosa* cultures respectively. The inoculated agar was then poured into petri dish and allowed to set. Four wells were created using a 6mm cork borer then 100ml of either undiluted oil or nutrient broth was added into these wells and the plates incubated at 37° C for 24hours then the zone of inhibition recorded.

5.5 Centrifugation Test

Centrifugal tests were performed for the 3 creams immediately after preparation. These tests were repeated for each cream after 24 hours, 7 days and 21 days. They were performed at 3000rpm for 30 minutes at room temperature after introducing 10g of the cream samples in each centrifuge tube.

5.6 Refractive Index Measurement

The light turned on and the cool water was ensured to be flowing and then the water temperature recorded using a precision thermometer to 0.1°C

The incident Prism (with the Prism Lock Knob) was opened and the prism face cleaned with acetone and then carefully blotted dry with a Klim Wipe.

Few drops of the liquid to be tested were placed on the polished surface of the lower refracting Prism. The hinged upper Incident Prism was closed and locked into place with the knob, so that the liquid is evenly distributed on the face of the refracting Prism. The lower large adjustment knob was scanned until a light and dark divided image was seen.

Also the dispersion was adjusted using the upper smaller dispersion correction Knob, until a sharp light /dark boundary was seen.

Then the boundary in the crosshairs of the telescope was centered using the lower large adjustment knob and the refractive index read from the green scale below the boundary.

5.7 Viscosity Test

Approximately 550ml of the sample (cream) to be evaluated was poured into a clean 600ml beaker then a temperature probe was attached to spindle guard leg and the viscometer lowered into the beaker until the spindle is fully immersed in the sample.

The procedure was repeated at different temperature while the LVDV II+ Pro motor was turned on to obtain a stable % Torque between 10% and 1000%, then the displayed viscosity was recorded in Centipose (Cp), rpm and %Torque on the viscosity data sheet.

5.8 Free Alkali Test

A 5g of the sample (cream) was weighed and placed in a 250ml flask. The samples were dissolved in 100ml of distilled water by warming gently. The resultant solution was cooled to room temperature, 3 drops of 0.1% methyl orange indicator was added and titrated with 0.05M H₂SO₄ solution.

Calculation:

$$\text{Free alkali (expressed as Na}_2\text{O)} = \frac{V \times 100 \times 0.0031\%}{W} \text{ ----- (7)}$$

Where:

V = Volume (ml) of 0.05M H₂SO₄ solution

W = weight (g) of the sample

5.9 Test for Sunscreen Activity

A 20% w/v solution of the creams was made and the absorbance of each cream formulation measured in a spectrophotometer at different wavelength of 280nm, 310nm, 330nm, 350nm, 370nm, 390nm, 410nm.

The same procedure was repeated for the oils and the standard sunscreen agent (zinc oxide).

A graph of wavelength against absorbance was plotted and the area under the curve determined. The values obtained were compared with the standard sunscreen agent.

5.9.1 Organoleptic Test

The physical characteristics such as: colour, odors, texture, appearance, rheology of the cream formulated were observed and recorded.

6. FORMULATION OF SKIN CREAM

6.1 Working formulae

Ingredients	Percentage composition (% w/w)
Emulsifying Ointment	2.8
Glycerin	7.2
Cetosearyl Alcohol	2.8
Carboxymethyl Cellulose	1.1
Tween 80	5.1
Sodium Lauryl Sulphate	2.8
Methyl Paraben	0.5
Avocado Oil (Olive Oil)	20.7
Water	57.6

6.2 Procedures

An emulsifying ointment was formed by the incorporation of 15g, 25g and 12ml each of emulsifying wax, white soft paraffin and liquid paraffin respectively, into a 250ml beaker and melted in a water bath and the mixture stirred until it was cooled and solidified.

The formulation of an o/w emulsion- based cream (semi solid formulation) was carried out by the adoption of the following procedure:

Firstly the preparation of the oil phase and this involves the mixture of glycerin (7.2%), cetosearyl alcohol (2.8%), emulsifying ointment (2.8%) and avocado oil (20.7%) all melted at 70° C. The second step involves the preparation of the aqueous phase and this involves the mixture of carboxymethyl cellulose (cmc) (1.1%), tween 80 (5.1%), sodium lauryl sulphate (2.8%), methyl paraben (0.5%) , water (57.6%) and heating at 70° C. The third step involves the gradual addition of the oil phase into the aqueous phase at same temperature (70° C) with continuous stirring using an electric mixer until cooling occurred. The procedure was repeated for other formulated creams involving olive oil and mixture of both oils.

7. RESULTS AND DISCUSSION

7.1 Physical Characteristics of Avocado pear oil

Form : liquid

Color : dark pale green

Odor : perceptible and characteristic

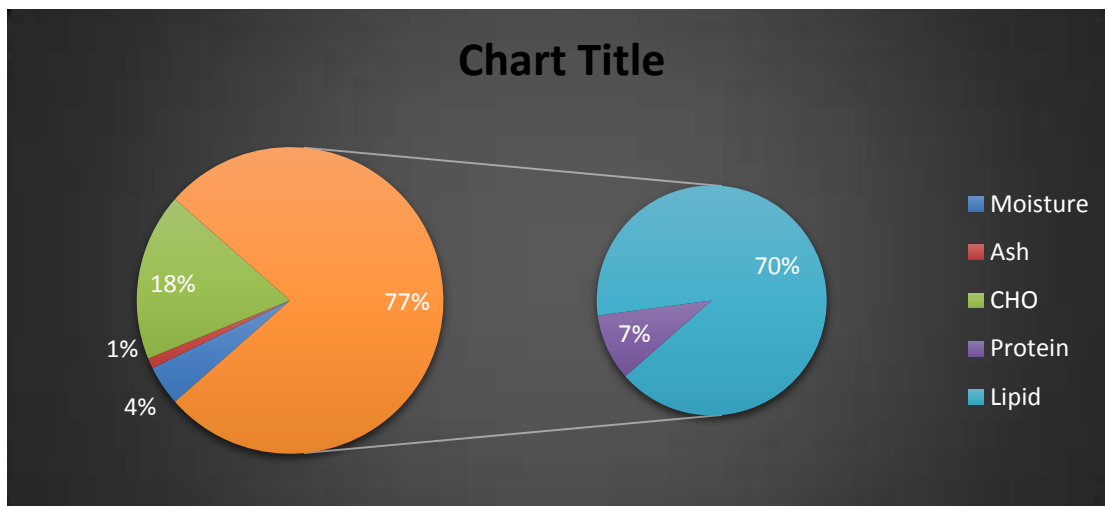


Fig3: Proximate analysis of Avocado pear oil

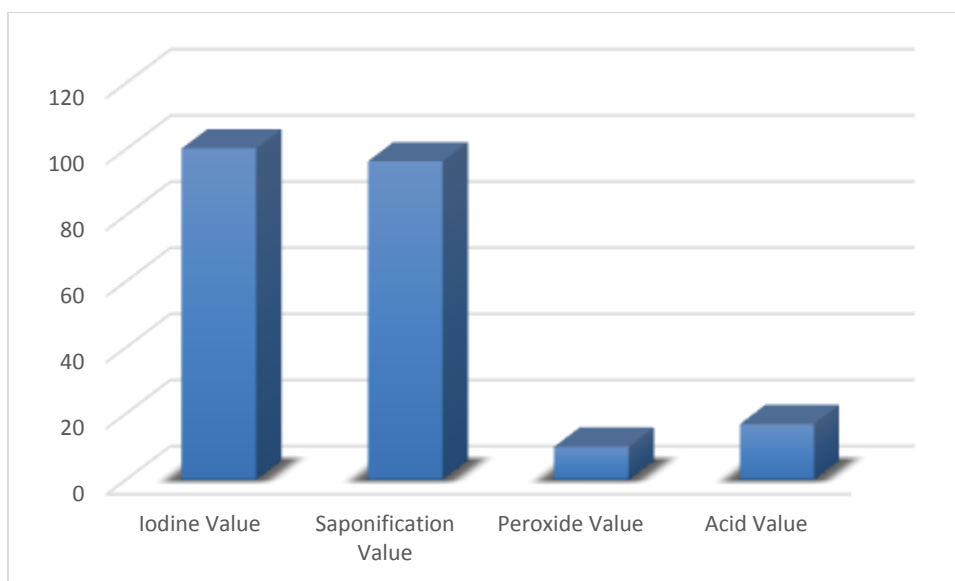


Fig4: Lipid profile of avocado pear oil

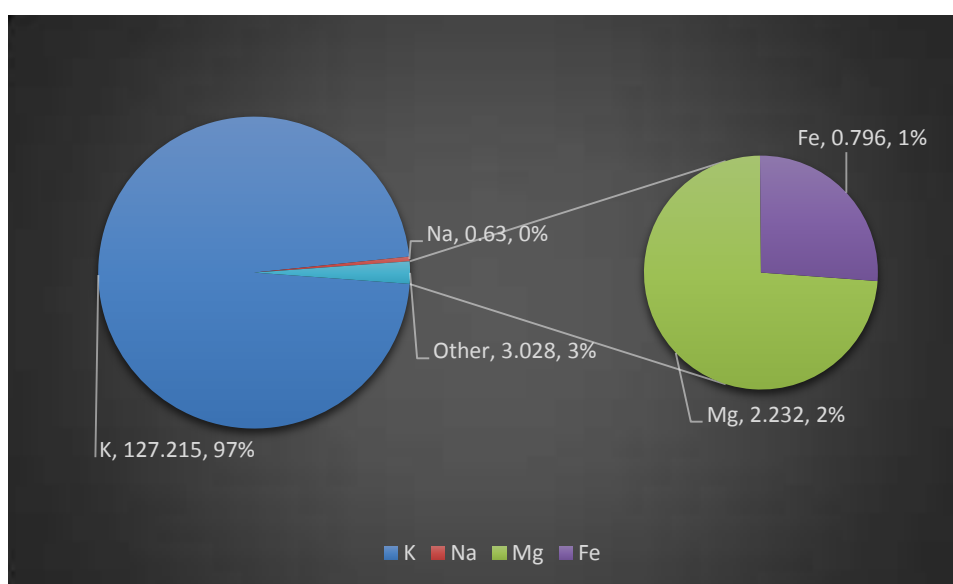


Fig5: Metal analysis of avocado pear oil

Table 1: Result of Extracted Oil Analysis

OIL	pH	CONDUCTIVITY (microS/cm)	SPECIFIC GRAVITY	REFRACTIVE INDEX	PERCENTAGE YIELD(%)
Avocado oil	4.56 at 29.1 ⁰ C	3.2	0.9107	1.47	7.3
Olive oil	6.0 at 29.0 ⁰ C	0.2	0.703	1.44	

CREAMS EVALUATION

Table 2: The Result of Creams Evaluation

CREAMS	pH	CONDUCTIVITY (micron hos/cm)	ALKALIS TEST	DENSITY (g/ml)
Avocado cream	5.36 at 28.6 ⁰ C	1444	0.0744	1.3
Olive cream	6.45 at 29.1 ⁰ C	1807	0.062	1.3
Mixture cream	5.53 at 28 ⁰ C	1556	0.062	1.4

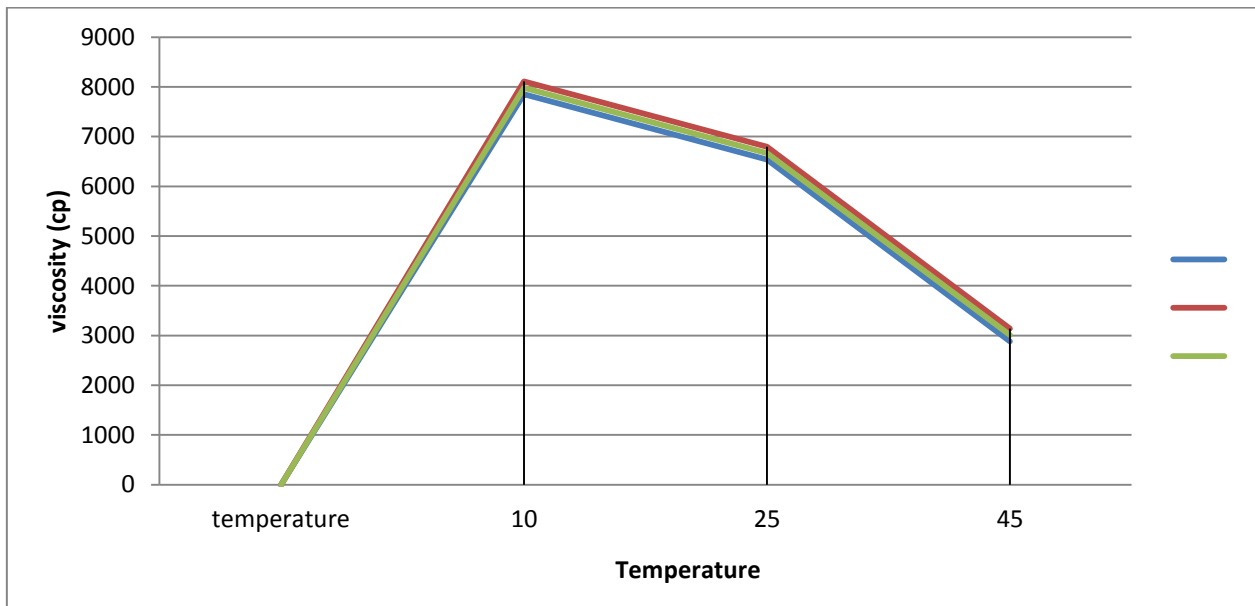


Fig 6: Viscosity Characteristics of Formulated Creams

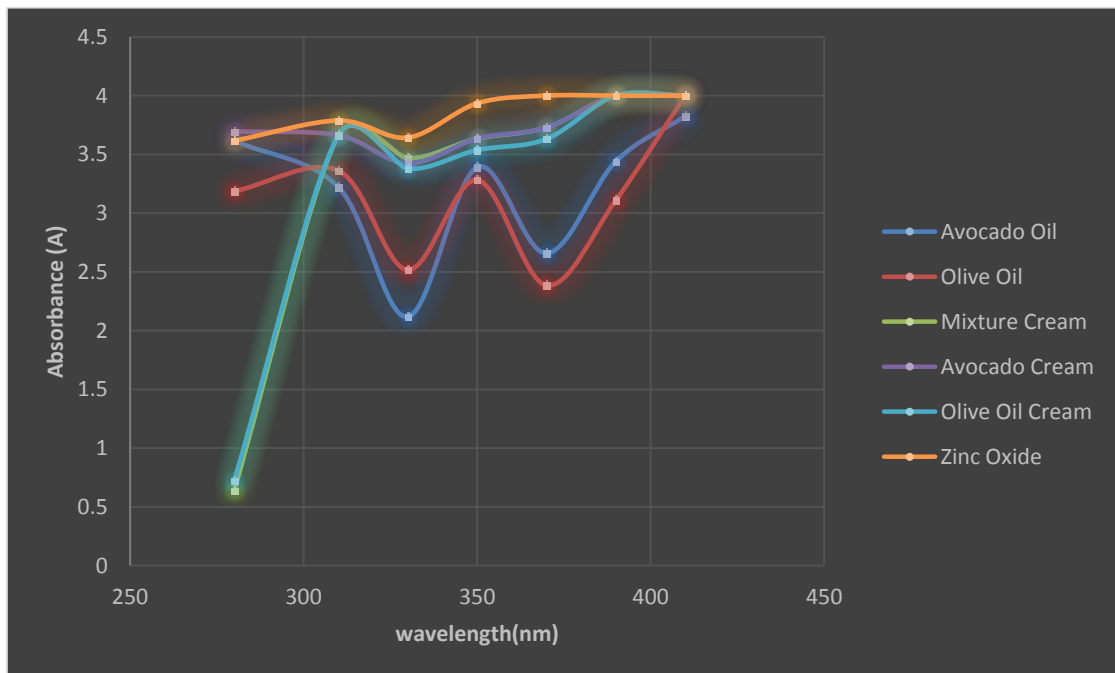


Fig 7: Sunscreen activities of Creams, Oils and Zinc Oxide used as standard

DISCUSSION

Avocado from several studies contains 73% of water and the oil content is estimated to be about 18%. This much is often obtained from species called Hass which is the specie known to contain the largest amount of oil. The percentage yield of the oil from the specie used for the research was 12%. This result is due to the type of species used therefore, percentage yield of avocado oil is species dependent although the species used for this research was not identified.

According to the proximate analysis in fig 3, avocado oil contains 18% of carbohydrate. Carbohydrate is very important for a healthy and glowing skin. Ageing is caused by the presence of free radicals which are produced in the body when certain substances or compounds undergo oxidation. These free radicals are called oxidant and can bind to cell surfaces to cause cell mutation which could lead to cancer, causing reduction in the life span of cells and resulting to cell death. By this same activities, ageing and wrinkling of the skin can also occur. The presence of carbohydrates therefore, facilitates the release of large amount of antioxidants which locates the free radicals and bind to them forming nontoxic or none harmful substances that can easily be excreted from the body. This action keeps the skin healthy, glowing and youthful, this therefore implies that use of avocado oil can help to prevent skin cancer as it is assumed to have high penetrating activity.

From the result in fig 4., it shows that iodine value of avocado oil is 100.24. Iodine value is defined as the mass of iodine in grams that is consumed by 100g of a chemical substance. It shows the amount of unsaturation in fatty acid. An iodine value of 100.24 therefore indicates high content of unsaturated fatty acid in the oil. , and this could be useful especially for human body as it could further help to reduce several heart conditions and it also shows that the avocado oil can be of use in soap and cream formulation.

From the result in fig 4, the saponification value is 196.35mgKOH/g. Saponification simply means hydrolysis and it is defined as the amount of KOH (potassium hydroxide) in mg that is required to hydrolyze 1g of an oil or fat. The higher the saponification value the shorter the fatty acid chain present and the higher the amount of carboxylic acid group and vice versa. The higher the molecular weight of the fat or oil the smaller the saponification value.

The result in fig 4, also shows that the peroxide value is 10mEq/Kg. This value is low as peroxide value indicates the degree of rancidity and further depicts the level of the stability of the oil. A peroxide value between 30 to 40mEq/kg will give a rancid taste while peroxide value of 10mEq/kg indicates good oil with degree of low rancidity.

In fig 5, concerning the metal analysis the avocado oil contains a large amount of potassium (127.215mg/L) and this is about 97% of the metal content in the oil. Potassium plays an important role in cell integrity by maintaining electrolyte balance and internal fluids consistency. It helps to keep the skin cells hydrated and internally moisturized hence when potassium is low in the body, the skin cells loses water and become dry. This makes avocado oil good for individuals with dry skin hence avocado oil is known to be a very good moisturizer due to the high percentage content of potassium.

According to the result of the microbial test a zone of inhibition of 1.3-1.7mm against *staphylococcus aureus* was observed but inactivity was observed against *Psuedomonas aeruginosa*. Olive oil on the other hand showed no zone of inhibition against both organisms. *Staphylococcus aureus* is a gram positive organism, and the result indicates that avocado oil could have antimicrobial activity against gram positive organisms than gram negative *psuedomonas aeruginosa*.

According to the result in Table 1, avocado oil had a pH of 4.56 which tends to be acidic, also similar observations was identified for olive oil which was 6.0. From the Table. 2, the three cream s formulated showed pH of 5.36, 6.45.5.53 at different temperatures for avocado, olive and mixture cream respectively. These pH values showed that they are acidic and are close to that of the skin pH which ranges from 4 to 6. This indicates that there would be no corrosion when the creams or oil is applied on the skin since their pH fall within the range as the skin.

As in same Table 2, all three creams formulated showed low amount of free alkaline contained in them. This free alkali test indicates the amounts of alkaline contained in the creams and since the alkaline content of all three(3) creams are 0.0744,0.062,0.062 for avocado, olive and mixture cream respectively which is very low. This means there would be limited foaming effect, bleaching or harsh reaction upon application of the formulated creams on the skin.

Conductivity shows the ability of a solution to conduct electricity by process of ion movement. The result of the conductivity tests of the oils and creams from Table 1 and 2 showed low degree of conductivity which is good for the skin and indicates limited ion migration of components of the creams and this could result to little or no chemical interaction between components of the cream and between the cream and the skin that it comes in contact with.

According to the result from fig 6, it showed that viscosity decreases as temperature increases but that of olive oil cream was not much affected and this indicates some inconsistency in the rheology of the creams although it shows that the viscosity of the creams are highly affected by temperature and environmental conditions.

Sunscreen activity is the ability of a substance to absorb ultraviolet light. From the result of the sunscreen test in fig 7, it showed an absorbance at wavelength within the ultra violet region of 280 to 400nm. The absorbance of the oils and creams show was equivalent to that of zinc oxide (a standard sunscreen agent) at wavelength of 390 and 400 nm. This shows that the avocado cream and oil could be useful as an effective sunscreen agent and can help to protect the skin against ultra violet light upon application.

CONCLUSION

Avocado pear is a rich source of oil and contains high mono unsaturated fatty acids and potassium. It is rich in carbohydrates and has sunscreen and antibacterial activity. It also highly penetrates into the skin and has moisturizing, antioxidant, emollient and toning effect keeping the skin glowing, healthy and youthful. The edible oil obtained from the pulp is said to be superior oil that compares favorably with olive oil. The result of the formulation have provided much justification for the use of avocado oil in cosmetic formulation and can serve as an alternative to other vegetable oils such as arachis oil, almond oil or olive oil used previously in cosmetic formulation.

The study therefore recommends that the oil obtained from avocado pulp should be considered for use in cosmetic industries and further work be done to determine its toxic or adverse effect since none was observed or reported during the course of the study.

Acknowledgements

Recognition is given to the laboratory staff of the Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, University of Port Harcourt, for their technical support and contribution towards the successful completion of this research work.

References

- 1: AD Daniel, U Sylvia and E Ufe. "A historical overview of natural prodrug in drug discovery". *Metabolites*, 2012; 2(2): pp303 – 336
- 2: MC Gordon and JN David. "Natural products a continuing source of novel drug leads". *Biochem Biophys Acta*, 2013; 1830(6): pp3670 – 3695
- 3: SD Jeroen." Biosynthesis and function of secondary metabolites." *Beistein J Org chem*, 2011; 7: pp1620- 1621
- 4: AF Miranda E Peter KM Miller and Howard M Howard." Intrinsic and extrinsic factors in skin ageing". A Review. *International journal of cosmetic science*, 2008; pp30(2): 87 – 95
- 5: AM Whiley, B Shafter, BN Wolstenholme " The avocado botany, production and uses". *CAB* , 2002; Pp 30. ISBN 0851999786
- 6: C Boning " Florida's best fruiting plants. Nature and Exotic Trees shrubs and Vines" Sarasota Florida pineapple press inc., 2006; pp3: 33-156
- 7: M Dreither. "Davenport AJ: Hass avocado composition and potential health effects" *Crit. Rev. food Sci. Nutr*, 2013; 53(7): pp738 – 50
- 8: VM Michael , WR Michael, W Guy and L Mary. "Hass" avocado tree grown on four root stocks in California: Shoot and root growth". *Scientia Horticulturae*, 2012; pp143: 205 – 210
- 9: T : Justra and R David. "Tips on how to grow and harvest avocado pear." *The Tribune*, 2016; 2: pp92-162
- 10: C Garcoma, B Matteo: "Avocado oil extraction: Method for cold pressed high quality edible oil production versus traditional production". *Journal of Agricultural Engineerig*, 2015; vol 46 issue3 pp 467
- 11: Q Xiaoli and Z Jinfeng." A review of extraction techniques of avocado oil" *Journal of Oleo sciences*, 2016; 11: pp881 – 889

- 12: W Dungen, L Xangum, H Xuergiong, S Wiemin and Z Mio.” Combined enzymatic and mechanical cell disruption and lipid extraction of Green algae” *Neochloris oleoabundans*. *Int. J. Mcb. Sci*, 2015; 16(4): pp7707 – 7722
- 13: AM Woolfe, L Wang, TM Eyres and CR Jacksman: “Avocado oil from cosmetic to culinary oil in Gaumet and Health – promoting speciality oils” R. Morecare and Kamaal A – Edim. Eds. AOCS press, Urbana, USA, 2000; pp73 – 125
- 14: P Lonseca, M Duarte, C Ahes, C, Borges D Carolina RB Madonca. “Avocado: Characteristics, health benefits and uses”. *Food Technology Clenca Royal Santa Maria*, 2016; 46(4): pp747 – 754.
- 15: MM Aluizouri, MJ Cmmanze, AE Henrera, CF Charez, GL Amezone.” Effect of avocado as a source of mono unsaturated fatty acids on plsma high levels” *Arch med Res*, 1992; 23: pp163 – 167
- 16: MS Buckley, AD Goff, WE Kapp.” Fish oil interaction with warfarin.” *A. M Pharmacother*, 2004; 38(1): pp1550 – 2
- 17: NZ Nnlu, T Bolm, SK Clinton, SJ Schuartz. “Carotenoid absorption from salad and salsa by hormones is enhanced by the addition of avocado or avocado oil”. *J. Nutri*, 2005; 135(2):pp 431 – 5
- 18: H Gylling, TA Mcettinen.” Plant sterols and artery disease in bioactive food as dietary intervention in cardio vascular disease”. *Encyclopedia of food sciences and Nutrition*, 2013; 2: pp681 – 691
- 19: Z Pietersie, JC Jerhima, W Oosthuizen, HS Knugger. SM Hanmerkon, CM Smutts, AE Scutte. “ Substitution of high mono unsaturated fatty acid avocado for mixed dietary fats during an energy restricted diet: effects of weight loss serum lipids, fibrinogen and vascular function” *Nutritio*, 2005 ; vol21 issue1: pp167 – 175
- 20: C Thitiphan. “ Formulation of skin cream from avocado oil and adlay seed extracts”. *Paccon*, 2017; 1: pp54-94
- 21: P Gues. “ Kjeldahl method – Estimation of protein in food”. *Discover food tech*, 2017; vol 1 pp340- 692.
- 22: Association of official analyticalchemists(AOAC): Agricultural, Chemical Contaminants, Drugs. 15th edition, 1990; Artinhtin Virginia 22201, USA
- 23: American oil chemist society (AOCS): Online version of two Aocs press journals.