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Production of Natural Antioxidant Concentrate Using Locally Available Raw Materials for Household Cosmetics

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Abstract

Background*: Most of the things that man uses (the food, cloth, cosmetics, drugs, etc are derived from plants or use or need a product derived from plants). With the increase in global population and industrialization, the whole world is devising means of producing products that are safe, cheap, readily available and environmentally friendly to meet the needs of the growing population. This, will therefore, lead to complete utilization of plant resources which will in turn help in achieving the sustainable development goals.*

Materials and Methods: *The leaves of three different plants, Kigelia Africana, Mitracarpus hirtus and Bryophyillum pinnatumwere investigated for the presence of secondary metabolites (flavonoids). The leaves Were defatted with n- hexane and was extracted with methanol. The methanol extract was purified by fractionation methods using solvent mixtures of water, n- hexane and t-butylmethylether (90:9:1), the aqueous phase was farther purified by successive extraction with n- hexane, ethyl acetate and acetone. The acetone extract was then used for the 1solation of flavonoids using liquid chromatography using n- hexane: ethylacetate mixture (20:80) as a solvent. The flavonoids isolated were phytochemically screened, tested for their antioxidant activity and antimicrobial activities against Salmonella typhi, Stapiylococcs aureus and candida albicans. Also, the isolates were used in the production of antioxidant concentrate which was applied in the production of a household cosmetic product (body cream).*

Results: *The results showed that all the isolates contain flavonoids which have varying antioxidant activities as compared to the standard (Quercetin). Also, Kigelia Africana had high level of antimicrobial activities against Candida albicans, Mitracarpus hirtus had good antimicrobial activity against Staphylococcus aureus while Bryophyillum pinnatumshowed no antimicrobial activity against all the test organisms.*

Conclusion: *The body cream produced using the antioxidant concentrate showed that it was good and acceptable within analytical test limits.*

Key Words: *Antioxidants, Cosmetics, Plant extracts, Sustainable development*

I. INTRODUCTION

A plant is any one of the vast number of organisms within the biological kingdom, plantae; these species are considered of limited motility and they manufacture their own food. They include a host of familiar organisms like trees, shrubs grasses, ferns, mosses etc. Conventionally, the term plant refers to a taxon that has multicellular cell structure containing cellulose cell wall and organelles capable of photosynthesis (Michael and Daniel, 2011) Most of the things that man uses (the food, cloth, cosmetics, drugs, etc are derived from plants or use or need a product derived from plants). With the increase in global population and industrialization, the whole world is devising means of producing products that are safe, cheap, readily available and environmentally friendly to meet the needs of the growing population. This, will therefore, lead to complete utilization of plant resources which will in turn help in achieving the sustainable development goals.

*Bryophyillum pinnatum*which is synonymous to *kalanchoe pinnata* and commonly called Life plant, Air plant, Maternity plant, Love plant and Cathedral bells in English. It is an ornamental plant that grows as weed around plantations and crops it is a perennial herb growing widely and used in folkloric medicine in tropical Africa, America, India, China and Australia. The plant flourishes throughout the Southern part of Nigeria. (Dalziel *et al.*, 1955).

Mitracarpus hirtus is a shrub plant that flourishes throughout the Northern part of Nigeria. It is commonly known as Googamasu in Hausa (Wagner ef al. 1999). Also the plant was commonly used externally for the treatment of eczema, pimples and rashes (Nassis, 1992)

Kigelia Africana occurs throughout tropical Africa, particularly in the drier regions. It is also found in South Africa (Northern Province, kwazulu-Natal) and Swaziland, it has been introduced as an ornamental to Cape Verde and Madagascar, as well as to Iraq, Pakistan, India, Chana, South-East Asia, Australia, Hawaii, Central and South America *Kigelia Africana* derives its name from the unusual "sausage shaped fruit that hangs from its long stalk which often grows over a meter in length and can weigh up to 10kg. The hard, grey fruit has a thin skin covering a firm, fibrous fruit pulp.

Natural products play a dominant role in the development of novel drug for the treatment and prevention of diseases. A large number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials in order to promote the use of medicinal plants as potential sources of antimicrobial compounds It is therefore quite necessary to thoroughly investigate their composition and activity in order to validate their use (lrawan *et al.*, 2014)

Some phytochemicals produced by plants have antimicrobial activity and are used for the development of new antimicrobial drugs. it has been shown that in-vitro screening methods could provide the needed preliminary observations to select crude plant extracts with potentially useful properties for further chemical and pharmacological investigations. The importance of these phytochemicals is seen in their application in the production of drugs, agricultural chemicals, industrial raw materials, food products and cosmetics (creams).

A cream is defined as “Any substance or preparation intended to be placed in contact with the various external parts of the human body (epidermis, hair system, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance and/or correcting body odors and/or protecting them or keeping them in good condition" (Cadenas and Packer, 2002).

For skin to remain in good condition, it is important to maintain an adequate level of moisture. This is affected more by external factors such as humidity rather than the amount of water intake. Moisture is constantly lost from the skin (Tans-epidermal Water Loss) and effective moisturisers should replace this lost moisture and or help protect skin from further dehydration (Cadenas and Packer, 2002)

Many substances, with more or less complex chemical structures, have been found to possess antiradical activity and have been introduced onto the market as anti-ageing products (Andreassi and Andreassi, 2004). There are synthetic antioxidants such as Butylated Hydroxy Toluene (BHT) and Butylated Hydroxy Anisole (BHA). Natural antioxidants (like tocopherols or Vitamin E) are defined as any substance, which inhibits or delays an oxidative change (Cadenas and Packet, 2002). These synthetic antioxidants are approved cosmetic additives, international regulations tend to establish more and more restrictions to their use and the consumer increasingly prefers to avoid synthetic additives in favour of those perceived as natural antioxidants. Cosmetological research has increasly focused on process leading to the formation anatomical-functional damage to the skin, identified with ageing At the same time, every possible means to counteract the injurious effects have been evaluated. Great interest in this topic has been aroused by the study of substances able to prevent cutaneous damage by tree radicals these substances are currently named as antioxidants.

The aim of this study is to produce an antioxidant concentrate from the isolates from the leaves of *Bryophyillum pinnatumKigelia Africana* and *Mitracarpus hirtus* and apply it in the production of body cream. The objectives of this work include:

a. To isolate flavonoids from the leaves of *Bryophyillum pinnatumKigelia Africana* and *Mitracarpus hirtus* plants.

b. To prepare a natural antioxidant concentrate using the flavonoids isolated from the leaves of *Bryophyllum pinnatum*, *Kigelia Africana* and *Mitracarpus hirtus* plants.

c. To produce a body cream using the flavonoids isolated from the leaves of *Bryophyillum pinnatum Kigelia Africana* and Mitracurpus hirtus plants as antioxidant.

d. To compare the results of a test cream produce using a natural antioxidant and a standard cream commercially sold in the market (control).

Since these cosmetic products are applied directly to the skin, it is very important to be cautious on the component to be used and also the end product. Therefore, the practical effects of some of the component of the product (cosmetics) have to be assessed to ascertain their safety and acceptability. This is necessary because many a times, product do not usually completely agree with theoretical result. They usually deviate either partially or completely from theoretical result (Sebastian et., 2017).

The scope of this research work involves the isolation of the antioxidants from the three different plants (*Bryophyllum pinnatum*, *Kigelia Africana* and *Mitracarpus hirtus* plants), phytochemical screening of the extracts, isolation of flavonoids from the extracts, antimicrobial activity lest of the isolates against test micro- organisms that affect the skin, production of an antioxidant concentrate using the isolates from these plants and the application of the antioxidant concentrate in the production of a body cream which is one of' the household cosmetics.

The *Bryophyllum pinnatum* Plant: Dalziel et al (1955) stated that *Bryophyllum pinnatum* is an introduced ornamental plant that is now growing as weeds or flowers around flowering gardens, plantations or crops. It is well known for its hemostatic and wound healing properties. It is rich in flavonoids which are water soluble polyphenolic molecules belonging to the polyphenol family found in most plants. The crushed leaves as well as the extracted juice are usually mixed with shear butler or palm oil and rubbed on abscesses or other swellings. This is also applied to ulcer, burns and on bodies of young children when they are ill (Agoha, 1974). *Bryophyillum pinnatum*is effective in the treatment of typhoid fever and other bacterial infections, particularly those caused by Staphylococcus aureus, E coli, K pneumonia, Salmonela typhi P. aeruginosa and B Subtills. If is also used in ethnomedicine for the treatment of car ache, bums, abscesses, ulcer, insect bites, diarrhea and lithiasis. In fact, the high saponin content justified the use of the extract trom the plant to stop bleeding and in the treatment of wounds. Research also carried out in the mid- 19S0s showed that Broyptyllum pinnatum leaves have antiviral, antifungal and antibacterial actives. The leaf Juice had demonstrated significant antibacterial activities towards Staphytlococcus, Escherichia coli Shigella, Bacilhus and Pseudomonas including several strains ot multt drugs resistant bacteria. (Obaseiki-Ebor, 1993)

The *Mitracarpus hirtus* Plant: The dried leaves of *Mitracarpus hirtus* plant along with leaves of nyctanthes were made and given oraly. The antihypertensive efficacy of the aqueous aond methanol extract of the leaves were examined in arterial blood pressure and heartbeat rates of normal spontaneous hypertensive rates using invasive and non-invasive techniques. Both extracts produced showed significant decrease in arterial blood pressure and hypertensive rats. The hypertensive effects of the leaves extracts were more pronounced in hypertensive rats than in non- intensive rats (Nassis, 1992).

The leave extract also produced showed a significant decrease in the rats and guinea pig isolated and inhibited electrical field stimulation. In addition to its antibacterial activities, the traditional use of *Mitracarpus hirtus* for upper respiratory condition and cough had been validated by studies demonstrating that the leaf juice has potent antihistamine and anti-allergic activities (Nassis, 1992)

The *Kigelia Africana* Plant: Invitro studies showed that *Kigelia Africana* plant has anti-allergic, anti-inflammatory, anti-cancer, anti-mierobial and anti-diarrhoea activities. Consumers and food manufacturers have become interested in 1lavonoid for their petulance role in prevention or cancer and cardiovascular disease. The flavonoids may also influence other organic and inorganic compounds such as coumarins, phenolic acids and micronutrients, Such as Copper, manganese, and zinc (Gadeotti et al, 2008).

Antioxidant Activity of Flavonoids

Oxygen is highly required by the body during cellular activities. It keeps the cells healthy and millions of processes in the body, functioning properly. However, oxygen can also cause problems to the body. It has provided humans with the benefit of fats, proteins and carbohydrates metabolisms for energy. However, oxygen being a highly reactive atom is capable of becoming part to potentially damaging molecules commonly called "tree radicals" Free radicals are capable of attacking the health cells of the body, causing them to lose their structure and function. Free radicals are formed as part of our natural metabolism but also by environmental factors, including smoking, pesticides, pollution and radiation. Free radicals are unstable molecules that easily react with essential molecules or our body, Like DNA, fat and proteins. All organic and inorganic materials are unstable because they have less than normal or too many electrons to attain a state of maximum stability. This arises as a result of giving away or receiving electrons from other atoms, thereby forming unstable molecules. Free radicals have high tendencies of attending chemical stability. When a free radical attacks a molecule, the molecule will itself become a free radical thereby causing a chain reaction which can result in the destruction of a cell. Cell damage caused by free radicals appears to be a major contribution to g and to degenerative diseases of aging such as cancer, cardiovascular diseases, cataracts, immune system decline and brain malfunction (Briviba and Sies, 1994). Antioxidants are chemicals that offer their own electrons to free radicals, thus preventing cellular damage. Although, the initial attack causes the free radical to become neutralized, another free radical is formed in the process, causing a chain reaction to Occur. Until subsequent free radicals are deactivated, thousands of free reactions can occur within the seconds of the initial reaction. Antioxidants are capable of stabilizing or deactivating free radicals before attacking cells (Halliwell and Gutterige, 1994).

Reactive Oxygen Species

Reactive oxygen species (ROS) is a term which encompasses all highly reactive, oxygen- containing molecules. This includes free radicals, hydroxyl radical, the superoxide anion radical, hydrogen peroxide singlet oxygen, nitric oxide radical, hydrochloride radical and various lipid peroxides. All are capable of reacting with membrane lipids, nucleic acids, protein and enzymes, and other small molecules, resulting in cellular damage (Harborne, 1979). In normal aerobic metabolism: approximately 90% of the oxygen utilized by the cell is consumed by the mitochondrial electron transport system (Halliwell and Gutterige, 1994).
Mechanism for the reaction of antioxidants with radicals

1. Hydrogen donation to free radicals by antioxidants:
a. R.+AH> RH + A.
b. RO.+AH-> ROH+A.
c. ROO.+ AH> ROOH+A.

2. Formation of a complex between the lipid radical and the antioxidant free radical acceptor:
R+ARA
RO.+A.-> ROA
ROO+A.-ROOA

3. Metal chelation:

Antioxidant+ O2 ⟶ Oxidized Antioxidant

Flavonoids have been shown to act as scavengers of various oxidizing species that is superoxide anions (O2), hydroxyl radical or peroxy radicals. They may also act as quenchers of singiet oxygen (Tomaire *et al.*, 1993). If a compound inhibits the formation of free alkyl radicals in the initiation step, or if the chemical compound interrupts the propagation of the free radical chain, the compound can delay the start or slow the chemical reaction rate of lipid oxidation. The initiation of free radical formation can be delayed by the use of metal chelating agents, singlet Oxygen inhibitors and peroxide stabilizers. The propagation of free radical chain reaction can be minimized by the donation of hydrogen from the antioxidants and the metal chelating agents. The major antioxidants used in Cosmetics are methyl para- benzoate and propyl –parabenzoate compounds

II. MATERIALS AND METHODS

Sample Preparation

The leaves of *Bryophyillum pinnatum* was collected from Mechanical engineering garden of the Federal Polytechnic, Bauchi while the leaves of *Kigelia Africana* and *Mitracarpus hirtus* were obtained from Dass Local Government Area of Bauchi State. The plants were identified by a botanist in Abubakar Tafawa Balewa University, Bauchi. The leaves were sun dried and pulerzied into fine powder and sieved using a laboratory test sieve of 212µm aperture

Extraction

Samples of *Bryophyllum pinnatum*, *Kigelia Africana* and *Mitracarpus hirtus* (30g of each) was weighed into a thimble, and it was defatted with n-hexane (250ml) using soxhlet extractor the extraction was carried out for about six hours at a temperature of 70'C. After the extraction, the thimble was removed, dried in an oven at 50'C. The defatted mare was further extracted with Methanol (250m) at 70'C. The Extract was dried using rotary evaporator.

Phytochemical Screening

The extracts were phytochemically screened using standard methods describe by Sotowora (985), Trease and Evans (1989) and Harbone 1979) in order to detect the presence of the active components or naturally occurring substances in the plant. The extracts were screened for the presence of Saponins, Flavonoids Alkaloids, Steroids, Tannins, anthraquinone and cardiac glycosides.

Preliminary Purification

The methanol extract (10 ml) was preliminarily purified using the method described by Marica *et al.*, (2004), It was dissolved in a solvent mixture of Water, n- Hexane and t-butyImethylether (90: 9: 1). It was shaken in a separatory funnel and allowed to stay overnight. The aqueous phase containing the polar components was separated and then dried with Rotary evaporator. The aqueous extracts were re-fractionated with n- hexane, ethyl acetate and acetone to reduce the complexity of the various components using laboratory separatory funnel after which they were then dried.

Thin Layer Chromatography (TLC)

The Acetone extract was selected (as target for glycosidic flavonoids) and analysed by means of TLC. Different solvents mixtures of Hexane ethyl acetate Methanol of different polarities were tried like 45:55:0, 40:60:0, 35:65:0, 30:70:0, 25:75:0, 20:80:0, 10:90:0, 5:95:0, 0:100:0, 0:95:5 and 0:90:10. The TLC plates were sprayed with vanillin solution (15g of vanillin in 250ml ethanol and 2.5ml conc. sulphuric acid). It was hexane: ethyl acetate (20:80) ratio that gave a better separation. And so it was chosen for the isolation of flavonoids using column chromatography

Isolation of Flavonoids Using Column Chromatography

The column was packed with silica gel (60:120 mesh), In each case the silica gel to sample ratio was about 30:1. The packed column was washed several times with n-hexane followed by ethyl acetate and finally with methanol. The mobile phase (Hexane: ethyl acetate) 20:80 was used and the fractions (Smi cach) were collected with the aid of a fraction collector (CF-2) and a peristaltic pump The extract (0.8g) was introduced into the column. At the end of the column, that fraction was re- analyzed by means of TLC again using n-Hexane and ethyl acetate (20:80) as mobile phase (with one drop of Acetic acid). The fractions that have the same Re values were merged together in a clean and dried container, and was then dried.

Antimicrobial Activity

Preparation of test organisms

Test organisms used were Staphylococcus aureus (S. aureus), Candida albicans, (C. albicans) and Salmonella typhi (S. typhi). They were aseptically purified, sub cultured and grown on 10ml sabouraud dextrose agar slants and thereafter kept in a refrigerator at a temperature between 2'C and 8^{O}C

Preparation of reference antifungal agents

Stock solutions of fluconazole was prepared by dissolving appropriate quantity or tne antifungal agent in dimethyl sulfoxide (DMSO) while ketoconazole was dissolved in methanol and later diluted to their required concentrations with broth.

Preparation of agar plate

Müller-Hinton Agar, MHA (6.84g) was weighed and dissolved in distilled water (200ml) in a 250ml conical flask and sterilized in an autoclave at 121°C for 15 minutes together with the petri dishes. The MHA was removed and allowed to cool to 45 C. The MHA was poured into the sterilized petri dishes of 10mm thickness and allowed to solidify at room temperature. Each bacterial strain was sub-cultured on the MHA and incubated for 24hrs at 37C after which the bacterial colonies were observed. Using a sterilized cork borer,

three (3) wells were punched on each plate and the pure stock solution of the antimierobial agent was poured (200ul/well) and also incubated at 37ºC for 24hrs. The zone of inhibition was observed and measured.

Dilution of stock solution

The pure solution of the samples of *Bryophyllum pinnatum*, *Kigelia Africana* and Miracarpus hirtus were serially diluted using 10-fold dilution, where 100µl was transferred in 900µl N/S in Eppendorf tubes and serially diluted to 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} µl respectively. It was securely covered and kept in a laminar flow hood to avoid possible external contaminants.

Another Mueller Hinton Agar (MHA) was prepared according to the dilution rate and the bacterial strains were also sub-cultured and incubated as described. After obtaining the bacterial colonies, holes were punched on the MHA containing the grown colonies using sterile cork borer and the Samples that had been serially diluted were inoculated. The Minimum Inhibitory Concentration (MIC) values were determined using MIC evaluator strip after incubation at 37C for another 24 hrs.

Determination of total flavonoid content

The total flavonoid content of crude extract was determined by the Aluminium chloride colorimetric method. The crude extract (2 mg/ml ethanol) were made up to I mL with methanol, mixed with distilled water (4ml) and then 5% NaNO2 solution (0.3 M). 10% AlCl; solution (0.3ml) was added after 5 min of incubation, and the mixture was allowed to stand for 6 min. Then, 1.0 Mol NaOH solution (2ml) were added, and the final volume of the mixture was brought to 10 ml with distilled water. The mixture was allowed to stand for 15 min, and absorbance was measured at l0 nm. 1he total flavonoid content was calculated from a calibration curve of rutin (10-250 µg) plotted by using the same procedure and total flavonoids was expressed as rutin equivalents in milligrams per gram sample (Nilesh *et al.*, 2012).

Measurement of antioxidant activities

The antioxidant activity of the fractions was determined on the basis of their scavenging activity of stable 1, 1-diphenyl-2-picryl hydrazyl (DPPH) free radical. To each solution (lml) of different concentrations (between 1-500µg/ml) of the isolates, 0.004% ethanol DPPH free radical solution (3ml) was added. After 30 minutes, the absorbance of the preparations were taken at 517nm using UV spectrophotometer. The absorbance of the standard (quercetin) prepared in the Same way as of the isolates (with concentrations between 1-500ug/ml) was taken. The method described by Hatano *et al.*, (1988) was used to measure the absorbance with some modifications.

Then the percentage inhibition was calculated by the following equation

$$\%\ Inhibition = \frac{Absorbance\ of\ blank - Absorbance\ of\ Sample}{Absorbance\ of\ Blank} \times 100\ \%$$

From the calibration curves obtained from different concentration of the fractions, the inhibitory concentration was determined. ICs0 value denotes the inhibitory concentration of the sample required to scavenge 50% of the DPPH free radicals (Gupta *et al.*, 2003).

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Attempted 1dentification of components isolated using Reversed-Phase (RP) high performance liquid chromatographic HPLC analysis

A selective and sensitive reversed-phase (RP) high-performance liquid chromatographic method was used for the quantitative analysis of flavonoids in the sample fractions taking the methods described by Marina *et al.*, (2016) and Mbamalu *et al.*, (2016) as a guide. Injection volume was 20ul, 0.9cm^3/mins and total runtime was 7mins for each fraction. The mobile phase was prepared from a 0.5% aqueous solution of orthophosphoric acid and methanol (ratio 45:55). Detection was at 350 nm. Separated flavonoid peaks were initially identified by direct comparison of their retention times with those of standards. Standard solution was then added to sample and peaks were identified by the observed increase in their intensity.

In addition, various concentrations (0, 1.2, 2.5, 5 and 10ug/cm^3) of rutin standard were prepared and run through the HPLC. The peak areas were plotted against concentration to form the calibration curve and the concentrations of the fractions were extrapolated from the graph.

GC-MS analysis of Isolate

The GC-MS analysis of various fractions isolated from the leaves of *Bryophyllum pinnatum*, *Kigelia Africana* and *Mitracarpus hirtus* was performed based on the method reported by Mooza *et al.*, with little modification. The GC-MS used was Agilent 7890B GC System, fitted with a 30 m x 250 um x 0.25 um Rtx-SMS capillary column using a maximum temperature of 325 °C, coupled to Agilent 5977A MSLD. Ultra-high purity helium (99,.99%) was used as a carrier gas at a constant flow rate of 1.0 mL/min. The injection, transfer line and ion source temperatures were all 290 °C. The ionizing energy was 70 eV. Electron multiplier voltage was

Obtained from auto tune. The oven temperature was programmed from 60 C (which was held for 1 min) to 110 °C at a rate of 15 °C/min and then ramped to 280°C at the rate of 5 °C/min.

The sample fractions were diluted with appropriate ethanol (1/100, v/v) and filtered and I µL was injected into the injector with a split ratio 30:1. All data were obtained by collecting the full Scan mass spectra Within the scan range. The percentage composition of the rude extract constituents was expressed as a percentage by peak area. The identification and characterization of chemical compounds in various crude extracts was based on GC retention time and by matching the mass spectra data generated with those of standards available in the mass spectrum libraries.

FTIR Analysis of isolates

A clean sodium chloride (NaCl) screen was used in this analysis. The empty screen (background) was scanned using eFTIR (model M530, Buck sci.) for 3 minutes' scan time. The sample fractions were dissolved in acetone and carefully smeared on the screen. The solvent was allowed to evaporate completely, until the screen became dry completely, leaving only the sample which was re-scanned again. The spectrum was obtained using Buck Scan-and-search software.

Preparation of Antioxidant Concentrate

The antioxidant concentrate was prepared by mixing all the three flavonoids isolated from the leaves of *Bryophyllum pinnatum*), *Kigelia Africana* and *Mitracarpus hirtus* in the same proportion (1:1:1) since all the isolates were found to have appreciable antioxidant activities.

Formulation of the Cream

The formulation of the cream was based on the standard method described by William Griffin (1949) and Irini *et al.,* (2012) which explains the composition that will give a good balance between the hydrophilic components and the lipophilic components in the cream. An emulsifier was used to bring the two phases together. The HLB of the lipophilic must agrees with that of the emulsifier so that it will give a good emulsion (Shinoda, 1969). The formulation was as follows:

Table 1: Phase A (Hydrophilic components)

Component	Proportion (g)
Water	68.00
Glycerin	2.00
Cetyl alcochol	2.00
Steatic acid	3.00
Total	75g

Table 2: Phase B (Lipophilic components)

Component	Proportion (g)	HLB	Required HLB
Olive oil	10	7	2.8
Shear butter	4	8	1.28
Bee wax	2	12	0.96
Paraffin oil	5	7	1.60
Propylene glycol	4	3.5	0.56
Total	**25g**		**7.20**

Table 3: Emulsifier

Component	Proportion (g)	HLB	Required HLB
Emulsifying wax	3.00	7	7.00

Table 4: Other Ingredients

Component	Quantity (%)
Antioxidant Concentrate	0.1
Perfume	0.5
Colourant	Nil

Production of the Body Cream

The body cream was produced by the following method:

All the components of phase A above were weighed in grams and mixed together in a beaker.

Also, all the components of phase B above were weighed and mixed into beaker B. The emulsifier was also added to the phase B. The two beakers were heated at the same time to 70°C with continuous stirring. They were then brought down and mixed together while the stirring continuous. When the temperature came down to 40'C, other additives like the perfume were added.

Evaluation of the body cream

The o/w cream produced was evaluated using a method described below by Purushotham et al, (2010) but with a little modification for pH, viscosity, Stability, Spreadability and skin irritation test.

a. Stability evaluation

The determination of the physical stability of the formulations was conducted in accordance with the Brazilian Cosmetic Products Stability Guide (Anvisa, 2004). Before the start of the stability tests, it is recommended that the formulation is subjected to centrifugation test. For this purpose, the samples were centrifuged at 1000 rpm for 30 minutes. After this test, the sample should remain stable, indicating that the product has been approved at this stage and may go forward to Subsequent tests, the cream samples were subjected to heating in an oven at 45°C and cooling in refrigerator at C and to room temperature (25°C). The cream (10g) was taken out for its physicochemical property tests.

b. Determination of pH

The cream (5g) was weighed in 100ml beaker. Water (45ml) was added and used to disperse the Cream in it. The pH of the suspension at 37 C was determined using the pH meter.

C. Determination of Viscosity

The method described by Mohammed (2010) for the determination of the viscosity was adopted. The viscosity of the cream was measured using PROLABO Viscometer (T30.014, N9007 of size 2. 5) and density bottle. The viscosity determinations were performed at 45°C under gravity. The principle of the PROLABO viscometer was to determine the time taken for a quantity of the sample taken to flow through the narrow width of the viscometer under the force of gravity. The time taken for the sample to flow is then compared with the time taken for the same quantity of water to flow. Als0, the density of the test cream was determined. The viscosity was then calculated using the formula

Viscosity, $V = \frac{t_1 \times d_1}{t_w \times d_w}$ where t_1 is the time taken for the cream to flow through the opening; t_w is the time taken for the water to flow through the opening; d_1 is the density of the cream; d_w is the density of water.

d. Homogeneity and Smoothness

The Cream formulations were tested for the homogeneity physically using visual appearance and by touch.

e. Rheological studies

The Rheological Studies on the formulated cream was carried out using the standard method described by Ravindran *et al.*, (2016). A fixed quantity (10g) of the test cream was taken in a 10ml beaker. It was kept for 1 hour at room temperature. The beaker was inclined to one side in order to see whether consistency has changed or not. The beaker was again tilted and checked for pour ability of the cream. The Rheological Studies on the formulated cream was found to be non-newtonian.

a. Psychometric Test

Psychometric assessment of the formulated body cream

A questionnaire was designed for the assessment of the two creams. It was designed to assess the colour, Homogeneity, Odour, Moisturizing effects and the Non- irritability of the creams as these are most important physical parameters that characterize such a cream. The creams were given to thirty (50) healthy adults, 1hey were requested to apply the creams and after twenty-four hours, they were to assess the creams and then filled in the questionnaire.

b. Analytical method

The filled questionnaire was collected, collated and the scores were analysed for the mean and standard deviation. Student t- test was used for the analysis of the test cream for 29 degrees of freedom.

III. RESULTS

The results of the phytochemical Screening for the active components present in the extract are shown in table 5. The result indicated the presence of flavonoids in the crude extracts. Table 6 shows the result for the thin layer chromatography of the crude extracts. The fraction that iso1ated more components was the n-hexane /ethyl acetate (20:80) traction. Also, Table 7 Snows how much was recovered from the crude extracts. *Kigelia Africana* had the highest percentage recovery followed by *Mitracarpus hirtus* and then Bryophylum pinnatum. The total flavonoid content of each of the extract is shown in table 8 as calculated using the calibration curve in figure 1. The result shows that *Kigelia Africana* had the highest flavonoid content followed by *Mitracarpus hirtus* and then *Bryophyllum pinnatum*. The results for the antioxidant activity and the Regression Correlations are shown in figures 2, 3 and 4. The minimum inhibitory concentration (MIC) of the flavonoids isolated from *Kigelia Africana*, *Mitracarpus hirtus* and *Bryophyillum pinnatum*leaves have been shown in tables 11 and 12.

The flavonoids isolated from the test plants were analyzed also to ascertain that the components were really flavonoids. Also, an attempt was made to determine their structure or the class of components present.

The body cream produced using the antioxidant concentrate was physically and psychometrically analyzed and the results were reported as shown in tables 24, 25 and 26. The results showed that the cream was good.

Table 5: Results for the Phytochemical Screening

Components	N-Hexane extract	Methanol extract
Flavonoid	+	+
Tannin	+	+
Saponin	+	+
Anthraquinone	+	+
Steroid	+	+
Alkaloid	+	+
Glycoside	+	+
Resins	+	-

NB: "+" indicates presence and "-" indicates absence

Table 6: Thin Layer Chromatography Data

Mobile phase	Ratio of solvents used	Number of components observed in the samples		
		K	M	B
Hexane: ethyl acetate	50:50	-	-	-
Hexane: ethyl acetate	40:60	3	-	-
Hexane: ethyl acetate	30:70	5	-	-
Hexane: ethyl acetate	20:80	7	5	2
Hexane: ethyl acetate	10:90	-	-	-
Hexane: ethyl acetate	20:50:30	5	0	2
Hexane: ethyl acetate: acetone	20:60:30	-	-	-

Note: B refers to *Bryophyllum pinnatum*, K refers to *Kigelia africana* and M refers to *Mitracarpus hirtus*

Table 7: Percentage Recovery of the Flavonoids from the Plant Extracts

Plant extract	Mass of extract	Mass of flavonoid isolated (g)	Percentage recovery (%)
Kigelia africana	0.800	0.164	20.19
Byophyllym Pinnatm	0.800	0.090	11.26
Matricarpus hirtus	0.800	0.109	13.63

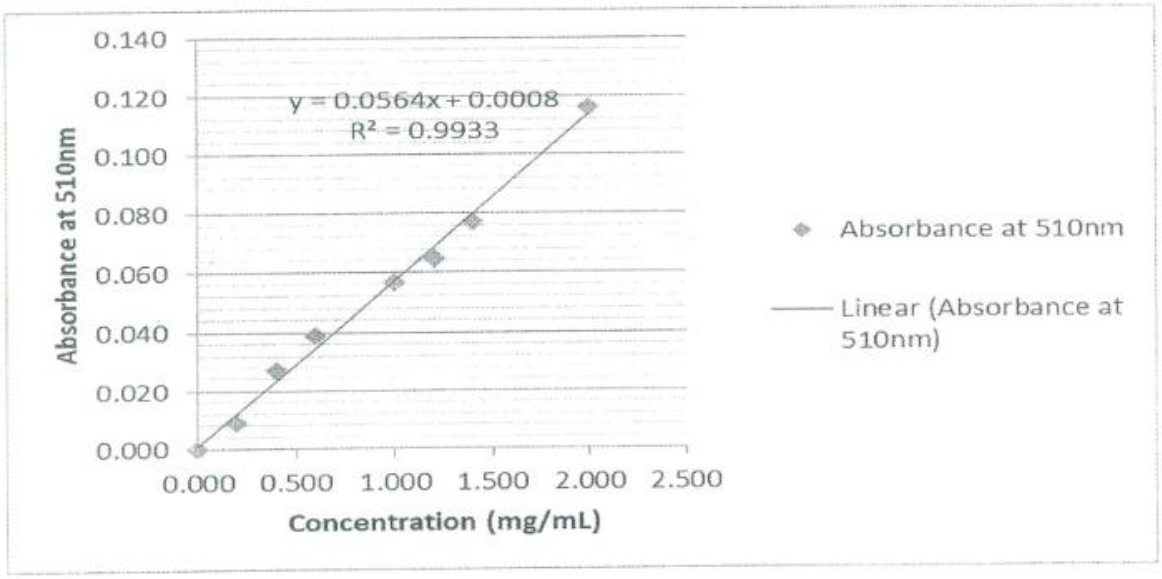


Figure 1: Rutin Standard Calibration Curve for Total Flavonoids

Table 8: Total Flavonoid Contents of *Bryophyllum pinnatum*, *Kigelia africana* and *Mitracarpus hirtus* Leaf extracts

Sample Flavonoid	Mean absorbance	Total
Kegelia Africana	0.031	52mg/g
Mitricarpus hirtus	0.021	40mg/g
Bryophyllum pinnatus	0.020	38mg/g

Table 9: Antioxidant Inhibitory Activity of Flavonoid Isolated from *Mitracarpus hirtus* (M) leat

Cone (ug/ml)	Antioxidant STD (querncetin)	Activity *Mitracarpus hirtus*
1	6.652	18.847
5	10.643	19.734
10	29.712	24.169
50	92.239	33.259
100	93.792	51.663
500	96.009	72.062

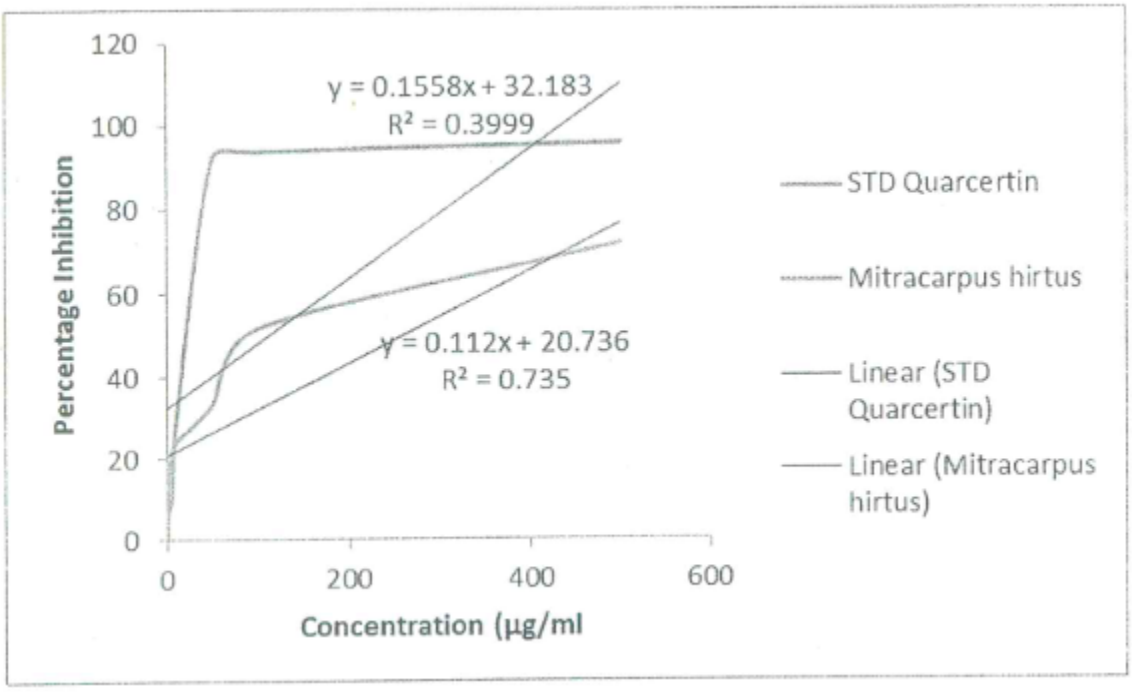


Fig. 2: Linear regression analysis for the correlation between the antioxidant activities of the flavonoids isolated from the extract of *Mitracarpus hirtus* and the standard (quercetin)

Table 10: Antioxidant Inhibitory activity of Flavonoid isolated from *Kigelia africana* (K) leaf

Cone (ug/ml)	Antioxidant STD (querncetin)	Activity *Kigelia africana* (K)
1	6.652	19.734
5	10.643	34.590
10	29.712	37.694
50	92.239	86.918
100	93.792	88.470
500	96.0089	94.013

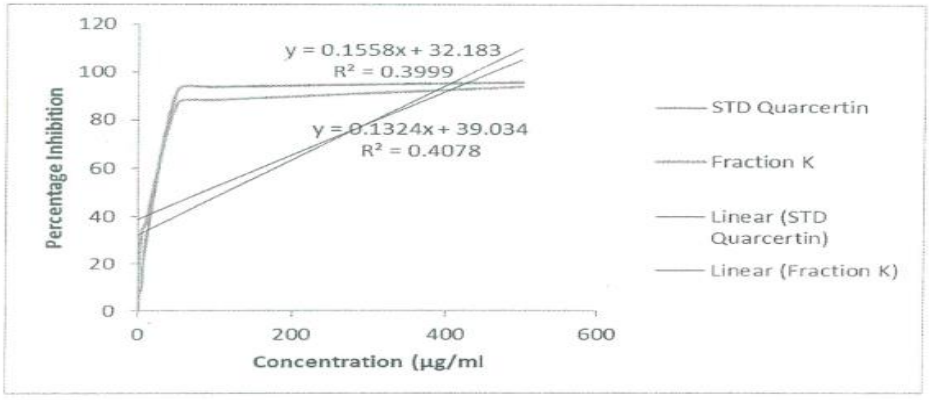


Figure 3: Linear regression analysis for the correlation between the antioxidant activities of the flavonoids isolated from the extract of *Kigelia africana* and the standard (quercetin).

Table 11: Antioxidant Inhibitory Activity of Flavonoid Isolated from Bryophyllum pinatum (B) leaf

Cone (ug/ml)	**Antioxidant STD (querncetin)**	**Activity *Bryophyillum pinnatum(B)***
1	6.6520	4.2130
5	10.643	20.621
10	29.712	31.929
50	92.239	56.985
100	93.792	81.375
500	96.009	8.2970

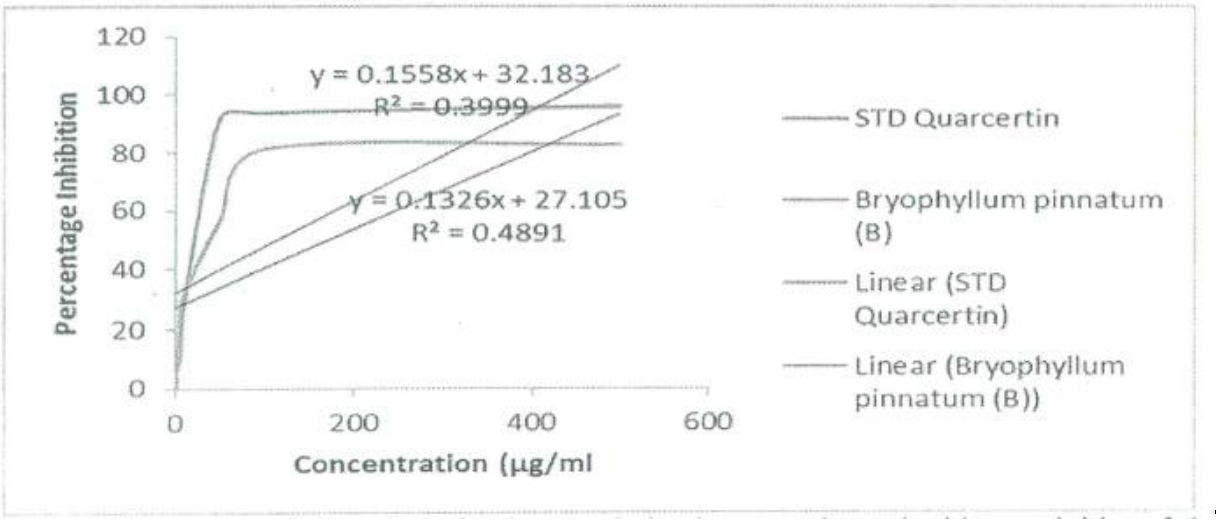


Fig. 4: Linear regression analysis for the correlation between the antioxidant activities of the flavonoids isolated from the extract of *Bryophyillum pinnatum* and the standard (quercetin)

Table 12: Minimum Inhibitory Concentration of Flavonoid Isolates against the test organisms after 24 hours

Test organism	Sample B		Sample K		Sample M	
	37°C	38°C	37°C	38°C	37°C	38°C
S. aureus	2.500	5.300	ND	ND	13.500	1.000
S. typhi	7.500	5.400	ND	ND	1.500	3.200
C. albicas	4.000	1.000	39.700	ND	ND	ND

NB: ND means Not Detected, B means Bryophyllum pinnatum, K means Kigelia africana and M Means Mitracorpus hirtus

Table 13: Minimum Inhibitory Concentration of Flavonoids Isolated from Sample B, K and M against the test Organisms after 48 hours

Test organism	Sample B		Sample K		Sample M	
	37°C	38°C	37°C	38°C	37°C	38°C
S. aureus	2.500	5.300	ND	ND	13.500	1.000
S. typhi	7.500	5.400	ND	ND	1.500	3.200
C. albicas	4.000	1.000	39.700	ND	ND	ND

NB: ND means Not Detected, B means *Bryophyllum pinnatum*, K means *Kigelia africana* and M Mean *Mitracarpus hirtus*

Physical properties of isolates

The characteristic properties of the isolates from the leaves of *Bryophyllum pinnatum*, *Kigelia africana* and *Mitracarpus hirtus* plant leaves are shown in Table 14.

Table 14: Physical characteristics of isolates from the leaves of Bryophyllum pinnatum, *Kigelia Africana* and *Mitracarpus hirtus* plants

Plant	Texture	Colour
Bryophyllum pinnatum	Fine texture	Light brown
Kigelia africana	Fine texture	Dark brown
Mitricapus hirtus	Fine texture	Dark brown

Fourier Transfer Infrared Radiation (FTIR)

The spectra for the FTIR of the three isolated components suspected to be flavonoids from the leaves of three different plants (*Bryophyllum pinnatum*, *Kigelia africana* and *Mitracarpus hirtus*) are shown in Appendix 1 - 3. Also, the functional groups observed to be occurring at the various absorption bands of these spectra of the integrated chromatograms are shown in Tables 4.1 - 4.3. Since this study was aimed at isolating flavonoids only, the functional groups that are related to the flavonoids were indicated with their frequencies.

Table 15: FTIR Spectral data for the isolate from *Bryophyllum pinnatum*

Frequeney of (Cm^{-1})	Bond type/ Vibration Absorption mode	Functional Group
3150-3500	O-H broad asymmetric	Ar-OH alcohol
2925.71	C-H Stretching	Saturated Methyl
2854.78	C-H Stretching	$-CH_2-$
1715.84	C=O Stretching	Ketone
1655.85	C=C Stretching	Aromatic
1462.79	C-H Bending	$-CH_2-$
1369.78	C-H Bending	CH_3-
1007.47	C-O	Ether
918.57		
780.65	C-O Stretching	Ether or Methyl groups
914	C-H Bending	Aromatic

Table 16: FTIR Spectral data for the isolate from *Kigelia africana*

Absorption bond	Vibration mode	Bond type/group
3200-3500	O-H Broad band	Aromatic OH
2921.91	C-H Asymmetric stretching	Alkanes
2854.93	C-H Symmetric Stretching	$-CH_2-$
1581.87	C-H Bending	$-CH_2-$
1408.86	C-H Bending	$-CH_3-$
1033.75	C-O Deformation	Ether

Table 17: FTIR Spectral data for the isolate from *Mitracarpus hirtus* (M).

Absorption bond	Vibration mode	Bond type/group
3200-3500	O-H Broad Asymmetric	Aromatic OH
2921.91	C-H stretching	$-CH_3-$
2854.78	C-H Stretching	$-CH_2-$
1581.87	C=C Stretching	Aromatic
1033.75	C-O Deformation	Ether

GC-MS Results

The GC-MS spectra of the isolated flavonoids from the leaf extracts of three different plants (*Bryophyllum pinnatum*, *Kigelia africana* and *Mitracarpus hirtus*) are shown in appendix 4-6. The results showed that only one type of flavonoid was present in two of the three isolates. At retention time of 25.976minutes, the spectra of *Bryophyillum pinnatum*isolate showed that peak 20 with a molecular mass of 405.1g/mol resembled the flavonolds. Also, at a retention time of 32.938 minutes, *Kigelia africana* isolate showed that peak 20 with a molecular mass of 405.1g/mol while the isolate from *Mitracarpus hirtus* had a peak at a retention time of 32.911 minutes showed that peak 9 with a molecular mass of 429.2g/mol resembled the flavonoids peaks.

Results for the HPLC analysis

The results for the HPLC analysis showed chromatograms for flavonoids of n-butanol fractions of leaves and their retention times in appendixes 7 to 9 and that of the standards are shown in appendixes 10.11 and 12. The data for the chromatograms suspected to be flavonoids are given in tables 4.5, 4.6 and 4.7. The HPLC results showed that there were two peaks in the Bryophyltum pinnatum with retention times of 2.681min and 2.801min. *Kigelia africana* showed four peaks at retention times of 2.527min, 3.165min, 3.878min and 4.899min. *Mitracarpus hirtus* showed about eight peaks with retention time of 0.061 min, 0.685 min, 1.146min, 1.514min, 2.049min, 2.763min, 3.922 min and 5.103min.

Table 17: Data for Calibration of the Standards

Concentration	Team peak area (mAUs)
0	0.00
1.2	8.89
2	98.88
5	136.20
10	397.87

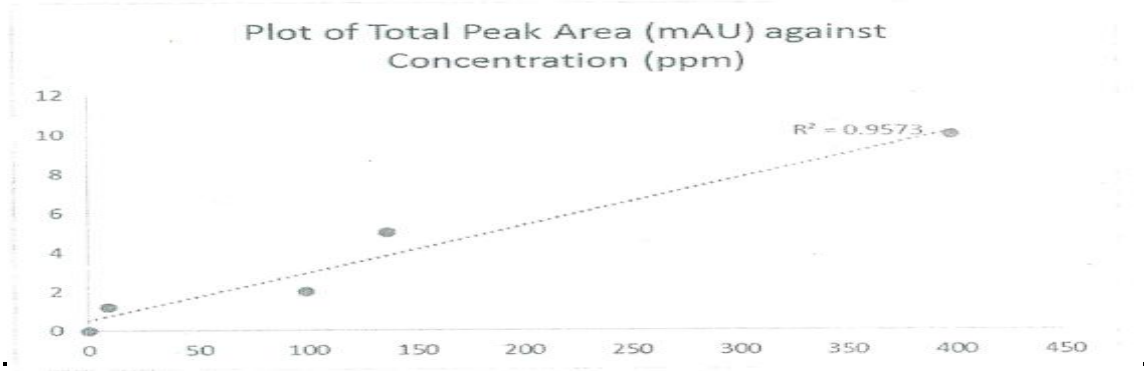


Figure 12: Calibration curve for the Standard solutions using HPLC

Table 18: HPLC Data for Flavonoids isolated from *Bryophyillum pinnatum* at 350nm using Diode Array Detector (DAD)

Peak Number	RetTime [min]	Type	Width [min]	Area [mAU*S]	Height [mAU]	Area %
1	2.681	BV	0.0879	24.51277	3.76683	51.5115
2	2.801	VB	0.0888	23.07424	3.50734	48.4885
Total				**47.58701**	**7.27417**	

Table 19: HPLC Data for Flavonoids isolated from *Kigelia africana* at 53Unm using Diode Array Detector (DAD)

Peak Number	RetTime [min]	Type	Width [min]	Area [mAU*S]	Height [mAU]	Area %
1.	2.630	BV	0.0759	521.75342	17.92587	63.4376
2.	3.165	BB	0.2479	171.70804	10.33198	20.8772
3.	3.878	BV	0.3053	120.96183	5.94600	14.7072
4.	4.899	VB	0.4136	8.04325	1.46851	0.9779
Total				822.46654	35.67237	

Table 20: HPLC Data for Flavonoids isolated from *Mitracarpus hirtus*at 350nm using Diode Array Detector (DAD)

Peak Number	RetTime [min]	Type	Width [min]	Area [mAU*S]	Height [mAU]	Area %
1.	0.061	BB	0.1171	289.26630	41.17075	3.5555
2.	0.685	BB	0.1623	70.72668	6.74889	0.8693
3.	1.146	BV	0.1859	50.24736	4.31347	0.6176
4.	1.514	VV	0.2229	294.28430	21.60048	3.6171
5.	2.049	VV	0.2642	5495.71582	333.59277	67.5494
6.	2.763	VB	0.4043	1734.61926	65.22649	21.3207
7.	3.922	BB	0.2356	88.98425	5.41818	1.0937
8.	5.103	BB	0.3777	112.00693	4.13982	1.3767
Total				**8135.85089**	**482.21087**	

Table 21: HPLC Data tor components concentration

Compound	Retention	Concentration (mg/ml)
Quarcetin	1.77	
Rutin	2.77	
Apigenin	3.97	
Bryophyllum pinnatum	2.68	0.1325
Kigelia Africana	2.63	0.1300
Mitracarpus Hirtus	2.76	0.1365
	3.92	0.1938

Table 22: Zone of inhibition of Flavonoids Isolated from B, K and

Samples	Test Organism		
	C. albicans	S. aureus	S. typhi
B	0.500	1.500	1.500
K	Not detected	Not detected	Not detected
M	Not detected	4.00	3.00

Table 23: Minimum Inhibitory Concentration of Isolates against the test organisms after 24 hours

Test Organism	Sample B		Sample K		Sample M	
	37^0C	38^0C	37^0C	38^0C	37^0C	38^0C
S. aureus	2.500	5.300	ND	ND	13.500	1.000
S. typhi	7.500	5.400	ND	ND	1.500	3.200
C. albicans	4.000	1.000	39.700	ND	ND	ND

NB: ND means Not Detected, B means *Bryophyllum pinnatum*, K means *Kigelia africana* and M Means *Mitracarpus hirtus*

The Household Cosmetie (Body Cream)

The skin care body cream (Test Cream) produced from the formulation earlier described in the methodology using the antioxidant concentrate is shown in appendix 1. Also, the photograph of the standard cream which is a well esteemed cream imported cream sold in most Nigerian market is shown in appendix 2.

The components used in the formulation of the cream (lest Cream) makes the cream to be unique, natural and highly medicinal.

Physical properties of the Cream (Test Cream)

The physical properties of the cream tested with different methods as described in chapter three are as shown in table 24.

Table 24: Physical properties of the cream produced

Parameters	Test Cream	Standard Cream
Appearance/Nature	Semi-solid cream	Semi-solid cream
Colour	Milky	Pink
Odour pleasantness	Good	Very good
Homogeneity	Homogeneous	Homogeneous
Non-Irritability	Good	Very good
Stability	Stable at $45^{O}C$	Stable at $55^{o}C$
Viscosity ($N.s/m^2$)	1.52	1.84
pH	5.7	6.1
Rheology	Non-Newtonian	Non-Newtonian

The psychometric or practical assessment or both the test cream and the commercial cream used as the standard or the control are shown in Figure 5. The data used were the averages for the 30 respondents who volunteered to assess the creams. Also, the body cream was statistically analyzed for its acceptability using student t-test for two independent variables and the result is shown in appendix.

Table 25: Average Grading of the Test cream and the Standard cream

Property	Standard	Test cream
Colour	4.00	3.30
Fragrance/Odour	4.10	3.13
Smoothness	4.30	3.87
Moisturizing effect	3.90	3.40
Non-Irritating effect	3.87	3.53
Homogeneity	4.30	3.50

Grading System: 1= Poor, 2 = Fair, 3 = Good, 4= Very good and 5 = Excellent

IV. DISCUSSIONS

The first extraction with methanol solvent gave a dark green extract which upon Screening showed the presence of flavonoids, tannin, glycoside and saponin only. This shows that the n-hexane has removed the steroids and other components. The preliminary purification with n- hexane/t- butylmethylether /water mixture reduced the intensity of the colour of the mixture which is a modification of the method described by Andersen and Markham (2006).

That implies that the amount of these components present has been greatly reduced. It may also imply that One or some of the components that are non- polar have been Completely removed. Further fractionation in n- hexane, ethyl acetate and acetone removed other components that are less or not soluble in acetone. Since flavonoids are highly soluble in acetone, it was the acetone fraction that was used for the isolation of the flavonoids.

All the extracts were tested for the best solvent that can be used for the isolation or flavonoids using thin layer Chromatography. It was observed that a mixture of n- Hexane/ ethyl acetate (20:80) isolated more components from all the extracts. From *Kigelia Africana* Seven components were isolated, from *Mitracarpus hirtus*, five components were isolated and two components were isolated from *Bryophyllum pinnatum*. That was why this solvent mixture was used for the isolation of flavonoids from the extracts of *Bryophyllum pinnatum*, *Kigelia Africana* and *Mitracarpus hirtus* using column chromatography. The result from the phytochemical screening Showed that flavonoids were present in all the extracts. Also, the results for the percentage recovery showed that *Kigelia Africana* had the highest percentage recovery followed by *Mitracarpus hirtus* which in turn was higher than *Bryophyllum pinnatum*. This agrees with the reports of Agoha (1974); Nassis *et al.*, (1992); Houghton (2002); Sunday and Olufunsho (2015).

The results for the antioxidant activities of the flavonoids isolated from all the extracts as compared to the standard Quercetin showed that all the isolates had antioxidant activities. The linear regression analysis for the correlation between the antioxidant activities of the flavonoids isolated from the extracts of *Bryophyllum pinnatum*, *Kigelia Africana* and *Mitracarpus hirtus* and that of the standard (quercetin) showed that *Kigelia Africana* had higher antioxidant activity followed by *Bryophyllum pinnatum*m which in turn has higher antioxidant activity than *Mitracarpus hirtus*. This agrees with Janmeda et. al, 2011; Sharma et. al, 2011; Sindhu and.

Although the flavonoids from *Mitracarpus hirtus* showed a better percentage regression while the extracts had lower percentages, the percentage regression of the standard (which is believe to be an excellent antioxidant) when compared with that of the various extracts it could be Seen that they have close values. Gupta (2013) explained that there are some exceptional cases that showed that low regression values does not mean that the value is not acceptable. He explained that when the graphical lines fit closely with the observed line of the standard. Surprisingly, the flavonoids isolated from *Kigelia Africana* with the higher antioxidant activity, could not inhibit the growth or Salmonella typhi and Staphylococcus aureus at all the temperatures tested. it was only able to inhibit the growth of Candida albicans at $37^{o}C$ and this agrees with the report of Sangita *et al.*, (2009). Candida albicans is the fungus most commonly detected in association with humans (Douglas, 2003 and Shirtliff et. al, 2009). Candida albicans is a type of yeast that is Commonly referred to as a dimorphicfungus since it grows both as yeast and filamentous cells. It is a common member of human gut flora and does not seem to proliferate outside mammalian hosts. It is detectable in the gastrointestinal tract and mouth in 40-60% of

healthy adults. It is usually a commensal organism, but can become pathogenic in immunocompromised individuals under a variety of conditions. It is one of the few species of the Candida genus that cause the infection candidiasis in humans. Overgrowth of the fungus results in candidiasis (candidosis) Sangita *et al.,* (2009). Also, the flavonoids of *Mitracarpus hirtus* was able to inhibit the growth of S. typhi and S. aureus and could not inhibit the growth of Candida albicans. The flavonoids isolated from *Bryophyillum pinnatum*had good inhibitory activity against all the test organisms. Staphylococcus aureus and Ent. faecalis entered the stationary phase at concentrations of 10^8-10^9 CFU ml^{-1}, the same as seen previously with the Gram-negative competitors. However, their entry into Stationary phase was not accompanied by inhibition of Salm. Typhimurnum multiplication.

Determining the total flavonoids by using aluminum chloride method was based on the formation of stable complex between aluminum chloride and keto and hydroxyl groups of the flavonoids. the Aluminium chloride colorimetric method uses wavelength scan of the complexes of the sample and standard with aluminum chloride showed that the complexes formed by flavonoids (Rutin) with C-3 or C-5 hydroxyl group revealing total flavonoids content in Ethanolic and aqueous extract of leaves of the *Kigelia Africana*, *Mitracarpus hirtus* and *Bryophyillum pinnatum*as 60 mg/g, 40mg/g and 38mg/g respectively as shown in figure 6.

The antioxidant activity of the methanol extracts of the various plants was evaluated by 1, 1- diphenyl-2-picrylhydrazyl (DPPH) method. The results indicated that methanol extract from the leaves of the plants possess considerable antioxidant activity. The highest radical scavenging activity was detected in *Kigelia Africana* followed by *Bryophyillum pinnatum*and the least was *Mitracarpus hirtus*. This reveals that the methanolic extract of this plants are attractive sources of flavonoid, especially the essential ones, as well as of effective natural antioxidants.

In a study on the diuretic activity of aqueous extract of the bark in experimental rats, Sharma et al reported that the extracts from Kigela Africana was safe up to 5g/kg. A determination of acute toxicity of the methanol fruit extract using male sprague- Dawley rats showed that the extract was well tolerated by the animals as there were no observable signs of acute toxicity effects like restiveness, seizure or dizziness after the administration of 400mg/kg. However, at 6400mg/kg, the animals showed signs of toxicity like Jerks and writhes with 60% death.

Peaks of the isolates from the leaves of *Bryophyllum pinnatum*, Kigelia and Metracarpus hirtus plants were initially identified by direct comparison of their retention times with those of standards using RP- HPLC. Also, standard solution was then added to the sample and peaks were identified by the observed increase in their intensity. In addition, various concentrations (0, 1.2, 2.5, 5 and 10mg/g) of rutin standard were prepared and run through the HPLC. The peak areas plotted against concentration formed the calibration curve and the concentrations of the fractions were extrapolated from the graph. It was observed that the detector gave a good resolution, good sensitivity and a good selectivity of the flavonoid bands at 350nm and so the detection was carried out at this point. This agrees with the reports of Fazilatun *et al.,* (2005) and Olszewska (2007).

The detected bands at 550nm were shown to occur between rention times of 2 and 4min. From the results, it can be seen that in all the peaks detected in the three samples two of the peaks resembled each other. The peaks of the isolates of *Bryophyillum pinnatum*and *Kigelia Africana* have close retention times. That implies that they may be containing the same type of flavonoids. Therefore, the flavonoids suspected to be present may be rutin. In the case of the isolate from *Mitracarpus hirtus*, two of the compounds detected had their retention times (2.76min and 3.92min) that resembled that of the flavonoids and the compound with the retention time of 3.92min was isolated (Olszewska, 2007).

The molecular masses of the isolates suggested by the library for the two compounds isolated were 342.34$gmol^{-1}$ and 385. 34$gmol^{-1}$ the molecular mass of 342$gmol^{-1}$ is Similar to that of quercetin 3 – 0 rutinoside (rutin) as reported by Jang *et al.,* (2016). The structure of the flavoid with the molecular mass of 342.34 $gmol^{-1}$ was as shown in figure 13 below.

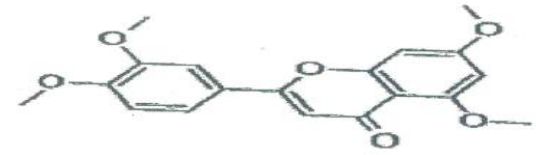


Figure 13: the structure of 3,7-Dimethoxy -3',4-methylenedioxyflavone

Molecular formular: $C_{19}H_{18}O_6$

The chemical structure of the compounds isolated from *Mitracarpus hirtus* with the molecular mass of 385. s4gmol-l is similar to that reported by CAS, 2018 and the structure suggested was as shown in figure 14 below:

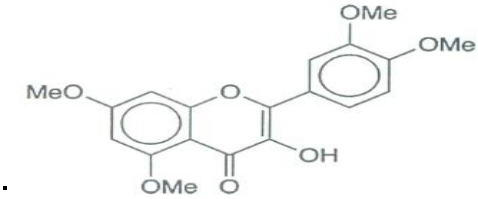


Figure 14: The structure of 3',4',3',7-Tetra-O-methylquercetin; Molecular formular: $C_{19}H_{18}O_7$

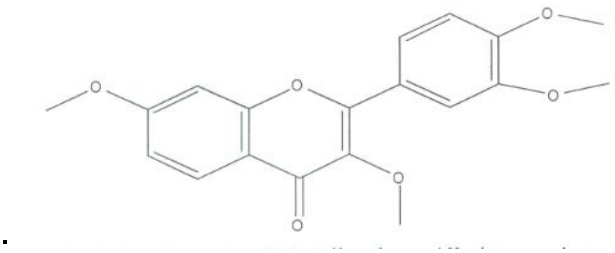
The FTIR results gave some absorption bands that correspond with the various functional groups expected in flavonoids. For instance, in the spectrum for the isolate from *Bryophyllum pinnatum*, there was Ar-OH (hydroxyl) at 3150- 3500cm^{-1}, -0-(ether moiety) at 1007.47 cm^{-1}, C=O (keto) at 1715.84 cm^{-1}, and (C=C) aromatic groups at 1655.85 cm^{-1} These together gave a strong Indication that the isolates were flavonoids. This conforms to the report of Nagaratna and Prakash (2015) who had earlier reported that *Bryophyillum pinnatum*contains flavonoids.

The FTIR Spectrum for the isolate from *Kigelia Africana* gave absorptions that were similar that of *Bryophyllum pinnatum*. That is, there was the presence Ar-OH (hydroxyl) at 3150 - a500cm", -0- (ether moiety) at l007.47 cm^{-1}, -O- (keto) at 1715.84 cm^{-1}, and (CC) aromatic groups at 1655.85 cm^{-1}. That implies that the isolate may contain a flavonoidthat is similar to that of *Bryophyllum pinnatum*. This agrees with the HPLC results reported above tor both plant isolates.

Also, the FTIR spectrum tor the isolate from *Mitracarpus hirtus* gave absorptions ot Ar-OH (hydroxyl) at 5150 3S00cm^{-1}, -O- (ether moiety) at 1007.47 cm^{-1}, C-O (keto) at 1715.84 cm^{-1}, and(C=C) aromatic groups at 1655.85 cm^{-1}". Although the absorptions were similar but the absorptions in *Bryophyillum pinnatum*and *Kigelia Africana* had a broader Ar-OH (hydroxyl) absorption at 3150-3500cm^{-1}". That implies that the isolate was a flavonoid and this agrees with the report given by John *et al.*, (2017).

The chromatographic spectra of the ethyl acetate isolated fraction from Bryophylum pinnatum was detected at a retention time of 35.980minutes. The result showed a spectra of a single pure isolate. Although, the percentage count was very low at this acquisition time, it corresponds to that obtained from the library.

The Mass Spectrum of the fraction showed a protonated molecular ion peak with m/z 342.10 $[M+1]^+$. This suggests the isolate obtained from the library with the molecular formula $C_{19}H_{18}O_6$. The IR spectrum of the compound is analyzed from the lR data. The presence of -OH group was seen from the absorption at the range 3330-3463cm^{-1}. A strong band at the range 1655-1617cm^{-1} was due to the presence of carbonyl (-C=O) group. The strong band at 2911 cm^{-1} was for a C-H asymmetrie stretching vibration. The bands at 2874 cm^{-1}, 291l cm^{-1}, 1110 cm^{-1} and 1095 cm^{-1} represents CH stretching vibrations.' The presence of ether functional group (-C-O-C-) indicated in the absorption at 1060cm^{-1}. Also, the absoption at the range 1580- 1600cm^{-1} was an indication of an aromatic ring. The presence of the above mentioned groups showed the functional groups present in f1avonoids. The propose structure of the flavonoid isolated from *Bryophyillum pinnatum*is shown in figure 15 below:



2-(3,4-dimethoxyphenyl)-3,7-dim ethoxy-4H-chromen-4-one; Chemical formula: $C_{19}H_{18}O_6$

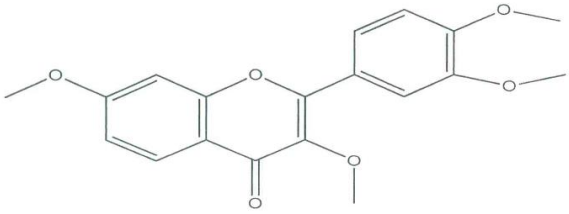
Molecular Weight: 342.34

m/z: 342.11 (100.00%), 343.11 (20.8%%), 344.12 (2.1%), 344.11 (1.2%)

Figure 15: Proposed structure of isolate from *Bryophyllum pinnatum*

The gas chromatography of the ethyl acetate isolated fraction from *Kigelia Africana* gave a signal with a retention time of 32.862mins. The Mass Spectrum of the fraction showed a protonated molecular ion peak at m/z 342.10 $[M+1]^+$. Suggesting the molecular formula $C_{19}H_{18}O_6$. The spectrums of the groups or flavonoid was analyzed from the IR data. The presence of -OH group known from the absorption at the band range 3330-3463em^{-1}. A strong band at the range 1655-1617cm^{-1} was due to the presence of –C=O group. Also, a strong band at 2911 cm was for a C-H asymmetric stretching vibration. Ihe bands between 2874 cm", 2911 Cm, 1110 cm^{-1}, 1095 cm^{-1} represent C-H stretching vibrations. The presence of -C-O-C indicated in the absorption 1600Cm^{-1} was for ether. Also the absoption at the range 1580-1600cm^{-1} was an indication of an aromatic ring. As shown above, the group at these absorption bands indicated the compound was a flavonoid. The fragmentation patern of the ions are shown in appendix 1. The

proposed structure of flavonoid isolated irom *Kigelia Africana* is shown in figure 16 below:



2-(5,4-dimethoxyphenyl)-3,7-dimethoxy-4H-chromen-4-one; Chemical Formula: C19Hs; Molecular Weight: 342.34; m/z: 342.11 (100.0%), 343.11 (20.8%), 344. 12 (2.1%), 344.11 (1.270)

Figure 16: Proposed structure of isolate from *Kigelia Africana*

The gas chromatography of the ethyl acetate isolated fraction from *Kigelia Africana* gave a signal with a retention time of 35.912mins. The Mass Spectrum of the fraction showed a protonated molecular ion peak at m/z 358.34 $|M+1]^+$ Suggesting the molecular formula $C_{18}H_{19}O_7$. The IR spectrum of the compound of flavonoid is analyzed from the IR data. The presence of – OH group known from the absorption at the range 3330-3463cm. A strong band at the range 1655-1617cm^{-1} is due to the presence of –C=O group. The strong band at 2911 cm^{-1} was for a C-H asymmetric stretching vibration. The bands between 2874 cm^{-1}, 2911 cm^{-1}, 1110cm^{-1}, 1095cm^{-1} represents C-H stretching vibrations. The presence of –C-O-C- indicates in the absorption at 1060cm^{-1}. Also, the absoption at the range 1580- 1600cm^{-1} was an indication of an aromatic ring. the fragmentation pattern of the ions are shown in appendix 1.

V. CONCLUSION AND RECOMMENDATION

From the results obtained and the discussions above, it can be concluded that the leaves of *Bryophyllum pinnatum*, *Kigelia Africana* and *Mitracarpus hirtus* plants have components that have some antioxidant activities at some appreciable levels that are significant enough to be used in the production of antioxidant concentrate that are usually needed in small quantities. These antioxidants were found to be useful as a substituent for synthetic antioxidants that that have side effects as seen in the body cream that produced using it as the only antioxidant.

We recommend that leaves of Bryophyllum pinmatum, *Kigelia Africana* and *Mitracarpus hirtus* plants should be studied further for development into a resource for industrial applications. It can be very useful achieving the sustainable development goals in the areas of industrial development and sustainable agriculture.

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