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RESEARCH ARTICLE

PRELIMINARY INVESTIGATION OF OIL FROM *PENTACHLETHRA MICROPHYLLA* SEEDS AS NEW EXCIPIENT IN COSMETIC/PHARMACEUTICAL CREAM FORMULATION

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ABSTRACT

Extraction of oil from African oil bean seed (*Pentaclethra macrophylla)* was, effected by soxhlet extraction using *n*-hexane and the extracted oil was, found to be brownish yellow with percentage yield of 38.09%. The extracted oil, analyzed for its proximate, elemental compositions and physicochemical properties, revealed presence of, lipid, moisture and protein at 53.14%, 46.86% and 13.44% respectively for proximate analysis while elemental contents included such minerals as, Mg, Fe, K, Na and small traces of lead (pb). Characterization of the oil shows the acid value as 1.40mg/KOH g-1, peroxide18.0 mEq, iodine 84.84 mg/100g and saponification value 193.12 mg/100g. Anti-microbial screening of the oil was, investigated on six pathogenic microorganisms as, *Candida albican, Bacillus cereus, Klesiella pneumoniae, Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa*. 0.1ml of the seed oil was found to inhibit growth of four out of the six-tested organisms including *E. coli, S. auerus, C. albicans and B. cereus*. Pharmaceutical screening as observed especially with the proximate and elemental compositions indicated medicinal implications of the extracted *P.macrophylla* oil.

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INTRODUCTION

Directly or indirectly, nature in its elegance and vastness has provided varieties of substance which could be harnessed to formulate several products for human benefit. Chemical substances obtained from plants, animals and other natural sources such as mushrooms and micro organisms, termed as natural products has been of great importance as ingredients used in pharmaceutical and cosmetic preparation since they could serve as lead compounds in pharmaceutical formulations judging that it is one of the oldest means for obtaining drug and drug related compounds [1]. Plant derived polymers(gums, mucilage and oils) have pharmaceutical applications as diluent, solubilizer, binder, disintegrant in tablets, thickeners in oral liquids, protective colloids in suspensions, gelling agents in gels and bases in suppository, viscosity enhancers, stabilizers and they could also be useful in cosmetics, textiles, paints and paper-making industries. Plant triglycerides (fats and oil) have also been, employed as oleaginous bases, solvents, levigating and flavoring agents [2]. Pharmaceutical formulation involves the combination of several of the chemical substances including, active and inactive ingredient (excipients) to produce a finished dosage form which may be dependent on, the type, physicochemical properties, compatibility and route of administration. Based on the route of administration therefore, formulations can, be divided into enteral, parenteral, and topical formulations. Topical Formulation: These are formulations intended for application on the body surface (skin) and mucous membrane. They include powders (finely divided solid), ointments (mixture of oil and water with oil being in higher proportion), lotions, pastes (mixture of oil, water and powder), foam, gel (in contact with skin it liquefies with alcohol as solvent) and creams (emulsion of oil and water in almost equal proportion).In essence, a formulation is onlyacceptable when the different ingredients are, mixed together in the right proportions to give a finished and elegant product [4]. Oil as a formulation excipient is one of the oldest form of natural herbal medicine, with majority, sourced from plants, animal or mineral. They are either, used solely or in combination with other forms of medicaments like powders, for the treatment of illness and diseases. Natural oil has found application in diverse sectors of production, can be used in foods and industries as, lubricants for coating,biofuel, and serve cosmetic or pharmaceutical relevance, where it could be incorporated in the production of locally made, creams, soaps and lotions, aimed at improving skin appearance [5].

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AFRICAN OIL BEAN SEED *(P. macrophylla)*


Fig. 1. The tree and seeds of *P. macrophylla*

This plantbelongs to the kingdom Plantae, subkingdom trachebionta, a vascular plant, super division spermatophyta a seed plant, division magnoliophyta a flowering plant and classmagnoliopsida a dicotyledon. It is, popularly known, by the Ibo tribes of Nigeria as Ugba and in Cameroun as Ebaye.
The plant is medium-sized up to 35 m tall; often crooked and low branching, with irregular, thick buttresses up to 3 m high, or without buttresses; outer bark is greyish to reddish brown, thin and flaking irregularly, inner bark is fibrous, yellow to orange and twigs brown, stellate and hairy [6]. It produces fruit that has obliquely linear-oblong pod up to 50 × 10 × 2 cm, woody, dark brown, tapering to the base, apex rounded, sides longitudinally ribbed, long persistent and opening explosively. The flat glossy brown edible seeds, sometimes averaging about 8,in number are contained in a brownish flattened pod.

The tree has been, identified by peasant farmers to improve soil properties and as a component of an agro-forestry system because of its nitrogen fixing activity. The edible seeds from the plant require tedious but careful processing and fermentation before taken as food supplement. The seeds are roasted or boiled when fermented andserves as a meaty flavoured snackeaten either alone or used as a condiment in soup, porridge and salad. The leaves, on mulching, improves soil fertility and the wood is suitable for making charcoal and fuel. Ash from its pods, wood or tannins are, used as a mordant for dyeing in Ghana. The wood is, carved to produce household utensils and decorations in Nigeria and Ghana. The seed of *P.macrophylla* analysed by some authors revealed an abundance of lipid; phospholipid, glycolipid, crude protein, fibre, moisture and carbohydrate with the oil analysis, revealing, presence of such elements as,Ca, Mg, Pb, Fe, Mn, P and Cu. The flowers are rich in nectar and often visited by honeybees and other insects and the seeds could be used for infertility, crushed seed for abortion, smoked or burnt leaf for convulsion, leaf/stem bark for diarhoea, stem bark prepared as, ointment, for itching, decoction for lactogenicity and as lotion for wound treatment [7].

Natural distribution of the plant suggests it to grow mainly on relatively acid soils and the species tolerates water logging as in the low altitudinal riverine areas of south-east Nigeria, Togo and Cameroon. The flowering season in West Africa is march-april with small flushes in june and november but in Liberia trees flower in february-april and fruit in september-december.The plantcould grow either wild or semi-wild with no organized cultivation in plantations or orchards in Nigeria and the mature dispersed seeds are harvested by gathering them manually from around the tree [8]. Several methods have been adopted in oil extraction from different plant and animal sources, however for oil bean seeds two main techniques involving mechanical expression (either cold or hot pressed) and solvent extraction are used. These conventional methods are time and solvent consuming. In modern times some innovative methods including, microwave assisted extraction (MAE), ultra sonic assisted extraction (UAE), supercritical fluid extraction (SFE), soxhlet extraction, aqueous enzymatic oil extraction (AEOE) and others has been introduced [9]. The aim of this study is to extract and characterize the oil from seeds of *Pentaclethra macrophylla* plant, for application as an excipient in pharmaceutical and cosmetic formulations.

MATERIALS AND METHODS

Ethanol, *n*-hexane, ethanolic KOH, 1% methyl orange solution, 0.5MHCl, diethylether 0.1M NaOH, carbon tetrachloride, wiji's solution, KI solution, sodium thiosulphate solution, glacial acetic acid (SIGMA-ALORICH 2.5L, LOT NO.83280, Germany), standard glucose and boric acid, seeds of *P. macrophylla* (Udi market, Udi ,Enugu state), extracted oil (Pharm Tech lab UniPort, Nigeria), oven (Scanfrost Germany), milling machine (Corona Landers, England),soxhlet apparatus (borosilicate glass, England), Heating mantle (PEC medicals, USA), Rotary evaporator, pliers, microcsope, pH meter (Helm reasinn, PHS 25 England), Sample Collection The *P.macrophylla*seeds were obtained from Udi, in Udi local government area, Enugu state, Nigeria. The seeds were dehulled and oven dried at 50°C before milling using a milling machine.

Extraction of oil: The soxhlet extraction method was employed using n-hexane as the solvent. A considerable amount of the milled seed cotyledon was, packed in the thimble of soxhlet apparatus with a cotton wool at the top and bottom of the thimble. The whole set up was, heated in a heating mantle at temperature of 50^0C. The n-hexane extract was concentrated using rotary evaporator and quality of the oil extracted determined gravimetrically.

Percentage Yield

$$\text{Percentage yield} = \frac{\text{weight of extracted oil (g)}}{\text{weight of the powdered seed (g)}} \times 100 \quad \text{--------- 1}$$

Physicochemical Properties of the Oil

Determination of Fatty Acid: 25ml diethyl ether was, mixed with 1.0ml 95% ethanol and 1 ml phenolphthalein (1%) then carefully neutralized with 0.1M NaOH. About 10g of the extracted oil was mixed with the neutral solvent and titrated with aqueous 0.1M NaOH, shaking intermittently, until pink color, which persisted for 15 seconds, resulted.

$$\text{Acid value} = \frac{\text{Titrevolume (ml)} \times 5.61}{\text{Weight of sample}} \quad \text{--------- 2}$$

The free fatty acid (FFA) figure is usually calculated as oleic acid (1 ml 0.1M NaOH=0.0282g oleic acid), in which case the acid value = 2 X FFA. For most oils, acidity begins to be noticeable to the palate when the FFA calculated as oleic acid is about 0.5 – 1.5%

Determination of the Iodine Value: The oil was, introduced into a small beaker, stirred with a small rod. A suitable quantity of the sample by difference was, weighed into a dry

glass- stoppered bottle of about 250ml capacity. The appropriate weight in grams of the oil to be, used was, calculated by dividing 20 by the highest expected iodine value. 10ml of carbon tetrachloride was dissolved with the oil then 20ml of wiji's solution added, then stopper (previously moistened with potassium iodine), was inserted and allowed to stand in the dark for 30 minutes. 15ml of potassium iodine solution (10%) and 100ml water was added, mixed and titrated with 0.1M thio-sulphate solution using starch as indicator to obtain the end- point (titration = a ml. Blank titration was carried out at the same time with content only of 10 ml carbon tetrachloride (titration = b ml).

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

where (b-a) is greater than b/2 the test was repeated using a smaller amount of the sample, noting that the less unsaturated fats with low iodine values are solid at room temperature, or conversely, oils that are more highly unsaturated are liquid (showing that there is a relationship between melting point and iodine value). Preparation of wiji's solution: 8g iodine trichloride was, dissolved in 200ml glacial acetic acid, 9g of the oil was dissolved in 300 ml carbon tetrachloride then the two solutions were mixed and diluted to 1000 ml with glacial acetic acid.

Determination of peroxide value: 1.0 g of oil was weighed into a clean dry test tube and while still liquid,1.0g powdered potassium iodide was added along with 20ml of solvent mixture (2ml glacial acetic acid + 1ml chloroform).

The test tube was, placed in boiling water so that the liquid boils within 30 seconds then boiling allowed vigorously for not more than 30 seconds. The contents was, quickly transferred into a flask containing 20ml of 5% KI solution and the test tube washed out into flask twice with 25 ml water then content of flask, titrated with 0.002M sodium thiosulphate solution using starch as indicator. The titer value obtained multiplied by 2 gives the peroxide value.

Determination of the Saponification Value: 2.0g of oil was, weighed into a conical flask and 25 ml of alcoholic KOH solution added. A reflux condenser was attached and the flask heated in boiling water for 1 hr, shaking frequently then, 1 ml of phenolphthalein (1%) solution was added into the hot excess alkali then titrated with 0.5M HCl (a ml). A blank titration was at the same time carried out (b ml) without oil.

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

Organoleptic Properties of the Oil. The physical characteristics (color, odor, texture, appearance and rheology of the oil were observed and noted.

pH of the oil: The sample 0.1ml was introduced into a beaker and the pH electrode inserted, the test was carried out in triplicates and the reading recorded when stable.

Viscosity of the oil: Using a Brookfield viscometer, the oil evaluated was, poured into a clean 250 ml beaker and a temperature probe attached to spindle guard leg and viscometer lowered into the beaker until the spindle is fully immersed. The procedure was repeated at different temperature and the displayed viscosity recorded in centipoise (cP), revolution per minute (rpm) and % torque on the viscosity data sheet.

Density of the Oil: An empty measuring cylinder was, weighed and known volume of oil transferred into the measuring cylinder and weighed. The density was, calculated using the formula.

Mass of oil = weight of cylinder and oil – weight of empty cylinder ------ 5

Density = mass/volume -------- 6

Conductivity of the oil: Using a, prob-conductometer, an electrode was, inserted into a beaker containing the oil sample. The test was, conducted in triplicates and the readings recorded when stable. Refractive Index of the Oil: Using the refractometer, light was turned on; cool water was ensured to flow while the water temperature was recorded on the precision thermometer to 0.1^0C. The incident prism was, opened with the prism lock knob and the prism face cleaned with acetone and carefully blotted dry with a Kim wipe. Few drops, of the oil to be tested was, placed on the polished surface of the lower refracting prism while the liquid was ensured to be evenly distributed and the lower adjustment knob scanned until a light and dark divided image was seen then the refractive index was read from the green scale below the boundary.

Specific Gravity determination: Empty specific gravity bottle with stopper was weighed, then,50ml of water and that of oil transferred into specific gravity bottle also weighed with stopper

The specific gravity was calculated:

$$\text{S.G} = \frac{\text{Wt of oil } (M_2-M_1)}{\text{Wt of water } (W_2- M_1)} \quad \text{----- 7}$$

Where M_1= mass of specific gravity bottle with stopper, M_2= mass of specific gravity bottle + oil, W_2= mass of specific gravity bottle+ distilled water

Proximate Analysis of the Oil: Carbohydrate content was determined using Cleg- Anthrone method

Absorbance of the standard glucose was, read and the value of the carbohydrate as glucose calculated as:

$$\% \text{ CHO as glucose} = \frac{25 \times \text{absorbance of sample}}{\text{Absorbance of standard glucose}} \quad \text{------ 8}$$

Protein Content was determined following Kjeldahl method involving: digestion, distillation, and titration.

$$\% \text{ Organic Nitrogen} = \frac{\text{Titre value} \times 1.4 \times 100 \times 100}{1000 \times 20 \times 0.1} \quad \text{--------- 9}$$

Where, Titre value= the volume of HCl used in titrating the ammonium distillate.

1.4= nitrogen equivalent to the normality of HCl used in the titration

100 = the total volume of digestion, 100 = percentage factor, 20 = conversion factor from gram to milligram, 0.1= the weight (g) of sample digested. Determination of Moisture Content: This was, carried out using the Air Oven Method

$$\% \text{ moisture} = \frac{\text{Weight of fresh sample} - \text{Weight of dried sample} \times 100}{\text{Weight of sample used}} \quad \text{------ 10}$$

Determination of lipid content: Using soxhlet extraction, 2.0g of sample was wrapped with filter paper and placedin a soxhlet extractor. The extractor was, introduced into a pre-weighed dried distillation flask. The solvent (acetone) was there after put into the distillation flask via the condenser end attached to the soxhlet extractor. The setup was, held in place with a retort stand clamped. Cooled water jet was, allowed to flow into the condenser and headed solvent refluxed. The lipid in the solvent chamber was, extracted in the process of continuous refluxing. When the lipid was, observed, to be completely extracted the condenser and extractor was, disconnected and the solvent evaporated to concentrate the lipid. The flask was there after dried in the air oven to constant weight to obtain the weight of lipid.

$$\% \text{ lipid} = \frac{\text{Weight of flask and extract} - \text{Weight of empty flask} \times 100}{\text{Weight of simple extracted}} \quad \text{------ 11}$$

Determination of Fibre content: This was obtained by difference using sum of other parameters from 100 hence Fibre content = 100 - ∑ (other parameters) ------ 12

Element Analysis of the Oil: The oil sample was placed and ashed in a muffle furnace at a temperature of 630°C for 3 hours. The ashed sample was dissolved in 10 ml concentrated HCl and heated on an electro-thermal heater hotplate. The solution of the ash was diluted to 50 ml with distilled water and analyzed for metal ion by atomic absorption spectrophotometer with lead ion (Pb) at wavelength of 283.3 nm, magnesium (Mg) 285.2 nm, sodium (Na) 589 nm, calcium (Ca) 422.7 nm and potassium (k) 766 nm.

Anti-Bacterial Effect

Susceptibility testing: Antimicrobial activity of the oil and reference standards was analyzed using Mueller Hinton agar and the agar plates inoculated with 0.1 ml broth culture of test organisms. Sterile cork borer was, used to make agar wells on the media and 2 drops of the varied oil introduced into the wells. The plates, was left to stand for 30mins to allow for pre-diffusion of the oil, and incubated, at 37°C for 24 h while the inhibition zone diameters was measured in millimeters (mm).

RESULTS

Fig. 2. *Pentaclethra macrophylla* oil

Table 1. Physiochemical characterization of *P. macrophylla* oil

P. macrophylla oil	Characteristics
Percentage yield (%)	38.09
Colour	Brownish-yellow
Odour	Agreeable
State at room Temperature (29.38°C)	Liquid
pH	6.2±0.00
Viscosity(cP)	39.83±0.29 at 31.1°C
Density (g/ml)	0.89
Specific gravity	0.90 ±0.002
Refractive index	1.431 ±0.001
Conductivity(µS/cm)	0.6 ± 0.11 at 29.2°C

Table 2. Chemical characterization of the *P. macrophylla oil*

Parameters	Values
Saponification Value (mgKOH g^{-1})	193.120
Iodine Value (mg $100g^{-1}$)	84.840
Peroxide Value (Meq kg^{-1})	18.000
Acid Value (mgKOH g^{-1})	1.404
Free Fatty Acid (mg g^{-1})	0.702

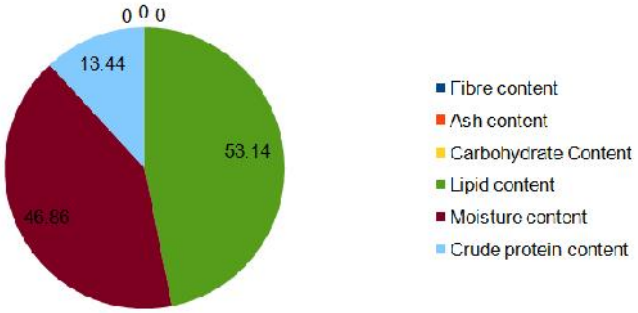

Fig. 3. Proximate Analysis of African Oil Bean Seed Oil

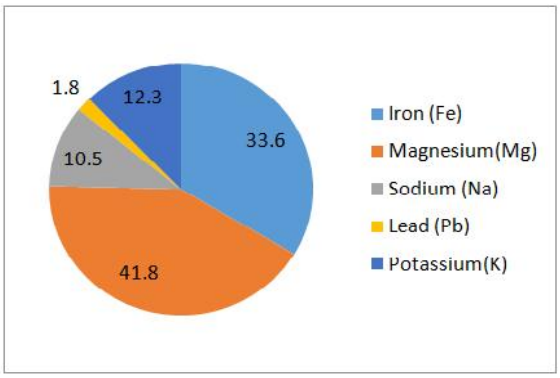

Fig 4. Elemental Analysis of *Pentaclethra macrophylla* seedoil

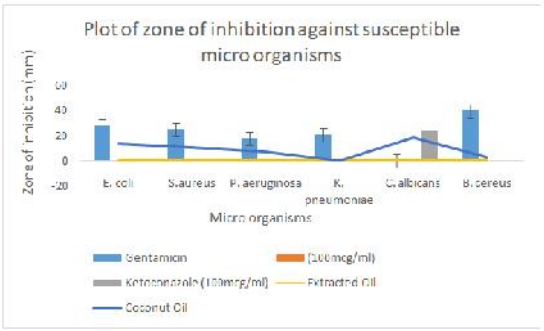

Fig 5. Antimicrobial effects of the Oils and standards on some isolates

DISCUSSION

The percentage yield of the oil obtained from the seeds was 38.09%, higher than 35.08% obtained by [9] and lower than 47.90% as obtained by [10]. This value seems quit appreciable and indicates that the seed could be a good source of oil and the energy contents suggest the seed to be a high-energy food.
The physical characteristics of the extracted oil as shown in table1 revealed that the extracted oil is liquid at room temperature, brownish yellow in color and has an agreeable odor. The pH of the oil was 6.2, which shows that it is slightly acidic hence can be of relevance in antimicrobial formulations. The viscosity was found to be approximately 39.83±0.29 cP at 31.1^0C, and appreciably viscous hence can exert stability and could be of relevance in pharmaceutical/cosmetic cream formulation, comparable to known naturally sourced ones such as coconut oil. The density of the oil was 0.89mg/ml and specific gravity 0.90 ±0.002, showing it to be less dense than water therefore could be associated with appreciable flow characteristic and such could enhance good spread ability especially when used in cream formulation. Refractive index value of the extracted oil was 1.431±0.001 confirming it to be liquid at room temperature, while the conductivity at 29.2^0C seems low with value of 0.6±0.11 µS/cm. This depicts a good safety profile of the oil more so, as it is revealed to contain little or no electrovalent ion hence could be suitable for human and animal physiochemical system. This characteristic property will also not permit high electro chemical conductance thus making it safe and compatible in the body system as, its presence will have little or no interaction with other elemental constituents in the system. Due to the low ionic composition and low conductivity, cream produced using this oil may not pose any negative effect on the skin thereby exerting good emollient property, soft and cool on the skin without any harsh, bleaching or deleterious effect. The chemical properties of *P. macrophylla* seed oil as shown in Fig 2, reveal the peroxide value as 18.00 meq/KOH although this is higher than 16meq/KOH as obtained by Okoye et al 2016. The peroxide value is an indication of deterioration of the oil caused by peroxide formation. According to study as revealed in [12], an oil with peroxide value of 10meq/KOH is a fresh and acceptable oil while those that are within 20 and 40meq/KOH are said to have gone rancid so the extracted oil is still stable but need to be properly stored to maintain its stability.

The acid value as obtained was 1.404mgKOH/g, this shows that the acid content is very low and hence the oil should be very edible and void of degradation as low acid values suggest stability of oil. More so, acid value have been identified as a measure of the extent to which triglycerides in the oil is decomposed by lipase, or drying hence a higher acid value may result to a proportional increased degradation. The acid value for the crude oil if high therefore, the oil extracts would need some form of purification (refining) to enhance stability and storage. It has been reported that acid value or free fatty acid (FFA) in crude oils, estimates the amount of oil that will be lost during refining. Thus, the higher the acid as FFA values the higher the amount of oil that could be lost during processing [13]. Saponification is a measure of level of adulteration of the oil, which includes free and chain bound fatty acids. The value obtained for *P. macrophylla* seed oil was 193.12mg/KOH/g. Saponification values approaching 200mg/KOH/g have been reported to be of high molecular weight oil useful use in industries for cosmetic, soap making and for hair shampoo [14]. The saponification values measure the amount of alkali required to combine with the fatty acids liberated by the hydrolysis of fats and oils from which weights of fatty acids could be determined. The iodine value is an index for assessing the ability of an oil to go rancid/deteriorate andthe extracted oil has value of 84.84mg/100g. Oil with iodine value less than 100 indicates low amount of unsaturated fatty acid and hence classified as a non-drying oil and the ash content of 2.30 % suggested a good source of minerals [15].In general, characterization of the extracted *P.macrophylla* seed oil, reveals, low acid value of 1.404mg/KOH g^{-1}, implying the oil to be edible. The oil was also found with peroxide value (18 mEq), meaning, it has not gone rancid, iodine value (84.84 mg/100g), giving the implication, that it is anondrying oil hence, may not be suitable in the polish and paint industry and saponification value (193.12 mg/100g), indicating its usefulness in the soap making industry.

With these, qualities therefore the oil, if properly harnessed stored and utilized can reduce over dependence on other known oils and especially the imported synthetic ones. According to the proximate analysis in Fig 3, *P. macrophylla* seed oil has 13.44%, 54.14%, and 46.86% of protein, lipid and moisture respectively. Protein is important in a healthy skin as it forms a film over the skin aiding hydration and moisturizing hence the oil if incorporated in cream formulation will impart good emollient and elegant property on the skin. In Fig 4, the elemental analysis showed the presence of iron (1.648mg/L), lead (0.086mg/L), sodium (0.516mg/L), potassium (0.601mg/L) and magnesium (2.050mg/L) which is about 33.6%, 1.8%, 10.5%, 12.3% and 41.8% respectively which is almost in line with that obtained by [7] as magnesium had the highest proportion in the elements analyzed. Magnesium is necessary for the enzymes that regulate DNA replication and repair as the skin is exposed to free radicals, help reduce inflammation caused by an excess amount of E-selectin and C-reactive protein, helps improve the overall skin appearance, reduce protuberance and other skin disorder by lowering cortisol levels. The presence of lead though in small concentration could be as, a result of environmental impact especially as the area where the seed was, sourced is, dominated by various local industries involved in both honey, crude oil and soft drink production. The *P. macrophylla* seed oil considering microbial analysis, was tested against G^{-ve}, G^{+ve} bacteria and fungi and shown in Table 3, its activity on the bacteria and fungi isolates were compared to that of coconut oil, gentamicin and ketoconazole as positive control. The control showed greater activity respectively in the isolates while zone of inhibition of coconut oil was greater than that of the *P. macrophylla* oil. The *P. macrophylla* oil showed activity against four out of six organisms which includes, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus and Candida albicans* while coconut oil had activity against *Pseudomonas aeruginosa*in addition. Although in majority of the isolates, coconut oil showed greater activity but for activity to be, observed on some of the microbial isolates using the extracted *P. macrophylla* oil as demonstrated, the oil can be, adjudged useful as antimicrobial agent in pharmaceutical/ cosmetic cream formulation.

CONCLUSION

P. macrophylla oil was successfully extracted using soxhlet extraction technique.

The extracted oil was more stable than coconut oil under stress condition. The *P. macrophylla oil* showed antimicrobial effect against tested organisms including *C.albicans, E. coli, S. aureus and B. cereus*, although its effect was less than that of coconut oil and other standards such as gentamicin and ketoconazole for bacteria and fungi respectively. Despite the selective microbial effect of the extracted *P. Macrophylla* oil, it could still, be harnessed as, a potential ingredient in pharmaceutical /cosmetic formulation especially for its envisaged emollient and spreadable properties. Therefore, emphasis should, be geared towards its commercial processing for the cosmetic/pharmaceutical industries, as it will help to cushion the economic implication emanating from high cost of imported and synthetic raw materials.

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