

Physicochemical, Antimicrobial, and Antioxidant Evaluations of *Pentaclethra macrophylla* Benth Seed Oil

Chioma N. Chinaka¹, George Awemu², Ibeabuchi J. Ali^{3,*}

¹Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Port-Harcourt, Nigeria; ²Department of Pharmaceutical Chemistry, Madonna University, Elele. Port Harcourt, Nigeria; ³Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Enugu State University of Science and Technology, Agbani Enugu Nigeria.

*Correspondence: ibeabuchi.ali@esut.edu.ng; +234-8064535760

Received 20 September, 2024

Revised 24 November, 2024

Accepted 25 November, 2024

Published 08 December, 2024

Copyright © 2024 the authors. This is an open access article distributed under the Creative Commons Attribution License which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. <https://doi.org/10.59493/advpbl/2024.1.5>

Abstract

Pentaclethra macrophylla seed oils have been used traditionally for treating wounds and burns and are of commercial importance in cosmetic and wellness industry. The physicochemical, antimicrobial and antioxidant properties of the seed oil were evaluated to validate its therapeutic potentials in this study. The exhaustive extraction of oil was done using a Soxhlet extractor at 50°C and n-hexane as the extracting solvent. The physicochemical analysis (color density, pH, acid value, ester value) was determined using standard methods. The antimicrobial determination was carried out by the Agar well diffusion method. The *in vitro* antioxidant activity was determined by potassium permanganate scavenging assay. The physicochemical analysis of the seed oil revealed an orange-colored, sweet odour oil, pH of 5.74 and density of 0.89 g/ml. The acid, saponification, hydroxyl and peroxide values of the seed oil were 5.67, 184.69, 114.6 and 5.46 respectively. The antimicrobial evaluation demonstrated a concentration- dependent effect on the test organisms at concentration range of 2:0.25-1:1 v/v of seed oil:water. The measured inhibition zone diameter (IZD) for *B. subtilis* and *K. pneumoniae* were 18.0 mm and 14.0 mm respectively while *C. albicans* had the least IZD of 8.0 mm. The antioxidant activity was also concentration-dependent (ranged 31.25- 1000 µg/mL) though not as effective as vitamin E. These results lend credence to the use of *P. macrophylla* oil for dressing wounds and burns due to the observed antimicrobial, and antioxidant activities. Our finding has established potential utilization of the seed oil in cosmetics industry as sunburn and skin protective agents.

Keywords: Extraction, Medicinal plants, Microorganisms, Oxidative stress

Introduction

The importance of vegetable seed oils cannot be overemphasized not only as an emollient in cosmetics but also in lipid drug delivery formulations (lotions, creams, emulsions and soft gel capsules) in pharmaceuticals [1]. They have a high medicinal value because of their high content of vitamin E (Tocopherol) which is an antioxidant and also a source of dietary lipids in the form of energy supply. The physicochemical properties of these fixed oils from seeds are determined not only to determine their quality for pharmaceutical, commercial or food purposes but also to avoid adulteration of the oil. Some of these properties depend on the geographical source (climate), time of harvest, method of extraction and storage, level of maturity of seeds, and general handling [2,3]. Chemically, oils are triglycerides which are a product of an esterification reaction between a triol (glycerol) and three molecules of fatty acid. The rich free fatty acid content of seed oils could be saturated (e.g. palmitic and stearic) or unsaturated (oleic and linolenic) due to the presence of unsaturation in the carbon chain. It is also beneficial in cosmetics and in the healing process of the skin (as anti-inflammatory and antimicrobial agents) [4-6]. The commonly known oil seeds are groundnut, soybean, palm kernel, castor, cotton, coconut, olive, sunflower, rapeseed, sesame, linseed, and safflower seeds, etc. These oils could be edible or non-edible depending on its level of free fatty acid content present in a given plant. Antioxidants are substances that protect cells from damage caused by reactive oxygen species such as singlet oxygen, superoxide ion, hydroxyl ion, and hydrogen peroxide. They prevent the oxidation of biomolecules (proteins, lipids, enzymes, and DNA) by interacting with, stabilizing and terminating the chain reactions (by removing radical intermediates) thereby hindering further oxidation reactions by sacrificing themselves [6].

Pentaclethra macrophylla (family- Fabaceae) commonly called African oil bean tree (Ugba in Igbo the language of Eastern Nigeria) are commonly found in tropical African countries especially Cameroon, Cote d'Ivoire, Ghana, Niger, Nigeria, and Togo [7]. The use of *P. macrophylla* seed traditionally in wound dressing and burns could be related to its antimicrobial, antioxidant, and emollient properties. The *P. macrophylla* seed have been investigated against number of pharmacological conditions such as antiulcerogenic, antioxidant, anti-inflammatory, antimicrobial, antisickling, and antihyperlipidemic activities [8 -10]. The results of the previous study showed that the seeds of *P. macrophylla* have therapeutic value at varying concentrations of the seed extracts. The phytochemical screening of the

extract from *P. macrophylla* as reported earlier showed that the seeds contained terpenoids, cardiac glycosides, saponin, alkaloids, flavonoids, and tannins [10]. The seed oil of *P. macrophylla* contains various classes of secondary metabolites which confer its pharmacological activities. The most abundant constituents are steroids: sitosterol, campstool, and stigma-sterol. The major terpenes present in the seed oil are γ -elemene, α -caryophyllene, and D-limonene. The seed oil also contains carotenoids, and tocopherols [11]. The abundant secondary metabolites of the seed oil coupled with its therapeutic potential instigated the present research for possible utilization of the seed oil in cosmetics and pharmaceutical industries for development of skin care products. Therefore, this study aimed to investigate the physicochemical, antioxidant, and antimicrobial properties of the seed oil of *P. macrophylla*.

Materials and Methods

Collection and preparation of plant material

The matured seeds of *P. macrophylla* were obtained from Nnewi North Local Government Areas of Anambra State, Nigeria. A sample of the plant parts and legumes was identified and authenticated in the Department of Pharmacognosy of Madonna University, Elele, Rivers State where a voucher specimen (MUPH 269511) was deposited in the herbarium. The cotyledon of the seeds was harvested manually, sorted, washed and oven dried at 50 °C for 12 h. These were pulverized to a fine powder.

Sourcing of test microorganisms

The microorganisms (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella species* and *Candida albicans*) were clinical isolates obtained from the Department of Medical Laboratory Science, Madonna University, Elele, Rivers State. The identities of the organisms were confirmed using standard morphological and biochemical tests.

Extraction of *P. macrophylla* seed oil

The oil was extracted by leaching in Soxhlet apparatus using *n*-hexane (b. p. 40- 60°C) as a solvent of extraction. The powdered seed sample (87 g) was placed in a thimble and loaded into a Soxhlet extractor. Six runs of the condensing solvent (lasting 6 h) were sufficient to extract all the oil from the sample. The process was stopped when the condensing solvent passing through the sample turned colourless. The solvent/oil mixture was separated at 40°C using a rotary vacuum evaporator Type 349/2 (Buchi, Germany) and finally air oven at 40-50°C. The resulting seed oil weight was expressed as a percentage of the powdered sample weight. The extracted seed oil was capped in an airtight dark brown vial and placed in a freezer at -2°C until used for further analysis.

Physicochemical analysis of *P. macrophylla* seed oil

The chemical analysis of the seed oil was done using previously developed methods [13]. The values of the parameters were measured in triplicate and expressed as mean $\pm$ standard deviation.

Specific gravity determination of *P. macrophylla* seed oil

The specific gravity bottle (50 mL volume) was washed, oven-dried at 60°C and then finally cleaned with acetone and air dried. The weight of the empty bottle and its fitted stopper (W) was taken, after which the bottle was filled to the brim with distilled water and the stopper was also inserted. The weight (W₁) was recorded for a specific gravity bottle and its water content. The distilled water was then removed and the bottle was again dried at 60°C to remove moisture. The *Pentaclethra* seed oil was finally loaded into the bottle and the stopper fitted in, after which the weight (W₂) was also taken and finally the specific gravity of the oil was determined.

Refractive index determination of *P. macrophylla* seed oil

The refractive index was determined using Abbey refractometer model A 80251 (BS). The glass prism of the refractometer was thoroughly cleaned and drops of the oil sample were placed on the lower prism and spread thinly. The equipment was adjusted using both coarse and fine adjustment knobs until the fine and thin demarcation between light and black shadow appears on the crosswire indicator. The reading of the telescope was recorded to give the value of the refractive index of the oil at room temperature.

Viscosity determination of *P. macrophylla* seed oil

A 20 mL volume of *Pentaclethra* seed oil was placed in a 50 mL beaker and the viscosity was determined using a Brookfield viscometer (DV2T, Brookfield Engineering Laboratories, Massachusetts, USA) by inserting the spindle down to a depth of 1 cm in the oil sample. Analysis was done using spindle #62 at 12 rpm for 5 min at 27.5°C.

Free fatty acid value determination of *P. macrophylla* seed oil

Approximately 1.0 g of the seed oil was added to an Erlenmeyer flask containing 20 ml of ethanol and 20 ml of diethyl ether and these were shaken for about 10 seconds. About 5 drops of phenolphthalein reagent (indicator) were added and titrated with a standard solution of 0.1 M KOH solution until a pink color appeared which persisted for at least 15 seconds. The procedure was done in triplicates. The acid value was calculated from the equation below:

$$\text{Acid Value} = \frac{56 \times \text{molarity of KOH} \times \text{Titration value}}{\text{weight of sample (g)}}$$

Saponification value of *P. macrophylla* seed oil

A 2 g quantity of *Pentaclethra* oil and 20 ml of 0.5 M KOH solution were placed in a distillation flask fitted with a reflux condenser and boiled for 1 h with occasional shaking. The amount of KOH remaining after saponification (refluxing) was determined while still hot by titrating with 0.5 M HCl using 2 drops of phenolphthalein as an indicator. A blank experiment was also carried out by repeating this procedure without the oil sample. The saponification value was calculated from the equation below.

$$\text{Saponification value} = \frac{(X-V) \times M \times 56.1}{W}$$

where X = volume of HCl used in the blank titration, V = volume of HCl used in the test titration, M = molarity of HCl (0.5M) and W = weight of the oil sample.

Peroxide value of *P. macrophylla* seed oil

A known weight (3.0 g) of *Pentaclethra* seed oil was dissolved in a solvent mixture (glacial acetic acid: chloroform, 3:2). Then 0.5 mL of saturated potassium iodide solution was added. After 1 min, 30.0 mL of distilled water was added and this was titrated with vigorous shaking with 0.01M sodium thiosulphate ($\text{Na}_2\text{S}_2\text{O}_3$) solution using freshly prepared starch mucilage as indicator. A blank titration was also carried out and the peroxide value was calculated using the formula below.

$$\text{Peroxide value} = \frac{F \times (A-B) \times 10}{W}$$

where F is the factor of 0.01M $\text{Na}_2\text{S}_2\text{O}_3$, A is the titre value of the oil sample and B is the titre value of the blank titration.

Hydroxyl Value determination of *P. macrophylla* seed oil

An approximate weight (2.0 g) of *P. macrophylla* seed oil in a distillation flask fitted with a reflux condenser was boiled under reflux with 1.0 ml of acetic anhydride for 5 minutes. This was cooled by placing in iced water. The mixture was titrated with 0.5 M ethanolic KOH using phenolphthalein as an indicator. A blank titration was also carried out. Hydroxyl value was determined using the equation below:

$$\text{Hydroxyl value} = \frac{A + 28.05 \times V}{W}$$

where: 'A' in the equation is the acid value of the substance, and V is the difference in ml between the titrations.

Iodine value determination of *P. macrophylla* seed oil

A quantity of *P. macrophylla* seed oil (0.5 g) was dissolved in 10.0 ml of carbon tetrachloride and then 20 mL of Wij's reagent was added, mixed and allowed to stand for 30 min in the dark. Then 15 mL of 10 % KI and 100 ml of distilled water were added. The resulting solution was mixed thoroughly and titrated with 0.1 M sodium thiosulphate using a few drops of 1% starch mucilage as an indicator toward the end-point. The detection of the end point is the disappearance of blue colouration. Blank titration was also carried out with the omission of the oil. The titre volumes for the determination and blank were noted as V_s and V_b respectively. The iodine value was calculated as follows:

$$\text{Iodine value} = \frac{(V_s - V_b) \times 1.269}{W}$$

Antimicrobial activity determination of *P. macrophylla* seed oil

The antimicrobial test of *P. macrophylla* oil against various clinical isolates was studied by the Agar well diffusion method [15,16]. Ciprofloxacin (100 µg/ml), fluconazole (100 µg/ml) and DMSO (10 %) were used as standards in the study for antibacterial, antifungal and negative control respectively. Nutrient agar plates and Sabouraud broth media were used for bacterial and fungal studies respectively. The plates were seeded with 100 µl indicator organism (equivalent to 0.5 Mac Farland turbidity standard) and 7.0 mm wells bored in the media were filled with 1.0 ml of oil mixed with water in different ratios (2:2, 2:1, 2:0.5, 2:0.25). The plates were incubated at 37°C for 24 h for bacteria and 28°C for 48 h for fungi. The antimicrobial capacity of seed oil was assessed by measuring the zone of inhibition around the well.

Antioxidant activity of *P. macrophylla* seed oil

The radical scavenging effect of *P. macrophylla* oil was determined using the KMnO_4 radical scavenging as reported [12]. About 1.0 ml of seed oil (31.25- 1000 µg/ml) was placed in a test tube with 3.0ml of 80 µg/l KMnO_4 solution in phosphate buffer (pH 9). The resulting test solutions were incubated at 25°C for 30 min and the absorbance of the residual KMnO_4 determined at wavelength 525 nm using a UV-VIS spectrophotometer. The blank solution, prepared under the same condition as the test solution comprised of a solution of KMnO_4 in phosphate buffer. The results were expressed as percentages of blank. The concentration required to cause a 50 % decrease in absorbance (EC_{50}) was calculated. The % KMnO_4 scavenging effect of the antioxidant was calculated as follows:

$$\% \text{KMnO}_4 \text{ scavenging effects} = \frac{A_c - A_t \times 100\%}{A_c}$$

where A_c is the absorbance of the control (KMnO_4) and A_t is the absorbance of the test sample (*P. macrophylla* oil).

Results and Discussion

The 32.79 % yield of the seed oil is an indication that processing *P. macrophylla* oil bean seed for industrial utilization has a good prospect for commercialization because of its substantial yield. The physicochemical properties are used to characterize oils, so they are important for the quality control and detection of adulterated oils. The physicochemical properties of the *P. macrophylla* seed oil are as

presented in Table 1. They indicated potential for the use of the seed oil for industrial purposes considering the physicochemical values obtained. The high viscosity of the seed oil describes the rheological properties of oil and measures the internal resistance to flow suggesting its emollient property. The acid value represents the amount of free fatty acids present in oil. The low content of peroxide value (1.9 ± 0.35 meq. O_2 /Kg) of oil indicates no rancidity while the staleness derived from partial breakdown of glycerides could be as a result of analyzing the seed oil immediately after extraction. This is not in agreement with other researchers that reported high peroxide value of 5.2 ± 0.12 meq. O_2 /Kg; based on their findings the analysis was not carried out on time making it go rancid [10]. It can be concluded that if the seed oil of *P. macrophylla* is stored in an air-tight container, the tendency for rancidity will not occur. The saponification value of oil is the number of potassium hydroxide required to neutralize the fatty acids resulting from complete hydrolysis of 1 g of the sample. The saponification value gives a measure of the average molecular weight of the fatty acids present, this is of importance when considering its usefulness in food or in the chemical industry especially skincare industries [13,14]. The saponification value of 184.69 ± 1.78 mg KOH/ g of oil obtained in the study corroborated previous reports with saponification value of 171 ± 1.54 mg KOH/ g of oil [15]. The iodine value is a measure of the proportion of unsaturated fatty acids in the oil. It quantifies the amount of double bond present in the oil and its susceptibility to oxidation. A high level is an indication of high nutritional value of the oil. The value obtained was 102.03 ± 2.45 g of iodine /100 g of oil which is in agreement with other vegetable oil, olive oil, cotton oil, groundnut oil and sunflower oil which ranged 86.23 ± 2.1 - 145.16 ± 2.45 g of iodine /100 g [16].

Table 1: The physicochemical properties of the *P. macrophylla* seed oil

Parameters	Value
Colour	Light yellow
Odour and taste	Characteristic
Specific gravity	0.89 ± 0.18 (g/ml) (25°C)
Refractive index	1.4575 ± 1.28
Viscosity	24.543 ± 1.03 (centipoise) (25°C)
Acid value	4.10 ± 0.43 (mg KOH/ g of oil)
Saponification value	184.69 ± 1.78 (mg KOH/ g of oil)
Peroxide value	1.09 ± 0.35 (meq. O_2 / Kg of oil)
Iodine value	102.03 ± 2.45 (g of iodine /100 g of oil)

The antimicrobial activity of *P. macrophylla* seed oil showed a concentration dependent effect on the test organisms at concentration range of 0.25-2 mL v/v of seed oil. The inhibition zone diameter recorded for the test microorganisms ranged 4-19 mm for *B. subtilis*, 2-17 mm for *S. aureus*, 2-15 mm from *P. aeruginosa*, 3-16 mm for *E. coli*, 1-15 mm for *K. pneumonia* and 1-8 mm for *C. albicans*. The *P. macrophylla* seed oil based on our study exhibited significant antimicrobial activity, as a consequence seed oil might have penetrated the cell wall of the test organism thereby inhibiting their metabolic processes. The study is in agreement with previous report which showed that different solvent extracts of *P. macrophylla* seed showed reasonable antimicrobial activity against *E. coli*, *S. aureus*, *P. aeruginosa*, *Klebsiella species*, *S. typhi*, *A. niger* and *C. albican*, though in contrast to another report that the *P. macrophylla* seed oil only had activity against *A. flavus*, a fungus and did not show any activity against *S. aureus*, *S. mutans*, *S. typhimurium* and *E. coli* and *C. albican* and *R. oligosporus*. The effect of the seed oil is more on the bacteria species as against the fungi, *Candida albican* as shown in Figure 1. This is scientific evidence for its use traditionally in management and disinfection of wounds and burns. It can therefore serve as an alternative source of ointment for skin infections.

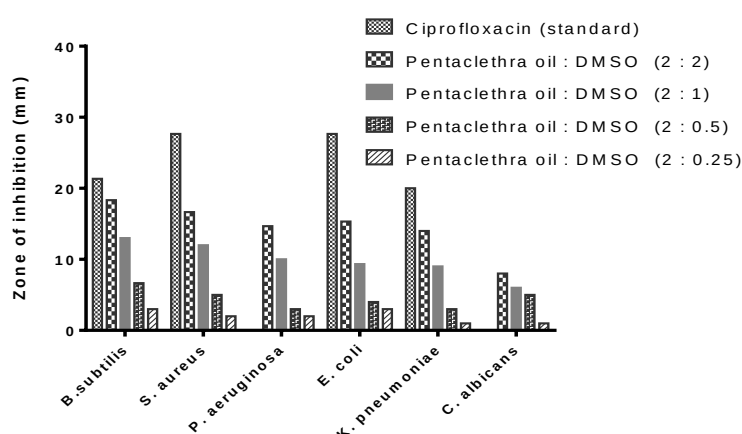


Figure 1: Antimicrobial activity evaluation of the seed oil of *P. macrophylla* against test microorganisms

The antioxidant activity of *P. macrophylla* seed oil showed concentration dependent activity ranging from 31.25- 1000 μ g/ml as shown in Figure 2. This observed activity is due to the fact that the seed oil contains constituents which inhibit the oxidative reaction by scavenging the reactive oxygen species preventing its deleterious effect to the cells. The *P. macrophylla* seed oil showed EC50 of 0.5319 g/ml against Vitamin E, 0.0029 g/ml indicating its application in protection of ailments associated with oxidative damage to cells.

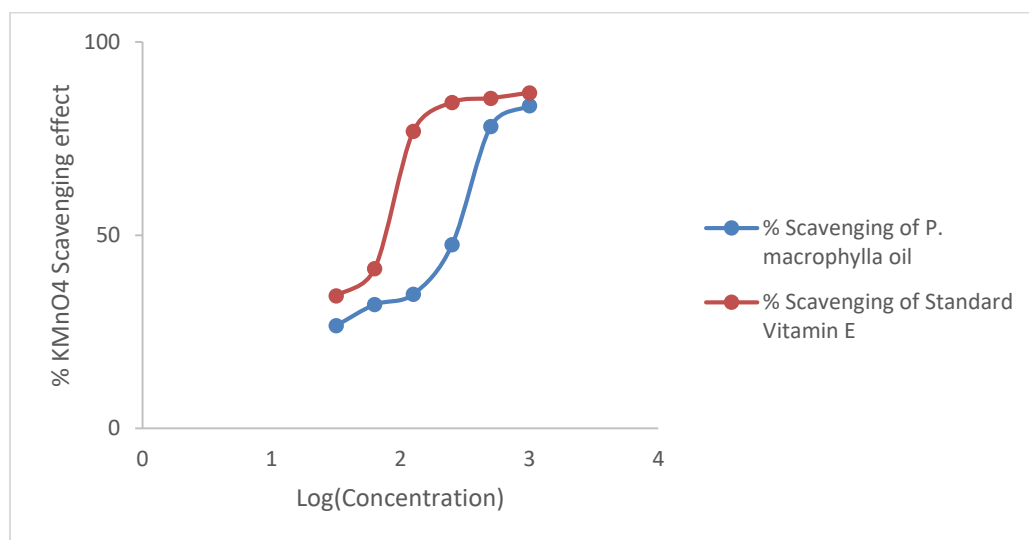


Figure 2: Potassium permanganate (KMnO_4) scavenging activity of *P. macrophylla* oil and Vitamin E

Conclusions

The seed oil extracted from *P. macrophylla* has demonstrated a significant antimicrobial and antioxidant activities which is attributed to the pharmacological active constituents of *P. macrophylla*. This research corroborates folkloric use of *P. macrophylla* seed oil to treat ailments associated with swelling and microbial infections. This has given insight on the utilization of the seed oil in cosmetics industries to produce skincare products that prevent effect of sunburns and skin diseases.

Conflict of interest

No conflict

Author's contributions:

The conceptualization, literature collection and curation were done by GA. The literature collection and experimentation and data analysis were done by CNC while IJA wrote the original draft and prepared manuscript.

Funding

Nil

References

1. Viljoen JM, Cowley A, du Preez J, Gerber M, du Plessis J, 2015. Penetration enhancing effects of selected natural oils utilized in topical dosage forms. *Drug Development and Industrial Pharmacy*, 41(12):2045–54.
2. Vermaak I, Kamatou GPP, Komane-mofokeng B, Viljoen AM, Beckett K, 2023. African seed oils of commercial importance — Cosmetic applications. *South African Journal of Botany* [Internet], 77(4):920–33. Available from: <http://dx.doi.org/10.1016/j.sajb.2023.07.03>
3. Chinaka CN, Awemu G, 2018. The physicochemical and antioxidant potential of the Theobroma cacao L. seed oil (malvacea) obtained from southern Nigeria. *World Journal of Pharmaceutical Research*, 7(6):1–11.
4. Junaid P, Dar A, Dash K, Ghosh T, Shams R, Khan S, Singh A, Kumar PV, Nayik G, Raj B, 2022. Advances in seed oil extraction using ultrasound assisted technology: A comprehensive review. *Journal of Food Process Engineering*, Vol. 46.
5. Ugbogu OC, Onyagba RA, Chigbu OA, 2006. Lauric acid content and inhibitory effect of palm kernel oil on two bacterial isolates and Candida albicans. *African Journal of Biotechnology*, 6:1045–7.
6. Aaser MA, Mosleh MA, 2024. Oxidative stress: an overview of past research and future insights, *All Life*, 17:1, 2316092
7. Olaitan JO, Kareem SO, Dada SO, 2009. Antimicrobial potency of Pentaclethra macrophylla seed extract on seven selected pathogens. *African Journal of Biomedical Research*, 12(2):140–4.
8. Guynani HC, Ezenwanze EC, 1985. Antibacterial activity of extracts of ginger (Zingider officinale) and African oil bean seed (Pentaclethra macrophylla). *Journal of Communicable Diseases*, 17:233–6.
9. Chinaka CN, Garuba AS, Ahmadu AA, 2017. Chemical constituents from the stem barks of Pentaclethra macrophylla Benth (Fabaceae). *Nigerian Journal of Pharmaceutical Research*, 13(1):37–44.
10. Akinlabu DK, Owioye TF, Owolabi FE, Audu OY, Ajanaku CO, Falope F, Ajani OO, 2019. Phytochemical and proximate analysis of African Oil bean (Pentaclethra Macrophylla Benth) seed. *Journal of physics: conference series*. 1378(2019)032057.
11. Ojmelukwu PC, 2024. Health Benefits and Medicinal Value of Pentaclethra Macrophylla. *Journal of Traditional Medicine and Applications*, 3(1), 01-10.
12. Amponsah IK, Orman E, Mensah AY, Sarpong FM, Armah FA, Sarpong LM, 2016. Development and validation of a radical scavenging antioxidant assay using Potassium permanganate. *Journal of Scientific and Innovative Research*, 5(2):36–42.
13. Aremu OM, Ibrahim H, Bamidele TO, 2015. Physicochemical characteristics of the oils extracted from some Nigerian Physicochemical Characteristics of the Oils Extracted from Some Nigerian Plant Foods – A Review. *Chemical and Process*

- Engineering Research, 7:36–52.
14. Talabi JY, Enujiugha VN, 2014. Physical and chemical evaluation of oils from selected underutilized oilseeds. *Der Chemica Sinica*, 5(6):9–12.
 15. Ugbogu OC, Akukwe AR, 2009. The antimicrobial effect of oils from *Pentaclethra macrophylla* Bent, *Chrysophyllum albidum* G. Don and *Persea gratissima* Gaertn F on some local clinical bacteria isolates. *African Journal of Biotechnology*, 8(2):285-287
 16. Oyedele OA, Odeyemi BA, Koleosho AO, 2019. Antimicrobial activity, spectroscopic and thermal characterization of oil from African oil bean (*Pentaclethra macrophylla*) seed. *Journal of Pure and Applied Sciences*, 1 (1): 182-187.