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Comparative Studies on the Elemental Analysis, Proximate Analysis, Antimicrobial Activity and Acute Toxicity Study of *Picralima nitida* Leaves and Seeds

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Abstract: In West African cultures, *Picralima nitida* has long been used in medicine for providing pain relief, controlling inflammation, reducing fever, and fighting infections. This study compares the elemental and proximate compositions, antimicrobial activities, and acute toxicity of the leaf and seed extracts of *P. nitida*. The samples were extracted using the cold maceration method. Elemental analysis revealed high levels of magnesium, calcium, and potassium in the leaf extract, while the seed extract contained lower amounts. The leaves also had a higher crude fiber content (15.5%), whereas the seeds were richer in crude protein (19.47%) and ether extract (14.00%). The leaf extract prevented the growth of *E. coli*, *S. aureus*, and *P. aeruginosa*, yet did not affect *C. albicans*. The seed extract exhibited a broader and stronger inhibitory effect against *P. aeruginosa*. The minimum inhibitory concentrations (MICs) of *E. coli* and *P. aeruginosa* were both 100 mg/mL (15.00 mm inhibition zone) when tested with the leaf extract, whereas S. aureus had an MIC of 75 mg/mL (13.00 mm inhibition zone). The MICs of the seed extract were 25 mg/mL (9.00 mm inhibition zone) for *E. coli* and *S. aureus*, 75 mg/mL (18.40 mm inhibition zone) for *P. aeruginosa*, and 25 mg/mL (11.20 mm inhibition zone) for *C. albicans*. For the toxicity tests, the leaf extract appeared safer for animals (mice) with no adverse effect at an LD50 of 800mg/kg, whereas the seed showed a notable adverse effect with an LD50 of ≥283 mg/kg. These experimental findings show the health benefit of *P. nitida* leaves and seeds in traditional medicines and its potential as an anti-microbial agent and use for dietary purposes.

Introduction

Medicinal plants contain a wide variety of bioactive compounds such as essential oils, saponins, flavonoids, tannins, alkaloids, and terpenoids; the combination of those with different contributions are what yield their much-sought antibacterial and therapeutic powers (1). As a result, there is growing interest in the bioactive chemical components of these plants and their potential in drug development. These plants provide three main types of benefits: medicinal benefits for patients who use them as remedies, economic benefits for those involved in harvesting, processing, and distribution, and social benefits for the communities that rely on them. In developing regions, particularly in rural areas, traditional medicine often serves as the only accessible form of healthcare. However, this dependence can be risky, as most products made from these plants had not been tested for their safety and efficacy and this is due to insufficient technology, poor practices involved in the processing of these drugs which can as well as contain dangerous contaminants which can stand as a threat to human lives hence the need for this study for proper evaluation of these plants. Toxicology theory explains that a medicinal agent's effect, therapeutic or toxic depends on its dose-response relationship, its toxicokinetics (absorption, distribution, metabolism, excretion), and the underlying mechanism, which means a medicine is safe at low doses but can be harmful at higher doses and this profile is essential for the safe use of any medicinal compound (1).

In that light, the safety of herbal medicines depends on the chemical arrangement of the plant, with the highest hazards usually occurring from prolonged or excessive usages of herbs otherwise thought to be mildly toxic (2). To understand the chemical arrangement of medicinal plants, scientists carry out elemental analysis, the process of determining the elements present and quantifying them in a sample, whether the sample be soil, water, minerals or plant extracts. This analytical technique plays a key role in pursuing the chemical characteristics of natural substances, being vastly applicable in medicine, nutrition and

environmental studies for instance (3). In the case of organic chemists, elemental analysis is one method they would use to determine the composition in a sample by measuring certain elements present such as carbon, hydrogen, nitrogen, sulfur and halogens (4). Though newer techniques employed in molecular structure determination, for instance, NMR spectroscopy and mass spectrometry, may often receive favor, elemental analysis remains a powerful ally (5). Proximate analysis also serves to determine nutritional and chemical composition of medicinal plants. This divides plant compounds into six groups: moisture, ash, crude protein, crude lipid, crude fibre, and nitrogen-free extracts, which are digestible carbohydrates, it is an equally relevant technique that provides data on composition with respect to nutrition and chemistry of medicinal plants (6). This additional understanding regarding nutritional constituents will further enrich the recognition and application of many medicinal plants, which are also used as food items (7).

Picralima nitida, first identified in 1896, belongs to Apocynaceae family. The plant is native to tropical Africa, spreading through countries such as Nigeria, Ghana, Ivory Coast, Cameroon, Gabon, Uganda, and the Central African Republic (8). *Picralima nitida* dried seeds have many medicinal applications: crushed or powdered, the seeds are taken against malaria, diarrhoea, and pain (9). The fruit is applied in gastrointestinal disorders, the leaves against tapeworm infections, and its sap is used in the ear for otitis (10). The bark has many applications such as laxative, anthelmintic, for curing venereal diseases and fevers, and hernias (11). It is also made into decoctions for jaundice and yellow fever (12). The root also possesses aphrodisiac properties and is used in the management of malaria, pneumonia, and digestion (2; 13). Given the multiple uses of *Picralima nitida* in traditional medicine for the treatment of various ailments, there remains a notable research gap in its scientific characterization. While some studies have explored its phytochemical constituents and ethnomedicinal applications, few have comprehensively examined its proximate composition, elemental content, antimicrobial properties, and toxicity levels particularly through a comparative analysis of its leaves and seeds. This lack of data limits the understanding of its nutritional, chemical, and safety profiles, which are crucial for validating its traditional uses and exploring potential therapeutic or dietary applications. Therefore, this study proposes an analysis of the proximate composition, elemental content, antimicrobial properties, and toxicity levels of the leaves and seeds of *P. nitida.*

Materials and Methods

Fresh leaves and seeds of *Picralima nitida* were collected in January 2024 from the Useh community in Benin City, Edo State, Nigeria. To confirm the identity of the plant, it was examined and authenticated by Prof. Akinnibosun Henry Adewale from the Department of Plant Biology and Biotechnology, University of Benin, and given the voucher number UBH-P424. In a properly ventilated area, the leaves and seeds were allowed to dry at room temperature (30±0.5℃) before being ground finely using a mechanical grinder in the manner prescribed by Wokocha and Okereke (2005) (14). For extraction, quantities of 500g and 200g of powdered leaves and powdered seeds respectively were each soaked in 1.4L of methanol for a period of 72 h. Filtering was done with cheesecloth, and the filtrates were reduced to dryness by evaporating with a rotary evaporator set at 40℃. The extraction yield was calculated using **Equation 1**.

$$\%\,\text{Yield} = \frac{\text{Weight of extract}}{\text{Weight of the powder sample}} \times 100$$

Equation 1 | Calculation of extraction yield (%).

Determination of the Elemental Composition

This involved digesting a 1-gram sample by the aqua regia method. The aqua regia comprised the mixture of concentrated hydrochloric acid and nitric acid in a ratio of 3:1 (400 mL of HCl and 133 mL of HNO_3). The solution was heated on a hot plate until digestion was complete, filtered through Whatman filter papers into a 100 mL volumetric flask, and made up to the mark with distilled water. The metal concentration in the sample was analyzed using an Atomic Absorption Spectrophotometer (AAS) (15).

Proximate Composition Analysis

Proximate analysis, as reported by Olomu (2011) (16), classifies compounds into six major groups: moisture (water), crude protein, ether extract (crude fat), ash, crude fiber, and nitrogen-free extract (NFE). These were analyzed for nutritional composition. Preparation of working solutions: For working solutions, four different amounts of the crude extract (0.25, 0.5, 0.75, and 1.0 g) were dissolved in 1 mL of DMSO in sterile capped test tubes. The prepared solutions were properly sealed and stored for later use.

Collection of Standard Cultures

Bacterial samples were collected in sterile containers. The bacterial strains tested in this study included *Proteus vulgaris, Staphylococcus aureus, Escherichia coli, and Pseudomonasaeruginosa*, while the fungal strain analyzed was *Candida albicans.* The isolates were obtained from the University of Benin Teaching Hospital (UBTH) in Benin City and characterized according to their cultural and morphological properties.

Preparation of Standard Culture Isolates

The characterization and identification done rendered pure bacterial and fungal isolates standardized to a 1.0 McFarland turbidity standard. This was to achieve uniformity in all the microorganisms studied. All glassware used during the process was thoroughly cleaned and sterilized in a hot air oven at 160°C for two h.

Media Preparation

40 g of Mueller-Hinton was accurately weighed, dissolved in water, and sterilized in the autoclave at 121°C for 15 min.

Assessment of Antimicrobial Activity

The screening of the antimicrobial potential of the crude extracts was done through the agar well diffusion method. After sterilization, Mueller-Hinton agar was melted and poured into sterile Petri dishes to allow for solidification. Standardized bacterial & fungal isolates were made using sterile cotton swabs on the agar plates. With the help of a sterile cork borer, four wells were made on each plate, and varying concentrations of crude extracts were added into the wells using a sterile pipette. The plates were labeled and then incubated.

Bacterial cultures were incubated at 37°C for 24 h and fungal cultures at 28°C for 72 h. After incubation, the antimicrobial activity was evaluated by measuring the zones of inhibition (clear areas around wells): their results were expressed in millimeters (mm) using a graduated ruler.

Determination of Minimum Inhibitory Concentration (MIC)

A loopful of each positive plate was transferred or mixed with an equal amount of nutrient broth with a corresponding concentration of the extract and incubated. The MIC of an extract was the minimum concentration that caused the reduced bacterial growth (turbidity).

Determination of Minimum Bactericidal Concentration (MBC)

For MBC, samples from the MIC tubes with positive results were streaked onto fresh agar plates and distilled nutrient broth was incubated afterwards. Subculture into agar plates: a silence of bacterial growth/turbidity after incubation demonstrated bactericidal effect: the MBC was noted.

Antibiotic Sensitivity Testing

Mueller-Hinton agar was prepared and poured into sterile Petri dishes, allowing it to solidify. Bacterial and fungal isolates were streaked across the plates using a sterile cotton swab. Antibiotic discs for both Gram-positive and Gram-negative bacteria were placed on the plates using sterile forceps. Bacterial plates were incubated at 37°C for 24 h while fungal plates were incubated at 28°C for 2-3 days. Following incubation, the zones of inhibition, which were the clear areas around the antibiotic discs, were measured in millimeters. Experimental Animals Swiss mice weighing between 18 and 24 grams were procured from the University of Benin, Edo State, Nigeria. The animals were normally housed in cages made of glass with normal lighting at room temperature and humidity. They were provided with a standard diet following the National Institutes of Health guidelines for the care and use of laboratory animals.

Ethical Approval

All the experimental procedures were reviewed and approved by the Ethical Review Board of the Life Sciences Research Faculty, University of Benin, with Approval Code LS23026.

Acute Toxicity Assessment

The acute toxicity study was conducted using a modified version of Lorke's method (1983) in accordance with the OECD guidelines (2001) for testing chemical substances in animals (17). A total of 12 mice were randomized into four groups of three. The crude extract was administered orally at doses of 200, 400, and 800 mg/kg. The animals were observed for signs of acute toxicity for 24 h, followed by further observation for mortality for 14 days. The parameters that were recognized include piloerection, responsiveness to sound and touch locomotion, aggression, fecal characteristics, salivation, urination, convulsions, coma, and mortality. LD50 was calculated according to the Globally Harmonized System (GHS) for chemical classification (OECD 1998). The lethal dose was determined based on the geometric mean of the lowest fatal dose and the highest nonfatal dose, using repeated experiments with a survival rate of 0% and 100%. The final value of LD50 was derived using **Equation 2**.

$$\mathbf{LD_{50}} = \sqrt{\mathbf{D_0} \times \mathbf{D_{100}}}$$

Equation 2 | D_0 = Highest dose with no mortality, D_{100} = Lowest dose with mortality.

Data Analysis

Experimental data were analysed using the Kruskal-Wallis test, followed by the Dunn-Bonferroni post-hoc test at a significance level of 0.05. Statistical analysis was conducted using SPSS software version 24 (IBM Analytics, USA) with a sample size of n = 3.

Results and Discussion

Percentage Yield of Extract

The percentage yields of the leaf and seed extracts of *Picralima nitida* were 15.912% and 6.500%, respectively. Compared to the studies reported by Haruna and Odunsi (2022), the percentage yield of the seed extract is relatively low, as the value obtained was 27.25% (18). Studies shown by Ololade *et al*., (2023) reveal that the leaf, however, is on the higher side (19). This may be due to differences in the extraction method and solvent used (18).

Elemental Analysis

As shown in **Figure 1**, variations in pollution, weather conditions, growth cycles, plant age, and soil composition can influence how plants absorb minerals. As a result, mineral uptake differs across environments and locations. This uptake is determined by several interacting factors, including species type, soil properties, climate conditions, and agricultural practices. Although many environmental and biological factors can cause fluctuations in mineral concentrations particularly within plant tissues mineral distribution within a single plant is generally non-uniform. *Picralima nitida* is notably rich in potassium, iron, magnesium, and calcium, which supports its potential health benefits such as blood pressure regulation, muscle function enhancement, and hypoglycemic activity, this suggests that *Picralima nitida* is a good pharmacology plant.

Proximate Analysis

According to **Figure 2**, which shows the proximate analysis, it is observed that nitrogen-free extract was the highest compared to all other compounds while ash content indicative of the total number of inorganic materials present in the sample was the lowest. It was observed that the leaf sample has the highest nitrogen-free extracts, crude fibers, and moisture content as compared to that of the seed. Previous studies on the proximate composition of *Picralima nitida* reported that the seed extract contained 13.92% crude protein, 7.13% ether extract, 7.15% crude fiber, 6.82% ash, and 58.09% nitrogen-free extract. In the same vein, the fruits of *P. nitida* were found to have low oil and moisture contents (6.05%) but higher fiber (23%), crude protein (7.87%), and ash content (9.50%) compared with the leaves (20). Proximate analysis is used to assess the purity of the plant specimen to standard values. Low moisture content generally curbs or prevents microbial contamination and chemical degradation (21). The high moisture content of crude drugs promotes the growth of microorganisms (yeast and fungi), leading to the destruction of active compounds (21). *Picralima nitida.* From the result in **Figure 2**, it shows

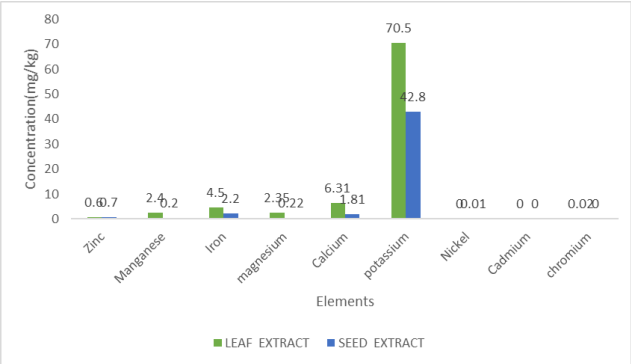

Figure 1. Comparative Elemental Analysis of the leaf and seed extract.

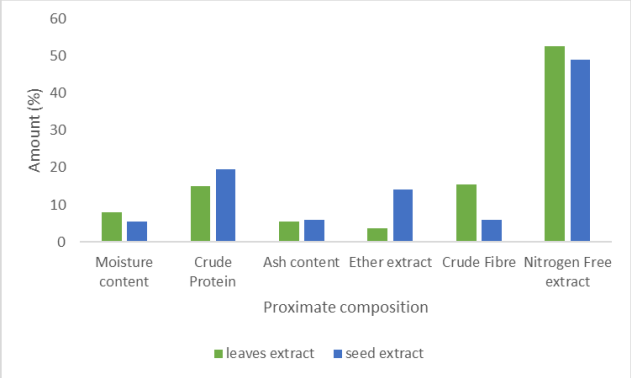

Figure 2. Comparative Proximate Analysis of the Leaf and Seed Extract.

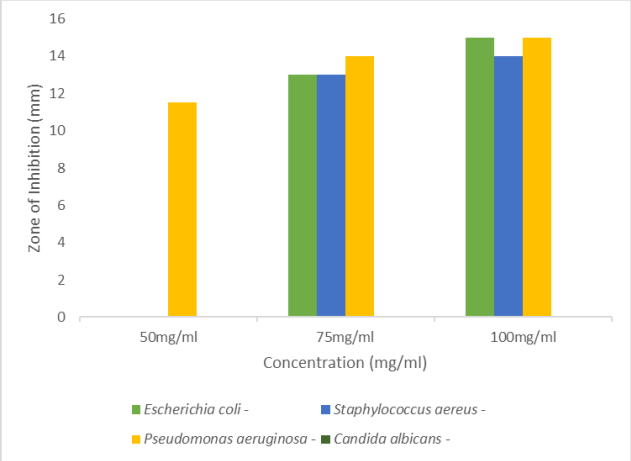

Figure 3. Zones of Inhibition of the leaf Extract.

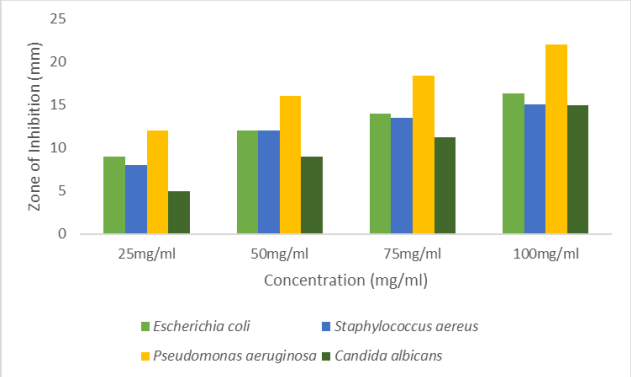

Figure 4. Zones of Inhibition of the seed Extract.

that the leaves and seed of *Picralima nitida* has a low moisture content of 7.83±0.62 and 5.50±0.50, showing that *P. nitida* prevent microbial contamination and chemical degradation and the plant extract and last for a long time before destruction of the active compounds and spoilage. The leaves and seeds has a crude protein of 14.88±4.30 and 19.47±0.67 showing it is a good source of protein and act as a building blocks of muscles, hormones immune function and enzymes and could be good for athletes and people with high protein needs. The leaves and seeds of the plant has a crude fibre of 15.5±1.87 and 6.00±1.00, showing the plant can supports motility and improve satiety.

Anti-microbial Activity of *P. nitida*

According to Sharma (2022), the classification of microbial activity divides it into three categories, a zone of inhibition less than 7 mm indicates resistance, whereas a zone of inhibition that is 7 to 9 is intermediate (22). In this comparative study, methanolic extracts from leaves and seeds of *Picralima nitida* showed that *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Candida albicans* were more sensitive to the seed extract than to the leaf extract. Notably, *Escherichia coli and Staphylococcus aureus* showed equal sensitivity to both extracts. The seed extract, however, showed antibacterial activity at a concentration of 25 mg/mL against *Escherichia coli* (9.00 mm), *Pseudomonas aeruginosa* (8.00 mm), and *Staphylococcus aureus* (12.00 mm), while *Candida albicans* showed a lesser 5.00 mm zone of inhibition. In comparison, the leaf extract showed no antibacterial activity at this concentration. The same leaf extract exhibited significant antibacterial activity against *Pseudomonas aeruginosa* at that concentration when taken up to 50 mg/mL while the seed extract was found to exhibit antibacterial properties against all tested isolates. From the result, the seed showed notable activity, having a zone of inhibition of 11.20 mm for *Candida albicans,* which was absent in the leaf extract, at 75 mg/mL. Still, at 100 mg/mL, the seed extract showed, without exception, the best antibacterial activity across all the isolates tested compared to the leaf extract. The above data provide evidence that indeed the seed extract had better antibacterial properties than the leaf extract.

The comparative study of the minimum bactericidal concentration and minimum inhibitory concentration of the methanolic extracts from the leaves and seeds of *P. nitida* has shown that the seed is a more potent antibactericidal agent than the leaf extract, perhaps because a low concentration is required with the seed to exhibit significant activity in comparison with the leaf, which requires a higher concentration to manifest its activity as shown in **Tables 1 and 2** and supported by the zones of inhibition observed in **Figures 3 and 4**.

Acute Toxicity Test

The comparative study on the acute toxicity of *Picralima nitida* leaves and seeds extracts reveals a significant difference in their toxic effects on living organisms. The seed extract exhibits toxicity even at low doses of 200 mg/kg, with mortalities observed at 400 mg/kg and 800 mg/kg, suggesting that the seed extract contains potent compounds that can be harmful to living organisms (**Table 5**). In contrast, the leaf extract shows lesser toxicity, with no deaths recorded at any dosage level (**Table 3**).

The phytochemical composition of the seed and leaf extracts may contribute to their differing toxicities. The seed

Table 1. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *P. nitida* leaves methanol extract in mg/mL.

Bacteria isolates	MIC (mg/ml)	MBC (mg/ml)
Escherichia coli	100	100
Staphylococcus aureus	75	75
Pseudomonas aeruginosa	100	100
Candida albicans	ND	NDs

Table 2. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *P. nitida* seeds methanol extract in mg/mL.

Bacterial isolates	MIC (mg/ml)	MBC (mg/ml)
Escherichia coli	25	25
Staphylococcus aureus	25	25
Pseudomonas aeruginosa	75	25
Candida albicans	75	50

Table 3. Acute toxicological effects of *P. nitida* leaf extract in mice.

Treatment/doses (mg/kg)				
Parameters	**Dw**	**200 mg/kg**	**400 mg/kg**	**800 mg/kg**
Number of mortality	0	0	0	0
Mortality	0	0	0	0
Adverse effects	Nil	Nil	Nil	Nil
Note: DW = Distilled water. n = 3 (total number of animals per group).				

Table 4. Observable acute toxicity effects of *P. nitida* leaf extract in mice.

Groups	Doses (mg)	Adverse effect
Control	DW	0
Leaf extract	200	0
Leaf extract	400	0
Leaf extract	800	0
Note: DW = Distilled water.		

Table 5. Acute toxicological effects of *P. nitida* seed extract in mice.

Treatment/doses (mg/kg)				
Parameters	**Dw**	**200 mg/kg**	**400 mg/kg**	**800 mg/kg**
Number of mortality	0	0	3	3
% mortality	0	0	100	100
Adverse effects	Nil	Nil	Nil	Nil
Note: Significant across the column. DW = Distilled water. n = 3 (total number of animals per group).				

extract contains alkaloids, terpenes, and phytosterols which may be responsible for its toxic effects observed.

Table 6. Observable effects of *P. nitida* seed extract in mice.

Groups	Doses (mg)	Adverse effect
-Control	DW	0
Seed extract	200	Writhing
Seed extract	400	Writhing, Hyper-respiration, Pilo-erection, Vomiting, Stooling blood, Restlessness, Jerking, Salivation, Lacrimation, Haemorrhage**,** Nausea, Diarrhoea, Motor-movement, Dizziness, Drowsiness, Convulsion, Cough, coma and Death.
Seed extract	800	Writhing, Hyper-respiration, Pilo-erection, Vomiting, Stooling blood, Restlessness, Jerking, Salivation, Lacrimation, Haemorrhage**,** Nausea, Diarrhoea, Motor-movement, Dizziness, Drowsiness, Convulsion, Cough, coma and Death.
Note: DW = Distilled water.		

On the other hand, the leaf extract contains different phytochemicals, such as phenolic compounds, that may be less toxic.

Research has also shown that the leaf extract has hepatoprotective properties, protecting the liver from damage caused by carbon tetrachloride (CCl4) (19). This suggests that the leaf extract may have a protective effect on living organisms.

The antibacterial and antifungal properties of the seed extract may contribute to its toxicity-related effects (23). These properties may be useful for developing new antimicrobial agents, but further studies are required to identify the specific compounds responsible for these effects and to evaluate their safety and efficacy.

The toxicity difference between leaf and seed extracts highlights the importance of careful consideration when using *P. nitida* for medicinal purposes (**Tables 4 and 6**). Further research is needed to determine safe dosage levels and potential interactions with other medications.

Overall, the study suggests that while *P. nitida* seed and leaf extracts have potential medicinal benefits; their toxicity profiles need to be carefully evaluated to ensure safe use, as reported by Ilenowa *et al*. (2024), who studied the phytochemical and proximate composition of fruit pulp (20).

Conclusion

The healthy benefit as well as the implication of the toxicity level of *P. nitida* was investigated in this study. From this study the elemental analysis of *P. nitida* extracts confirmed that essential elements existed within the limits allowed, and the proximate analysis indicated that both the seed and leaf extracts can be used for dietary purpose. For the antimicrobial activities evaluation, the seed extract was the most effective against *P. aeruginosa* which indicates the plant potency as an antimicrobial agent. The toxicity evaluation further confirmed the leaf was safe at 200, 400 800mg/kg, revealing no observable effects and mortalities. However, the seed extract warrants further consideration for safety when used at high dosage owing to its high level of toxicity.

Declarations

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Conflict of Interest

The authors declare no conflicting interest.

Data Availability

The necessary data are available in the manuscript.

Ethics Statement

All procedures of the experiment were approved by the Ethical Review Board of Faculty of Life Sciences of University of Benin (LS23026).

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Additional Information

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