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RESEARCH ARTICLE

PHYTOCHEMICAL COMPOSITION, ANTIBACTERIAL ACTIVITY, PROXIMATE AND MINERAL ANALYSIS OF METHANOL EXTRACT FROM COMBINED SEEDS AND PEELS OF *PICRALIMA NITIDA*

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Abstract

Background and Aim: This study explores the untapped potential of the discarded seed and peel of *Picralima nitida*, a plant with numerous ethno-medicinal uses. This study was aimed to conduct a comprehensive investigation into the phytochemical, antimicrobial, proximate, and mineral composition of the combined seed and peel to maximize its utilization.
Methods: Phytochemical screening method was used to identify the variety of biological active constituents in the seed and peel, proximate composition analysis technique measured the basic nutritional content of the seed and peel, mineral composition analysis was used to determine the levels of essential minerals and trace elements and antibacterial susceptibility testing was performed against *Escherichia coli* and *Staphylococcus aureus*, assessing the extract ability to inhibit microbial growth, with a focus on the sensitivity of *E. coli*.
Results: Phytochemical analysis showed the presence of some compounds like: flavonoids, saponins, tannins, alkaloids, and polyphenols, while proximate composition analysis showed the following values: moisture (12.80%), carbohydrate (59.71%), crude protein (5.85%), ash (2.53%), crude fat (4.64%), and crude fibre (14.05%). The caloric content was 265.8 kcal/100 g, indicating a high nutritional content suitable for use as a feed additive. Mineral composition analysis revealed significant levels of essential elements. Sodium-to-potassium ratio (0.69) aligns with World Health Organization recommendations for cardiovascular health.
Conclusions: These findings highlight the promising pharmacological and nutritional value of *Picralima nitida* seed and peel, suggesting its potential as a bioactive additive in both ethno-medicine and industrial applications, including as a complementary food source and poultry feed additive.
Keywords: Antimicrobial activity, *Escherichia coli*, *Picralima nitida*, phytochemical, proximate composition, *Staphylococcus aureus*.

INTRODUCTION

Picralima nitida is a plant with significant interest due to its multiple ethno-medicinal uses, particularly in the management of different health diseases like: microbial infections, fever, hyperglycemia and gastrointestinal disorders[1-3]. Traditionally, different parts of the plant, such as leaves, roots and fruits, have been utilized for their therapeutic properties[4,5]. However, certain parts of the plant, such as the seeds and peels, are often discarded, despite their potential value[6]. Recent trends in sustainable resource utilization have called for the exploration of these overlooked plant parts to maximize the benefits of *P. nitida*[7].

Phytochemical compounds are essential in determining the medicinal value of plants, as they possess biological activities that can be exploited for therapeutic purposes[1,8-10]. Previous studies have highlighted the rich presence of alkaloids, flavonoids, tannins, saponins, and polyphenols in various parts of *P. nitida*, which are known for their antibacterial, antioxidant, and anti-inflammatory properties[11]. However, there is limited research specifically focused on the phytochemical compounds and bioactive

properties of the combined seeds and peels, both of which are usually discarded during processing.

In addition to their bioactive properties, the nutritional value of *P. nitida* seeds and peels remains largely unexplored. Proximate analysis provides valuable information on the composition of crucial dietary components including proteins, lipids, carbohydrates, fiber and moisture content[12], which are critical in evaluating the plant's potential as a supplementary food source or animal feed additive[13,14]. Mineral composition analysis further reveals the presence of essential macro- and micronutrients, like calcium, magnesium, iron, zinc and potassium, which are vital for human and animal health[15].

This study sought to analyze the phytochemical profile, antibacterial potentials, proximate composition, and mineral content of methanol extracts from the combined seeds and peels of *P. nitida*. By identifying the bioactive compounds and nutritional properties of these discarded parts, this research aims to highlight their potential for use in both industrial and medicinal applications, contributing to the sustainability of this valuable plant resource[3]. Furthermore, the antibacterial activity of the methanol extract was tested against common pathogens, which could provide insights into the plant's utility as a natural antimicrobial agent[13,16].

The present study aims to address the knowledge gap by providing a comprehensive investigation of the combined seed and peel extracts of *P. nitida*, with a focus on their potential for broader applications in medicine, agriculture and food industries.

MATERIALS AND METHODS

Sampling and authentication process

The fruits of *P. nitida* were harvested from Benin city, Nigeria, during the semi-rainy season in April 2023. The seeds and peels were separated from the fruits, then dried at room temperature in the laboratory for one month. The plant material was identified by Prof. Garuba Omotosun of Plant Science and Biotechnology Department, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

Sample preparation and extraction

The powdered seeds (900 g) and peels (900 g) of *P. nitida* were mixed, submerged in methanol and distilled water (160:40 v/v) for 72 hours with periodic shaking. Following filtration with Whatman No. 1 filter paper, and the resulting filtrate was concentrated using a rotary evaporator under reduced pressure at 40°C to obtain the dry plant extract.

Phytochemical Analysis

Qualitative phytochemical screening: The presence of certain compounds was investigated in crude plant extracts for the following phytochemicals using standard methods as modified by Imran *et al.*[17], reducing sugars, hydrogen cyanide, carbohydrates, tannins, alkaloids, steroids, terpenoids, phenols, flavonoids, saponins and glycosides.

Proximate composition analysis: The extract's proximate analysis were determined including moisture, ash, protein, fat, crude fiber and carbohydrate content using standard methods outlined by the Association of Official Analytical Chemists[18].

Moisture content determination: A 2.0 g sample of the powdered seeds was transferred into a pre-weighed crucible and oven-dried at 105°C to achieve a constant weight and then cooled in a desiccator before using reweighed to determine the content. The moisture content was determined by measuring the weight loss after drying.

Crude ash content determination: A 5 g sample incinerated in a muffle furnace at 550–600°C for 2 hours to determine ash content with final weighing performed after desiccator cooling.

Crude fat content determination: The Soxhlet automated system as described in a previous study was utilized to extract 10g sample and determine the crude fat content with 25 ml of petroleum ether solvent which was subsequently evaporated[18].

Protein content determination: A 3g sample was subjected to digestion with sulfuric acid and a catalyst mixture and protein content was determined by weight difference before and after combustion. Protein content was determined using the Kjeldahl method[18].

Crude fiber determination: Crude fiber contents was analyzed by boiling a 5 g sample in 100 ml of TCA digestion agent for 40 minutes. The residue was filtered and washed, dried overnight at 105°C. Incineration at 550°C for 6 hours and the weight difference before and after combustion was used to calculate crude fibre content.

Carbohydrate content determination= 100% - (moisture + ash + protein + fat + crude fiber) was calculated.

Determination of mineral elements: The mineral elements in the *P. nitida* extracts were analyzed using dry ash extraction followed by wet digestion, as outlined in AOAC. The elements analyzed included calcium, magnesium, potassium, sodium, iron, zinc, copper, and selenium[18].

Test organisms: The research utilize a selection of bacterial strains, including clinical isolates of *S. aureus*, *E. coli* and *Salmonella* species. These strains were reactivated by sub-culturing on Mueller Hinton Agar (MHA).

Reactivation of stock culture of test organisms: The test organisms were reactivated from nutrient agar (NA) slants onto fresh NA plates by streaking, followed by incubation at 37°C overnight. A 0.5 McFarland turbidity standard was achieved by adjusting the turbidity of a cell suspension prepared from 3-4 colonies in sterile physiological saline.

Media preparation: According to the manufacturer's instructions, Mueller Hinton Agar (MHA) was prepared and sterilized by autoclaving at 121°C for 15 minutes at 15 psi, and then poured into plates.

Preparation of stock solution of extracts: The methanol extract stock solution of 200 mg/ml was obtained by dissolving 0.4 g of the plant extract in a sufficient volume of 2 ml of dimethyl sulfoxide (DMSO). This solution was serially diluted to prepare concentrations of 100 mg/ml and 50 mg/ml for antimicrobial testing.

Screening of extracts for antibacterial activity: The antibacterial activity of the plant extracts was determined using the agar well diffusion method[19]. Sterile 6 mm wells were created using a cork borer and 50 µl of each extract concentration (200, 100, 50, and 25 mg/ml) was added. DMSO served as the negative control, while gentamicin was used as the positive control. The plates were incubated at 37°C for 24 hours, and the zones of inhibition were measured to determine the antibacterial efficacy of the extract.

Minimum Inhibitory Concentration (MIC): The MIC was determined by plating the contents of MIC tubes onto agar plates and incubated overnight at37°C. The lowest concentration that kill 99.9% of the bacterial cells, as confirmed by the sub-culturing onto agar plate.

Minimum Bactericidal Concentration (MBC): The MBC was analyzed by sub-culturing an aliquot from the MIC tubes onto fresh MHA plates and incubating at 37°C for 24 hours. The MBC was determined as the lowest concentration of the extract that completely inhibited bacterial growth on Muller-Hinton Agar MHA plates.

RESULTS

Table 1 shows the result on the qualitative phytochemical present in the methanol extract of Mixed of *P. nitida* seed and peel. The result revealed the presence of seven (10) major phytochemicals (saponin, tannin, carbohydrates, reducing sugars, flavonoid, alkaloid, glycosides, steroids, fat and oil and protein) in various proportions and indicated the non-dictation of one (1) phytochemical (hydrogen cyanides). Table 1 presents the quantitative phytochemical analysis of extracts from combined *P. nitida* seed and peel, with results expressed as percentages. Saponins and flavonoids are notable due to its potential health promoting properties such as antioxidants and anti-inflammatory capacities. The presence of tannins, carbohydrates, alkaloids, and glycosides indicates the diverse chemical profile of this plant, which may contribute to its medicinal and nutritional value. Reducing sugars, steroids, fats, oils, and proteins which are crucial to the biological activity of phytochemicals.

Notably, the analysis shows no detectable hydrogen cyanides. All values represent the mean of triplicate determinations, highlighting the reliability of the results. Table 4 presents the proximate analysis of extracts derived from combined *P. nitida* seed and peel. The values indicate the mean percentage composition of each component, along with their standard deviation, reflecting the nutritional profile of the material studied. Moisture content is critical for assessing the stability and shelf life of plant extracts, while the proportions of ash, fat, fibre, protein and carbohydrates provide insight into their potential nutritional and pharmacological applications.

Table 1÷ Quantitative phytochemical analysis of extracts of combined *P. nitida* seed and peel.

S. N.	Phytochemical	Quantity (%)
1	Saponin	3.45
2	Tannins	2.02
3	Carbohydrates	2.01
4	Reducing sugars	0.98
5	Flavonoids	3.46
6	Alkaloids	2.03
7	Glycosides	2.01
8	Steroids	0.89
9	Fat and oil	2.00
10	Protein	2.01
11	Hydrogen cyanides	-

Table 5 provides the macro-mineral composition of extracts from combined *P. nitida* seed and peel, expressed as mean concentrations in mg/kg, accompanied by standard deviations. Sodium (Na) and potassium (K) are essential for various physiological processes, while calcium (Ca) and magnesium (Mg) play pivotal roles in bone health and metabolic functions. Phosphorus (P) is also critical for energy transfer and bone formation.

Table 2: Mean diameter zones of inhibition (mm) produced by extracts of combined *P. nitida* seed and peel against selected clinical isolates.

Test organisms	Combined seeds and peels concentration (mg/ml)			Control Gentamicin/Fluconazole
	200	100	50	
E. coli	12.5±0.70	11.0±1.41	8.0±0.00	27.0±1.41
S. aureus	0.0±0.00	0.0±0.00	0.0±0.00	21.5±0.70
Salmonella species	10.5±0.70	0.0±0.00	0.0±0.00	24.0±0.00
C. albicans	0.0±0.00	0.0±0.00	0.0±0.00	19.5±0.70

Values in the table are the mean±standard deviation from the results of two replications of each experiment.

Table 3: MIC and MBC values (mg/ml) of extracts of combined *P. nitida* seed and peel against the isolates.

Plants	Organisms	100	50	25	12.5	6.25	3.12	1.56	0.78	MIC	MBC
Stem	*E. coli*	-	-	-	-	+	+	+	+	6.25	12.5
Bark	*S. aureus*	-	-	-	+	+	+	+	+	12.5	25
	Salmonella species	-	-	+	+	+	+	+	+	25	50
Stem	*E. coli*	-	-	-	+	+	+	+	+	12.5	25
	S. aureus	-	+	+	+	+	+	+	+	50	100
	Salmonella species	-	+	+	+	+	+	+	+	50	100

+: growth of the organism indicated by turbidity in the broth medium; - = Absence of growth of the test organism shown by no form of turbidity in the medium

This result highlights the mineral content that could contribute to the dietary significance and potential health benefits of *P. nitida* extracts.

Table 6 details the micromineral composition of extracts derived from combined *P. nitida* seed and peel, with values reported as mean concentrations in mg/kg and standard deviations. Selenium is known for its antioxidant properties, while iron is essential for oxygen transport in the body. Copper plays a role in iron metabolism and the formation of connective tissue, and zinc is important for immune function and enzymatic reactions. This result shows the potential nutritional value of *P. nitida* as a source of vital trace minerals.

DISCUSSION

Phytochemical screening of the methanol extract of *P. nitida* seeds and peels (Table 1) indicated the presence of ten major phytochemicals: saponins, tannins, carbohydrates, reducing sugars, flavonoids, alkaloids, glycosides, steroids, fat and oil, and proteins. Among these, flavonoids, saponins, and alkaloids were present in high concentrations, while hydrogen cyanides were absent. These results align with previous studies where flavonoids and alkaloids, particularly in the seeds of *P. nitida*, have been linked to antioxidant, antimicrobial, and anti-inflammatory properties[19-22]. Flavonoids are widely recognized for their ability to neutralize free radicals, contributing to the plant's potential as a natural antioxidant[23]. The presence of saponins is notable as they possess both antimicrobial and anticancer properties[24].

Table 4:Proximate analysis of extracts of combined *P. nitida* seed and peel.

S. N.	Components	% Composition
1	Moisture	12.70±0.04
2	Ash	2.53±0.20
3	Fat	4.64±0.17
4	Fibre	14.00±1.33
5	Protein	5.85±0.07
6	Carbohydrate	59.71±0.07

Table 5: Macro-minerals extract of combined *P. nitida* seed and peel.

S. N.	Components	Mean (mg/kg)
1	Na	9.40±1.53
2	K	13.62±1.09
3	Ca	36.40±0.55
4	Mg	27.40±0.61
5	P	1.69±0.07

In the quantitative analysis (Table 1), flavonoids and saponins had the highest concentrations (3.46% and 3.45%, respectively), which further corroborates their potential as the main bioactive compounds in *P. nitida*. The presence of tannins and alkaloids in moderate concentrations (2.02% and 2.03%, respectively) suggests additional bioactivity, particularly their roles in antimicrobial activity as described in the literature[23]. This supports the importance of these compounds in traditional medicine, where *P. nitida* has traditionally been employed to alleviate conditions such as microbial infections[25]. The antibacterial activity of the extracts of *P. nitida* was evaluated against four clinical isolates: *E. coli*, *S. aureus*, *Salmonella* species, and *C. albicans* (Table 2). The highest inhibition was observed at 200 mg/ml for *E. coli* (12.5±0.70 mm), with a significant reduction in the zone of inhibition at lower concentrations. *Salmonella* species showed moderate inhibition at 200 mg/ml (10.5±0.70 mm), while no inhibition was observed for *S. aureus* and *C. albicans* at any concentration. These results are consistent with earlier studies on *P. nitida*, which also exhibited substantial antibacterial potency against Gram-negative bacteria, particularly *E. coli* and *Salmonella* species[26]. The absence of inhibition for *S. aureus* and *C. albicans* suggests that the extract's antimicrobial spectrum may be limited to certain bacterial strains, which is typical for plant extracts rich in flavonoids and alkaloids[27].

Table 6: Micromineral composition of extracts of combined *P. nitida* seed and peel.

S. N.	Components	Mean (mg/kg)
1	Selenium	0.28±0.11
2	Iron	0.78±1.29
3	Copper	0.09±0.08
4	Zinc	0.49±0.10

In the context of therapeutic applications, the moderate antimicrobial activity observed against *E. coli* and *Salmonella* species highlights the potential for *P. nitida* extracts to serve as natural antimicrobial agents, especially in the treatment of gastrointestinal infections, where these pathogens are commonly implicated[25]. The MIC and MBC values (Table 3) further confirm the extract's antimicrobial potency, with the MIC values obtained for *E. coli* and *Salmonella* species were as 6.25 and 25 mg/ml. For *S. aureus*, the MIC was 12.5 mg/ml, suggesting a moderate activity against this strain, while *C. albicans* did not show significant inhibition at the tested concentrations. The MBC values for *E. coli* and *Salmonella* species were 12.5 mg/ml and 50 mg/ml, respectively, indicating bactericidal properties at these concentrations. The bactericidal effects of plant extracts, especially those containing alkaloids and flavonoids, have been well-documented[20], suggesting that *P. nitida* could potentially serve as an alternative to synthetic antibiotics in treating bacterial infections.

The proximate composition (Table 4) reveals that the combined *P. nitida* seed and peel extract is rich in carbohydrates (59.71%), which is an important energy source. The protein content (5.85%) indicates its potential as a nutritional supplement. The fat content (4.64%) and fiber content (14.00%) are also noteworthy, as these components contribute to the plant's potential as an animal feed additive. Similar findings were reported by Okwu[20,28], who emphasized the nutritional value of *P. nitida* in terms of its high carbohydrate and protein contents. Additionally, the low ash content (2.53%) suggests that the plant is relatively low in inorganic material, further supporting its suitability for consumption. The mineral composition (Table 6 and Table 7) indicates that the

combined seed and peel extract contains essential macro-minerals such as calcium (36.40 mg/kg), magnesium (27.40 mg/kg), and potassium (13.62 mg/kg), which are vital for bone health, muscle function, and electrolyte balance. The trace minerals, including iron (0.78 mg/kg), zinc (0.49 mg/kg), copper (0.09 mg/kg), and selenium (0.28 mg/kg), are also present in modest amounts, underscoring the plant's potential as a source of essential micronutrients. The presence of these minerals in *P. nitida* is consistent with its use in folk medicine for centuries, for treatment of different diseases, including supporting the immune system and preventing deficiencies[29].

Limitation of the study

This study was based on *in vitro* antimicrobial testing which may not accurately predict in *in vivo* efficacy or clinical relevance. The study only focused on a specific range of phytochemicals. The study did not also investigate potential interactions between nutrients, potentially overlooking synergetic effects. These are the limitations of the study which may needs detailed analysis in the subsequent studies.

CONCLUSIONS

The results of this study contribute meaningfully to our knowledge of phytochemical, antimicrobial and nutritional properties of *P. nitida* seeds and peels, which are often discarded despite their potential. The bioactive compounds such as flavonoids, alkaloids, saponins and tannins were detected along with significant antimicrobial activity against *E. coli* and *Salmonella* species, suggests that these plant parts could be harnessed for medicinal purposes, particularly in the development of natural antimicrobial agents. Additionally, the plant's favourable proximate and mineral composition supports its potential as a supplementary food source or animal feed. Given these findings, further research on the industrial applications of *P. nitida* is warranted to explore its full potential in both the pharmaceutical and food industries.

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AUTHOR'S CONTRIBUTIONS

Alaebo PO: designed and supervised the study. **Achi NK:** Wrote the original draft. **Ezurike PU:** Reviewed and corrected the manuscript. **Abuchi FJ:** conceptualization. **Nwuke CP:** literature search and data collection. **Egbuonu ACC:** reviewed and co-supervised the study. **Chukwuka EW:** statistical analysis. **Onochie AU:** results interpretation. Final manuscript was checked and approved by all authors.

DATA AVAILABILITY

The dataset will be available to anyone upon request from the corresponding author.

CONFLICT OF INTEREST

None to declare.

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