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Evaluation of the Laxative, Hematological and Biochemical Effects of *Picralima nitida* Stem Bark Extract in Albino Rats

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ABSTRACT

Picralima nitida, a plant belonging to the Apocynaceae family is widely used in traditional medicine for treatment of various ailments**.** This study investigated the effects of aqueous *Picralima nitida* stem bark extract on liver enzymes, antioxidant activity, hematological parameters, serum electrolytes and laxative properties in albino rats. Forty-nine rats, with weights ranging from 120 to 130 g were divided into seven groups. The control group (1) received normal saline, while the remaining groups were administered varying doses of the extract 200 - 600 mg/kg) or reference drug (Bisacodyl) for 14 days. The results showed a significant dose dependent increase in wet faeces production in animals treated groups (extract and reference drug). Marginal increase in AST (41.97 and 41.20 U/L) and ALT (33.28 and 34.62 U/L) were observed at doses from 200-400mg/kg body weight while ALP (70.08 U/L) was only observed at 200mg/kgbody weight. Additionally, the extract increased conjugated bilirubin levels (0.23 – 0.25 mg/dL) and serum sodium concentrations. Hematological analysis revealed a marginal increase in hemoglobin (12.38 -12.42 g/dL) and packed cell volume (36.42 -37.26 %), while white blood cell count decreased in treated groups. This study suggests that the aqueous *Picralima nitida* stem bark extract is effective as a laxative agent for treating constipation with potential hepatic stress and dose-related side effects on Na^+ and antioxidant status.

Keywords: Laxative property, antioxidant activity, hematological, serum electrolyte and *Picralima nitida*

Q1Introduction

Traditional medicine and the use of medicinal plants have played a crucial role in healthcare systems worldwide, especially in developing regions where they serve as the primary source of treatment for most of the population (Femi-olabisi *et al*., 2020). According to the World Health Organization (WHO), 65–80% of people in these areas rely on herbal remedies for managing illnesses, highlighting the global reliance on natural products for therapeutic Purposes (Chikezie & Ojiako, 2015) . Medicinal plants are not only a cornerstone of traditional medicine but also a significant source of modern pharmacological agents, offering affordable, sustainable, and culturally accepted treatment options (Ogunnowo *et al*., 2010). Aside from providing a cost-effective alternative to synthetic drugs, medicinal plants also preserve cultural heritage and biodiversity. Furthermore, the increasing interest in natural products has led to resurgence in exploring traditional remedies,

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highlighting the need for scientific validation of their efficacy and safety.

Picralima nitida, a species in the Apocynaceae family, is one such plant widely used in traditional medicine across West Africa, including Ghana (Nwaogu *et al.,* 2015), Ivory Coast (Nwaogu *et al*., 2015; Obasi *et al*., 2012) and Nigeria (Creed *et al*., 2020; de Campos *et al*., 2021). Commonly referred to as "Akuamma," the plant has been employed to treat a variety of ailments such as malaria, fever, hypertension, and gastrointestinal disorders (Erharuyi *et al*., 2014; Mabeku *et al.,* 2006). Its stem bark is known for its therapeutic potential, including reported anti-inflammatory, antioxidant, and analgesic properties (Mabeku *et al*., 2006). Phytochemical investigations have revealed the presence of bioactive compounds such as saponins, alkaloids, flavonoids, tannins, and anthracene glycosides, which contribute to its diverse pharmacological effects (Chibuzo *et al*., 2024). Despite its broad use in traditional medicine, scientific studies on the stem bark of *P. nitida* have primarily focused on identifying its phytochemical composition (de Campos *et al*., 2021; Chibuzo *et al.,* 2024) rather than elucidating its broader pharmacological effects on major organ systems and physiological functions. This study seeks to address this gap by evaluating the laxative, hematological and biochemical effects of *Picralima nitida* stem bark extract on Albino rats.

Liver and kidney health are critical indicators of the safety and efficacy of potential therapeutic agents, as these organs are central to metabolism, maintaining homeostasis, and detoxification (Olugbodi *et al*., 2023). Damage to these organs can lead to severe health complications, making it imperative to explore natural products that may affect their function. Additionally, the hematological parameters, which reflect the overall health and functionality of the blood, are essential indicators of physiological status and can be influenced by various phytochemicals (Zarkawi & Soukouti, 2024). These hematological analyses provide insight into the systemic effects of the extract, including its impact on red blood cells, white blood cells, and platelet function, which are crucial for maintaining overall health and immune response (Zarkawi & Soukouti, 2024). Oxidative stress, caused by an imbalance between reactive oxygen species (ROS) production and antioxidant defenses, contributes to the progression of various diseases, including liver and kidney disorders, inflammation, cardiovascular disorders, and neurodegenerative conditions (Olufunmilayo *et al*., 2023). Antioxidants in medicinal plants can play a significant role in neutralizing ROS, thereby mitigating oxidative damage (Dumanović *et al*., 2021).Beyond its other pharmacological properties, the laxative effect of *P. nitida* stem bark extract remains underexplored. Laxatives are essential for managing constipation and bowel irregularities, a common condition affecting millions globally, and identifying natural alternatives with minimal side effects to conventional laxatives is crucial for improving gastrointestinal health. By investigating the biochemical effects and functional impacts of *P. nitida* stem bark extract, this study aims to validate its traditional use, identify potential mechanisms of action, and provide a scientific basis for its therapeutic applications.

The findings from this research will thus contribute to understanding the pharmacological potentials of *P. nitida* stem bark extract and its role in addressing critical health concerns. Ultimately, the findings may pave the way for the development of effective herbal formulations that can enhance health outcomes and promote the sustainable use of natural resources in healthcare.This study not only aims to validate the traditional uses of *Picralima nitida* but also to encourage further research into the pharmacological profiles of other underexplored medicinal plants, thereby enriching the field of ethnopharmacology and contributing to the global discourse on natural medicine.

2.0 Materials and Method

Equipment

Samples were weighed using an analytical weighing balance (G&G-JJ1000) after excision from the animals. All samples and chemicals were preserved in the deep freezer (HTF-319H) and refrigerator while the chemicals and samples were properly mixed using the vortexing machine (QL901). The chemical reactions were monitored

spectrophotometrically using a UV-visible spectrophotometer (England752S). Separations were performed using cold ultra-centrifuge (H-103NR) and micro-hematocrit centrifuge. Heparinized bottles, ethylenediaminetetraacetic acid (EDTA) sample bottles, and capillary tubes were used for the collection of whole blood samples, while plain tubes were used to collect tissue samples. Tissue samples were homogenized with a Teflon homogenizer. A water bath (TT42D) was also used to apply a certain amount of temperature to the samples during analysis.

Drug, Chemicals, and Reagents

All used chemicals and reagents in this study were obtained from certified suppliers such as Qualikems Laboratory reagents, BDH laboratory supplies, England, etc, and were of the best analytical standard. They were all used without further purification. Reagent kits for Alanine aminotransferase, Aspartate aminotransferase, and Bilirubin were bought from Randox laboratories, United Kingdom, while reagent kits for sodium, potassium, and Alkaline phosphatase were bought from Teco Diagnostic, USA. The standard stimulant laxative "Bisacody" (Dulcolax) was a product of Rock Pharmaceutical Laboratories (PVT) Ltd., Risalpur, Pakistan.

Plant material

The stem bark of *Picralima nitida* was obtained at Isuikwuato in Abia State. The plant was identified and authenticated at the Department of Forestry, Michael Okpara University of Agriculture Umudike, Umuahia Abia State, Nigeria.

Preparation of Extract

The plant material was sun-dried. The dried stem bark of *Picralima nitida* was pulverized into powder using an Arthur Miller Machine, sealed in cellophane bags to avoid the effect of humidity, and then stored at 27°C until used. One-fifty grams (150g) of the powdered sample was extracted with 1000ml of water by cold maceration for 48 hours with occasional stirring and the extract was filtered through Whatman no.41 filter paper. This was followed by evaporation to dryness in a boiling water bath for 8 hours. The obtained dark brown residue was kept in an airtight container and stored at 4°C for further use (Evans, 2002).

Experimental Animals

Forty-nine(49) healthy male albino rats aged about 8 weeks and weighing between 120g-130g were purchased from the animal house of the Department of Veterinary Medicine, University of Nigeria, Nsukka.The animals were kept in a well-ventilated house condition (temperature: 22±3°C; photoperiod: 12h/12h light/dark cycle; humidity: 45-50 %). They were allowed to acclimatize for two weeks at the experimental site with access to food (standard rat feed) and water *ad libitum* for a complete period of 30 days.

Experimental Design

Based on the weight of the animals, they were randomly divided into seven groups of seven animals each. Administration was done orally with the aid of an orogastric cannula for fourteen (14) days. The different doses of bisacodyl and stem bark extract of *Picralima nitida* were all dissolved in normal saline and administered to the rats once daily throughout the experiment. Based on the dose and weight of the animals, 0.5 ml of the stock solution was administered to the animals. The extract administration according to the groups was carried out as follows:

Group 1 served as the control group receiving normal saline as a placebo.

Group 2 received the reference standard laxative "bisacodyl"(200mg/kg body weight).

Group 3 received the reference standard laxative "bisacodyl"(400mg/kg body weight)

Group 4 received the reference standard laxative "bisacodyl"(600mg/kg body weight).

Group 5 received stem bark extract of *Picralima nitida* (200mg/kg body weight).

Group 6 received stem bark extract of *Picralima nitida* (400mg/kg body weight).

Group 7 received stem bark extract of *Picralima nitida* (600mg/kg body weight).

The choice of the doses 200mg/kg, 400mg/kg, and 600mg/kg body weights used in this study

was as a result of the findings from an ethnobotanical survey. Ethnobotanical survey was conducted between September 2013 and March 2014 in Isuikwuato Local Government Area of Abia State Nigeria. The Local Government is inhabited primarily by the Igbo ethnic group, and agriculture is the major economy. Ethnobotanical information was obtained from participants using a semi-structured questionnaire.

The participants were purposively selected, comprising herb sellers, traditional health practitioners, farmers and individuals with indigenous knowledge. The questionnaire was prepared in English. However, to facilitate efficient communication, informal discussion with herb sellers and traditional health practitioners was done in Igbo, the local language in the local government area. A total of 36 participants were interviewed, and the participants were interviewed individually to maintain confidentiality. Data collected include age, gender, occupation, educational background, residence, plant part used, method of preparation/extraction, dosage, and administration method.

The study and data collection were done following the regulations made by the International Society of Ethnobiology (available at https://www.ethnobiolgy.net.: accessed on 20 August 2013). Grouped animals were placed in individual cages lined with filter paper at the bottom. They were observed for the passage of faeces over 12 hours. The form of the stool for each group was noted. The property of wet faeces was considered a measure of the laxative activities of the extract (Elujoba *et al*., 1999; Fairbairn & Moss, 1970; Yagi *et al*., 1997).The rats were weighed daily from the period of acclimatization to the last day of the experiment when they were sacrificed. Forty-eight hours after treatment with the stem bark extract, the animals were starved overnight; anesthetized with chloroform, and euthanized. Liver and kidney markers, alongside the antioxidant and hematological analysis, were assayed in the serum and respective tissues of the animals.

Determination of Laxative Potency

The laxative effect of the aqueous extract of *P. nitida* stem bark was determined by comparing the wet and dry weights of the faecal samples. Stools were collected to determine the faecal mass (Abo *et al.,* 1999; Chen *et al.,* 2006). Bisacodyl, a known laxative agent, was used as a reference drug. The nature of faeces produced in 12 hours by the animal groups was examined hourly for wetness using the "wire adhesion" method. The wire adhesion technique that distinguishes dry from wet faeces is based on the principle that a 17g hypodermic needle will pick up the dry faeces but not the wet faeces (Elujoba *et al*., 1999). Hence, in these experiments, those faeces that were not picked by the needle were classified as wet faeces. The results were expressed as the percentage of wet faeces against the total faeces and then with reference, to "Bisacodyl" tablets. The percentage (%) wet faeces as a function of dose as well as the percentage (%) bisacodyl activity as an index of comparison was deduced from the results.

Blood Collection

Forty-eight hours after treatment with the stem bark extract; the animals were starved overnight; anesthetized with chloroform, and euthanized. Blood samples from each animal were collected by cardiac puncture. The blood samples were divided into two. The first part was dispensed in ethylenediaminetetraacetic acid (EDTA) collection bottles for hematological analysis. The second part of the blood sample was allowed to stand in a sterile plain sample bottle for about 15 minutes to clot and further spun in a centrifuge. The serum was separated from the clot with a Pasteur pipette into sterile sample bottles for the measurement and evaluation of liver enzymes, antioxidant activities, and serum electrolytes.

Liver Function Tests

Determination of Aspartate Aminotransferase (AST) & Alanine Aminotransferase (ALT)

These tests were carried out spectrophotometrically on the serum of the rats used for this experiment according to the method of (Reitman & Frankel, (1957) as described by the Randox diagnostic kit manual.

Determination of Alkaline Phosphatase (ALP)

The test was carried out spectrophotometrically on the serum of the rats used for this experiment according to the method ofKing *et al.*, (1951) as described by the Teco Diagnostic kit manual with little modification.

Determination of Bilirubin

Both total and conjugated bilirubin levels were carried out spectrophotometrically on the serum of the rats used for this experiment according to the method of Jendrassik, (1938) as described by the Randox diagnostic kit manual.

Antioxidant Assays

Determination of Reduced Glutathione (GSH)

The GSH level was determined using the method described by Ellman, (1959) with slight modifications. Supernatant liver protein Homogenate (0.2ml) was added with 25% Trichloro Acetic Acid (TCA) and centrifuged at 3000 rpm for 10 minutes. The supernatant (0.2ml) was added with 10mM of 5, 5'-dithiobis-(2-nitrobenzoic acid) (DTNB) in the presence of phosphate buffer (0.IM, pH 7.4). Absorbance was read at 420nm.

Determination of Superoxide Dismutase (SOD)Activity

The method of Sun and Sigma as described by Ogbunugafor *et al.,* (2012) was adopted. Test tubes were labeled to correspond with the serum. Sodium carbonate buffer (2.95ml, 0.05M, pH 10.20) was dispensed into the labeled test tubes. Serum (0.02ml) was pipetted into the tubes followed by 0.03ml of epinephrine in 0.005N HCl. The reaction mixture of 3ml was thus initiated. The reference cuvette contained 2.95ml buffer, 0.03ml of substrate (epinephrine), and 0.02ml of distilled water. And extinction coefficient for epinephrine at 480nm of $4020M^{-1}cm^{-1}$was used in calculating activity.

Determination of Catalase Activity

The catalase activity of the supernatant fraction from the liver was determined according to the method of Sinha, (1972).The supernatant fraction was properly diluted (50 times). H_2O_2 (4ml, 0.2 M) was added to 5ml of phosphate buffer. The properly diluted supernatant fraction (1ml) was added to the H_2O_2/buffer mixture and the resultant mixture was gently mixed at room temperature. 1ml portion of the reaction mixture was withdrawn and dropped into 2ml dichromate/acetic acid reagent at one-minute intervals. The steady absorbance reading was taken at 570nm.

Determination of Vitamin C (Ascorbic Acid)

The method employed involves the titration of 2, 6-dichlorophenolindophenol (Harris & Ray, 1932). Equal volumes of plasma (separated immediately from blood) and trichloroacetic acid were mixed and centrifuged. The filtrate (supernatant) was collected. The diluted dye was pipetted into a test tube and titrated with the filtrate until the reddish color disappeared and the ascorbic acid level was calculated.

Hematological Evaluations

Determination of Hemoglobin (Hb)

The method of cyanomethemoglobin was employed as described by Sood, (2006). 5 ml of Drabkin's solution was put into a test tube followed by 0.02ml of whole blood. The test tube was covered and inverted several times. It was allowed to stand at room temperature for 10 minutes to ensure a complete reaction. The colorimeter was zeroed with Drabkin's solution and the absorbance was taken at 540nm. Hemoglobin level was read off in a standard curve treated the same way as the samples.

Determination of White Blood Cells (WBC)

The method described by Cheesbrough, (2006) was employed. Turk's solution of 0.38ml was mixed gently with 0.02ml of well-mixed whole blood drawn with a pasture pipette. The solution was allowed to stand for 3 minutes, and a drop was used to charge the counting chamber. The counting was carried out using the x10 magnification objective of the microscope. The white blood cells were counted uniformly in the four corners' $1mm^2$ areas and the white blood cells were calculated using the formula below:

Total number of cells counted X 50 = cells/mm^3 or cells/cu.mm

Determination of Packed Cell Volume (PCV)

The centrifuge method as described by Cheesbrough, (2006) was employed. Here, special non-graduated glass capillary tubes were used. These tubes are 7cm in length and 1mm in internal diameter. They can be purchased pre-coated internally with dried heparin for capillary blood. The capillary tube was filled two third with well-mixed blood. One end of the capillary tube was sealed with plasticine. The filled tubes were then placed in the micro-hematocrit centrifuge and spun at 12000 rpm for 5 minutes. The spun tube was then placed into a well-designed scale (PCV reader) and read as a percentage.

Renal Markers (Electrolytes)

Determination of Serum Potassium

The method employed was a modification of the spectrophotometric method described by Terri &Sesin, (1958), according to the Teco Diagnostic kit manual.

Determination of Serum Sodium

The method employed was a modification of the spectrophotometric method described by Maruna, (1958) and Trinder, (1951), according to the Teco Diagnostic kit manual.

Determination of Serum Chloride

A modification of the spectrophotometric method described by Skeggs & Hochstrasser, (1964) according to the Teco Diagnostic kit manual was used.

Determination of Serum Bicarbonate

The method employed was a modification of the enzymatic procedure described by Forrester *et al*., (1976).Test tubes were labeled to correspond with the serum, standard, blank, and control. Carbon Dioxide reagent 1.0ml was pipette into each tube. The tubes were incubated for 3 minutes at 37°C. The spectrophotometer was set at a wavelength of 340nm, and temperature to 37°C.Water (5µl) was pipetted into the blank. Standard (5µl) was pipetted into the standard tube and 5µl of serum was pipetted into the respective tubes. The tube contents were mixed gently by inversion and incubated for 5 minutes. The absorbance of all cuvette was read at 340nm.

Statistical Analysis

The results were pooled and expressed as mean ± standard deviation. Analysis of variance (ANOVA) was used to analyze the results and Duncan multiple tests (DMRT) were used for the post hoc analyses. DMRT was used to compare the treatment means for significant differences and to test specific number of comparisons besides comparing them to the controls. DMRT was also used because of the large pairs of means being compared on a table format. The significance level was set at $p < 0.05$.

Results

Acute Oral Toxicity (LD_{50}) Test

No death was recorded up to the highest dose of 5000mg/kg body weight as shown in Table 1a and 1b. No visible adverse reactions were observed after throughout the 24-hour period of acute toxicity test. This shows that *P. nitida* is of low mortality and therefore safe as a pharmacological agent.

Table 1a: Acute Toxicity study (LD_{50}) of *P. nitida* aqueous stem bark extract (Phase 1)

Dose (mg/kg)	Number of Death	Behavioural changes
10	0/3	Calm and Resting
100	0/3	Calm and Resting
1000	0/3	Calm and Resting

Table 1b: Acute Toxicity study (LD_{50}) of *P. nitida* aqueous stem bark extract (Phase 2)

Dose (mg/kg)	Number of Death	Behavioural changes
1600	0/1	Calm and Resting
2900	0/1	Calm and Resting
5000	0/1	Calm and Resting

Laxative effect of stem bark extract of Picralima nitida in albino rats

The result of this study as presented in table 2 and 3 showed a significant dose dependent increase in wet faeces production in animals treated with stem bark extract doses at 200, 400 and 600 mg/kg of *P. nitida* with mean defecation of 2.64±0.11, 5.16±0.26 and 11.09±0.24 g respectively. The effect of the extract had a significantly ($p<0.05$) higher laxative activity when compared to the control with a mean defecation of 2.41±0.21after 12 hours of administration.

Table 2: Mean wetness of faeces (g) produced by experimental animals at different time intervals

Time (Hrs)	Group/Bisacodyl (mg/kg) Treatment				*P. Nitida (*mg/kg*)*		
	Normal saline (10mls/kg)	**200**	**400**	**600**	**200**	**400**	**600**
2	0.43±0.01[a]	0.51±0.0 1[b]	0.60±0.01[c]	0.90±0.01[d]	0.48±0.01[e]	0.70±0.06[f]	0.60±0.06[g]
4	0.41±0.01[a]	0.55±0.01[a]	1.2±0.40[b]	1.50±0.01[c]	0.38±0.01[a]	0.75±0.01[a]	1.60±0.06[d]
6	0.40±0.01[a]	0.50±0.01[b]	0.65±0.06[c]	1.60±0.06[d]	0.35±0.01[e]	1.21±0.06[f]	1.80±0.05[g]
8	0.41±0.10[a]	0.47±0.01[b]	0.80±0.06[c]	2.60±0.06[d]	0.49±0.06[e]	0.80±0.06[f]	2.40±0.05[g]
10	0.38±0.02[a]	0.47±0.01[b]	0.80±0.06[c]	2.61±0.01[d]	0.47±0.01[e]	0.86±0.06[f]	2.35±0.01[g]
12	0.38±0.01[a]	0.46±0.01[b]	0.76±0.01[c]	2.60±0.00[d]	0.47±0.01[e]	0.84±0.01[f]	2.34±0.01[g]
Total	**2.41±0.21**	**2.96±0.06**	**4.81±0.60**	**11.82±0.10**	**2.64±0.11**	**5.16±0.26**	**11.09±0.24**

The results represented are mean ± SD of triplicate determination at (P<0.05). Mean in the same row, having different alphabet are statistically significant (P<0.05) compared with the control (group one).

Table 3: % Wet faeces produced by *P. nitida* and Bisacodyl at different doses

	Doses (mg/kg)/% wet faeces		
	200	**400**	**600**
P. nitida	23	39	40
Bisacodyl	24	34	42

The laxative potency of *Picralima nitida* stem bark extract investigated at different doses and presented in table 4 and 5 shows that the extract gave laxative activities comparable to that of the reference drug "bisacodyl' at varying doses.

Table 4: Biological Bisacodyl Equivalent of *P. nitida* at different doses

	Doses (mg/kg)/ Bisacodyl Equivalent		
	200	**400**	**600**
P. nitida	0.96	1.15	0.95
Bisacodyl	1.00	1.00	1.00

Table 5: % Bisacodyl-Activity of *P. nitida* at different doses

	Doses (mg/kg)/Bisacodyl-activity		
	200	**400**	**600**
P. nitida	96	115	95
Bisacodyl	100	100	100

Effect of Picralima nitida Stem bark extract on serum liver enzymes (U/L)

P. nitida exhibited non-significant values in serum liver enzyme activity on experimental rat as compared with the control group. Marginal increase in AST (41.97 and 41.20 U/L) and ALT (33.28 and 34.62 U/L) were observed for dose 200 mg/kgb.w and 400mg/kgb.w, respectively while ALP (70.08 U/L) was only observed at 200mg/kgb.w for *P. nitida* stem bark extract treated rat whereas for control group AST, ALT and ALP were found to be 36.46, 25.85 and 67.70 U/L, respectively (Table 6).

Effect of *Picralima nitida* stem bark extract on serum Bilirubin

The extract showed a significant increase ($p<0.05$) in levels of conjugated bilirubin (0.23 – 0.25 mg/dL when compare with the control (0.12 mg/dL) but no significant difference was observed in total and non-conjugated bilirubin when compared with controls (Table 7).

Effect of *Picralima nitida* stem bark extract on serum renal biomarkers (Electrolytes)

The extract showed no significant difference ($p.<0.05$) in the concentrations of HCO_3^-, K^+ and Cl^- however, an increase in Na^+ (151.33 – 156.71 mg/L) level was observed when compared with the control Na^+ (137.95 mg/L) (Table 8).

Table 6: Effect of *Picralima nitida* Stem bark extract on serum liver enzymes (U/L)

Liver enzyme (U/L)	Group treatment	Bisacodyl (mg/kg/bw)			*P. Nitida (mg/kg/bw)*		
	Normal saline (10mls/kg/bw)	200	400	600	200	400	600
AST	36.46±1.44[a]	34.94±1.42[a]	34.26±1.96[a]	38.90±4.88[a]	41.97±6.34[a]	41.20±1.20[a]	0.60±0.06[a]
ALT	25.85±9.48[a]	32.50±5.63[a]	32.97±5.59[a]	32.46±4.52[a]	33.28±2.50[a]	34.62±3.69[a]	1.60±0.06[a]
ALP	67.70±11.43[a]	55.08±0.79[b]	58.67±1.44[a]	65.75±5.71[a]	70.08±4.65[a]	66.98±6.42[a]	67.43±4.54[a]

The results represent mean ± SD of group serum results obtained (n=5).Mean in the same row, having different alphabet are statistically significant (p<0.05) compared with the normal control (group one).
Legend: AST = Aspartate Aminotransferase, ALT = Alanine Aminotransferase, ALP = Alkaline Phosphatase and U/L = Unit per litre

Table 7: Effect of *Picralima nitida* stem bark extract on serum Bilirubin (mg/dL)

Bilirubin(mg/dL)	Group treatment	Bisacodyl (mg/kg/bw)			*P. Nitida (mg/kg/bw)*		
	Normal saline (10mls/kg/bw)	200	400	600	200	400	600
Total Bilirubin	0.91±0.13[a]	0.65±0.09[a]	0.76±0.16[a]	0.99±0.67[a]	1.07±0.05[a]	1.20±0.20[a]	1.17±0.24[a]
Conjugated Bilirubin	0.12±0.02[a]	0.20±0.06[a]	0.25±0.03[b]	0.21±0.08[c]	0.23±0.05[d]	0.24±0.05[e]	0.27±0.03[f]
Non-conjugated Bilirubin	0.80±0.12[a]	0.45±0.07[b]	0.51±0.18[a]	0.96±0.43[a]	0.97±0.18[a]	0.76±0.43[a]	0.89±0.25[a]

The results represent mean ± SD of group serum results obtained (n=5). Mean in the same row, having different alphabet are statistically significant (P<0.05) compared with the normal control (group one).

Table 8: Effect of *Picralima nitida* stem bark extract on serum renal biomarkers (Electrolytes)

Electrolyte (mg/L)	Group treatment	Bisacodyl (mg/kg/bw)			*P. nitida (mg/kg/bw)*		
	Normal saline (10mls/kg/bw)	200	400	600	200	400	600
K^+	3.99±0.33[a]	4.75±0.57[a]	4.56±0.40[a]	5.25±0.54[b]	3.88±0.76[a]	4.32±0.79[a]	4.39±0.79[a]
Na^+	137.95±2.18[a]	148.55±3.40[b]	151.33±1.93[c]	156.70±2.57[d]	151.33±6.19[e]	157.04±1.99[f]	157.71±2.52[g]
Cl^-	102.98±8.53[a]	104.35±6.12[a]	106.46±5.01[a]	109.91±7.21[a]	103.56±9.35[a]	107.40±6.33[a]	108.54±5.63[a]
HCO_3^-	29.58±2.75[a]	29.82±4.99[a]	31.57±3.13[a]	28.99±5.29[a]	31.02±6.39[a]	29.75±6.32[a]	28.52±6.73[a]

The results represent mean ± SD of group serum results obtained (n=5). Mean in the same row, having different alphabet are statistically significant (P<0.05) compared with the normal control (group one).
Legend: K^+ = Potassium ion, Na^+=Sodium ion, Cl^- =Chloride ion, HCO_3= Bicarbonate ion,MEq/L = Milliequivalent per litre and Mmol/L= millimole per litre

Table 9: Effect of *Picralima nitida* stem bark extract on haematological parameters

Haematological parameter	Group treatment	Bisacodyl (mg/kg/bw)			*P. Nitida (mg/kg/bw)*		
	Normal saline (10mls/kg/bw)	200	400	600	200	400	600
Hb (g/dL)	12.02±0.94[a]	10.22±147[a]	9.98±0.84[b]	10.66±0.66[a]	12.38±0.65[a]	12.32±0.64[a]	12.42±1.04[a]
WBC (x10^3/UL)	5.94±1.42[a]	6.70±2.70[a]	5.24±1.05[a]	5.68±1.19[a]	6.28±0.72[a]	5.28±0.54[a]	5.48±1.14[a]
PCV (%)	36.20±2.77[a]	31.80±4.09[a]	30.88±1.90[b]	32.54±1.78[a]	36.42±1.89[a]	36.80±1.92[a]	37.26±3.29[a]

The results represent mean ± SD of group serum results obtained (n=5). Mean in the same row, having different alphabet are statistically significant (P<0.05) compared with the normal control (group one).

Legend: Hb = Haempglobin,WBC = White blood cell**,** PCV = Packed cell volume

Effect of *Picralima nitida* stem bark extract on Liver Tissue antioxidant activity

Table 10 shows the effect of *P. nitida* stem bark extract on liver tissue antioxidant activity. Catalase 0.15, 0.17 and 0.21 kcat/mg protein), SOD (11.12, 10.93 and 11.23 nm/mgprotein) and vitamin C (15.69, 15.56 and 17.98 nm/100ml) were observed to reduced significantly in all groups administered with extract of *P. nitida* when compare with the control catalase (0.27 kcat/mg protein), SOD (24.21nm/mg protein) and Vitamin C (18.48 nm/100ml)

Table 10: Effect of *Picralima nitida* stem bark extract on liver tissue antioxidant activity

Antioxidant	Group treatment	Bisacodyl (mg/kg)			*P. Nitida (mg/kg)*		
	Normal saline (10mls/kg)	200	400	600	200	400	600
GSH (nm/mg protein)	21.70±1.17[a]	19.94±0.80[a]	21.81±1.25[a]	19.21±0.95[a]	21.94±1.95[a]	22.34±2.42[a]	22.33±1.63[a]
SOD (nm/mg protein)	24.21±1.86[a]	11.79±1.91[a]	12.23±1.04[c]	12.80±2.00[d]	11.12±1.44[e]	10.93±1.13[f]	11.23±0.79[g]
CAT (kcat/mg protein)	0.27±0.01[a]	0.12±0.08[b]	0.12±0.08[c]	0.12±0.08[d]	0.15±0.08[e]	0.17±0.08[f]	0.21±0.02[g]
Vit. C (nm/100ml)	18.48±1.25[a]	16.58±1.44[a]	17.36±0.98[a]	17.53±0.58[a]	15.69±0.47[c]	15.56±0.52[c]	17.98±1.20[a]

The results represent mean ± SD of group serum results obtained (n=5). Mean in the same row, having different alphabet are statistically significant (P<0.05) compared with the normal control (group one).

Legend:*GSH = Glutathione peroxidase, SOD = Superoxide dismutase, CAT = Catalase, Vit. C = Vitamin C (Ascorbic acid).*

Discussion

For long, plants have been crucial sources of medications against several illnesses including constipation. In Africa including Nigeria, more than fifty percent of the population depends on plant for medicines due to the expensiveness and undesirable side effects of orthodox medicines (Ashafa*et al*., 2011).

The work of Chibuzo *et al*. on phytochemical and Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the stem bark of *Picralima nitida* revealed the presence of bioactive compounds, including saponins, alkaloids, flavonoids, tannins, oxalate, phytate, anthracene glycosides, cyanogenic glycosides, phenols, ketones, alcohols, thio compounds, fatty acid esters (FAEs) and vitamin E (Chibuzo *et al*., 2024). These compounds had been reported to possess various pharmacological activities, including antimicrobial, antioxidant, hepatoprotective, anti-inflammatory and laxative properties (Aghedo *et al.,* 2021).

Acute toxicity studies provide critical insights into the safety of medicinal plants. The toxicity profile of *P. nitida* stem bark extract indicates a high safety margin, with no observed lethality at doses up to 5000 mg/kg body weight in experimental models (John-Africa, 2019). This suggests its potential for safe pharmacological applications.

The present study has obviously demonstrated that the extract of *P. nitida* has a laxative activity, which is comparable to Bisacodyl. Laxation was indicated by the excretion of wet faeces in considerable quantities and recognized by their rounded, irregular shape, soft consistency and presence of brown stains on white paper while the normal, dry faeces are elongated, regular in shape, hard and dry with no stains on the filter paper (Elujoba and Iweibo, 1988). The biological bisacodyl-equivalent at any given dose of *P. nitida* is defined as the quotient of the % wet faeces produced by *P. nitida* and that of bisacodyl produced within 12 hours of oral administration into rats under the same experimental procedures, and serves as an index of comparison. Similarly, percentage bisacodyl-activity at a particular dose of*P. nitida* is defined as the relative quantity of bisacodyl-activity possessed by *P. nitida* when assayed at the same dose under the same experimental conditions. Hence in table 5, *P. nitida* possessed 115% and 95% of bisacodyl activity at 400mg/kgb.w. and 600mg/kgb.w. respectively. The significant laxative activity of *P. nitida* stem bark extract may be attributed to its anthracene glycoside content, which readily undergoes hydrolysis and oxidation by β-glucosidase in the intestinal flora in the presence of oxygen to produce anthraquinones, known for their strong laxative effects (Sakulpanich & Gritsanapan, 2009). Anthraquinone laxatives stimulate intestinal peristalsis, increase water retention in the colon, and promote bowel movements, similar to synthetic laxatives like bisacodyl (Schiller, 2001).Furthermore, the extract contains vitamin E, which at high doses has been associated with mild gastrointestinal disturbances, including diarrhea (Brigelius-Flohe, R., & Traber, M. G. (1999). This suggests that the synergistic effects of anthraquinones, vitamin E, and other bioactive compounds may contribute to the observed laxative properties.

The reduction in catalase and superoxide dismutase (SOD) activity observed in extract-treated groups may suggest a compensatory mechanism where exogenous antioxidants replace endogenous enzymatic antioxidants (Valko *et al*., 2006).

The treatment with extracts of *Picralima nitida* gave a slight increase in AST and ALT activities at 200 and 400 mg/kg/b.w. respectively while a slight increase in ALP was at 200mg/kg/b. w. was also observed. However, a drastic decrease in the activities of AST and ALT was observed at 600mg/kg/b.w. while non-significant difference in ALP activity was observed at 600mg/kg/bw. This dose-dependent alterations in liver enzyme activities are indicators of side effects of *P. nitida* extract.

The increase in conjugated bilirubin after the administration of the extract is likely due to the presence of alcohols known to influence bilirubin metabolism (Sean & Rebecca, 2004). Bilirubin is a lipid-soluble byproduct of heme metabolism. The rise in conjugated bilirubin levels is an indication that the process of conjugation is integral but however, points to the inability to effectively excrete conjugated bilirubin. This may be attributed to influx of bilirubin into the blood stream as a result of biliary obstruction and abnormal function of the liver.

The haemoglobin (Hb) pigment is a compound with high molecular weight established within the red blood cells which formed oxyhaemoglobin when bound to oxygen (Strippoli *et al.,* 2010, Nwankwo *et al*., 2017). The result of the effect of the stem bark extract of *Picralima nitida* on haemoglobin count (Hb) of rats showed that the extract was able to increase the haemoglobin count (Hb) in rats administered with the extract when compare with the control and reference drug. The increase in Hb count may be a signal that the plant extract could also enhance blood production in addition to it laxative property. This observation is in conformity with the previous work of Nwankwo *et al*., (2017) who reported a significant increase in Hb count among rats administered with 20, 40, and 80mg/kg body weight of *P. nitida* seed extract. Likewise, the result also shows that PCV count slightly increased when compared with the control and

reference drug. The confirmation that the extract-treated groups show an increase in PCV of rats exposed the fact that the extract had the ability to keep the integrity of red blood cells by boosting their level percentage in the blood. Since the test groups show slight increase PCV and Hb values when compare to the control, we may therefore suggest that this plant can also be use as possible haematenic agent in the management of anaemia.White blood cell (WBC) count quantifies the number of white blood cells present in the blood (Nwankwo *et al*., 2017). A slight increase in WBC count was observed in the group administered with 200mg/kg/b.w of the extract, while the group administered with 400 and 600 mg/kg/b.w of the extract showed non – significant increase. The main function of WBC as phagocytes is to defend against invading microorganisms or foreign substance. The slight and non-significant increase in WBC count observed at the administered doses to effect laxative activity indicate that the doses administer was at a safety range, because it does not trigger overproduction or induction of WBC. Since the body system naturally produced WBC in response to any foreign substances in the body

The result of the effects of the extract on K^+ and Cl^- electrolytes concentration of rats administered with stem bark extract of *P. nitida* showed slight progressive increase in K^+ and Cl^- concentrations whereas Na^+ electrolyte concentration of rats treated with the stem bark extract depicted higher Na^+ concentration when compared with the normal control. However, HCO_3^- electrolyte concentration of extract-treated rats showed non-significant value. The reference drug (bisacodyl) showed higher K^+ concentrations than the extract-treated groups at the group administered with 600mg/kg/b.w. These findings compare well with those of Nwankwo *et al.* (2017) who reported a significant increase in serum sodium electrolyte of malaria infected rats treated with the seed extract of *P. nitida*. This research work also agrees with the work of Arise *et al*. (2013) who noted that there were significant decreases in some serum electrolytes (bicarbonate, chloride and potassium) of malaria infected rats treated with some extracts of *P. nitida*. Increase in Na^+ concentration and non-significant value in K^+, Cl^- and HCO_3^-concentrations as result of extract administration suggest that the extract could lead to dehydration (loss of water) or damaged kidney tissues.

Conclusion

The findings of this study show that the extract of *Picralima nitida* stem bark exhibits strong laxative potential, supporting its traditional use in the treatment of constipation and gastrointestinal motility disorders. However, the observed signs of hepatic stress, suppression of antioxidant activity and electrolyte imbalance suggest that its chronic use may cause potential risks to liver health, oxidative balance and kidney health, particularly with prolonged or high-dose administration. Therefore, while *P. nitida* shows promise as a potent natural laxative, further studies on its dose-dependent toxicity, mechanism of action, and safe therapeutic window are recommended to ensure its safe and effective medicinal usage.

Conflict of Interest

The authors declared no conflict of interest.

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