*IOSR Journal Of Pharmacy And Biological Sciences (IOSR-JPBS)*
*e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 15, Issue 3 Ser. V (May –June 2020), PP 41-55*
*www.Iosrjournals.Org*

# Pharmacognostic Screening And Antidiarrheal investigation of Methanol seed extract of *Picralima nitida* Linn (Apocynaceae)

Odoh, Uchenna E.[1], *Osuala Felix .N[2], and Okwubuasi Lisa Isioma[2],
[1] *Department of Pharmacognosy and Environmental Medicine, Univerity of Nigeria, Nsukka, Nigeria.*
[2] *Department of Pharmacognosy, Madonna University, Elele Campus, Rivers State, Nigeria.*
**Corresponding Author: Osuala Felix N.*

***Abstract***
***Aim:*** *There have been claims that the seeds of Picralima nitida possess anti-plasmodial, anti-inflammatory, antipyretic properties. The aim of this research is to screen the pharmacognostic profile of Picralima nitida, and investigate the anti-diarrhea activity of the methanol seed extract of Picralima nitida (Family: Apocynaceae) commonly known as Akuamma in West African folk medicine.*
***Method:*** *The seeds of the P. nitida were collected, dried, pulverized and macerated with methanol and condensed to give methanol extract. The macroscopic, microscopic and phytochemical analysis was carried out by standard procedures. Extract was subjected to dry ash digestion and the resultant supernatants were used for macro- and micronutrients determination using the emission flame photometer and the absorption spectrophotometer. The proximate analysis and the acute toxicity in Wister rats were also studied. The anti-diarrheal activity was tested by monitoring the number and frequency of wet and dry droppings following castor oil induced diarrhea in Wister rats. Also the reduction of rat gastrointestinal motility by the extract was examined following a charcoal meal.*
***Result:*** *The microscopy of the powdered seeds revealed the presence of sclereids, parenchyma and epidermal cells, calcium oxalate crystal and fat globules. Qualitative and quantitative phytochemical analyses of the extract showed the presence of alkaloids (5.84 g), tannins (0.065), flavonoids (7.03 g), glycosides (4.58 g), terpenoids (4.08 g), protein (11.63 g), steroids, resins, reducing sugars, carbohydrates, fats and oils. The analytical standards gave 3.0, 11.5, 3.5, 4.0, 8.1, 20.4 and 8.0 % for moisture content, total ash, acid insoluble ash, water soluble ash, sulphated ash, alcohol soluble and water soluble extractives respectively. Microscopic analysis revealed the presence of lignin, starch, cellulose, calcium oxalate crystals present. $K^+$, $Na^+$, $Fe^{2+}$ and $Zn^{2+}$ ions were relatively higher than $Ca^{2+}$ and $Mg^{2+}$ in the test for metals in the extract. The extract was not toxic at a higher dose of 5000mg/kg body weight as no death was recorded. The methanol seed extract of Picralima exhibited significant anti-diarrheal activity evidenced by the significant reduction in the rate of wet and dry droppings in the Wister rats. The gastrointestinal motility in Wister rats was significantly reduced (9.21%) following the oral administration of 400 mg/kg the extract. The protective activity of Picralima nitida was better than that of the standard drug, loperamide (16.58 %).* ***Conclusion:*** *From the research carried out, Picralima nitida possess anti diarrheal effect and can serve as a lead to production of antidiarrheal drugs.*
***Keywords:*** *Antidiarrheal, phytochemical analysis, microscopy, macroscopy, proximate analsis.*

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Date of Submission: 23-06-2020 Date of Acceptance: 11-07-2020

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## I. Introduction

Diarrhea from the Ancient Greek means *dia* "through" and *rheo* "flow".It is defined by the World Health Organisation is the condition of having three or more loose or liquid stools per day,or as having more stools than is normal for that person (WHO 2013).1 Diarrhea is characterized by loose, watery bowel movements that may occur frequently and with a sense of urgency. It can also be defined as the frequent passage of liquid feaces, a condition generally accompained by abdominal cramps and sometimes nausea and vomiting (Rang *et al*, 2007) 2. Diarrhea is usually short-lived, lasting no more than a few days but, when diarrhea lasts for weeks, it usually indicates that's there's another problem. If one has diarrhea for weeks or longer, one may have a condition such as irritable bowel disorder, or a more serious disorder, such as a persistent infection or inflammatory bowel disease

Diarrhea can have causes that aren't due to underlying disease. Examples include a liquid diet, food intolerance, stress, anxiety or use of laxative.

The Symptoms of diarrhoea are:Increased frequency of bowel movement, Loose watery stools, Urgency(having to go right away), Incontinence( leakage of stools), Bloating,Rectal pain, Lower abdominal

pain or cramping, Nausea, Vomiting, Fever, Blood or flecks of mucus in the stool, Loss of appetite,Weight loss,Dehydration (WHO 2013).3

*Picralima nitida* (Apocynaceae) known as the akuamma is a known specie native to tropical Africa(Benin, Ghana, Ivory Coast, Nigeria, Gabon, Cameroon, Central African Republic, Republic of Congo, Zaire, Uganda) ( Kouitcheu *et al* 20054, Harris, *et al*, 2002).5 It is a tree up to 35m tall, with white latex in all parts, glabrous: bole up to 60cm in diameter; bark hard, brittle, pale to dark greyish black or brown, smooth to slightly rough or finely striped. *Picralima nitida* bears white flowers (about 3cm long) with ovoid fruits which at maturity are orange-yellow to brownish yellow in colour. The flowers are bisexual. The corolla has fleshy cylindrical tube 25-45mm long, hairy inside and narrowed below the insertion of the stamens. The leaves are opposite, simple and entire; broad (3-10 cm) and oblong (6-20 cm long) with tough 14 to 20 pairs of thin lateral nerves (Adjanohoun 1996).6 The fruits consisting of two obviod to ellipsoid follicles 11-20cm long, smooth, brown to orange, embedded in soft white pulp. Seedling with epigeal germination; cotyledons ovate or oblong, 10-13mm long, base slightly cordate to rounded, apex obtuse to rounded.

The dried seeds are used in traditional medicine throughout West Africa, particularly in Ghana as well as in the Ivory Coast and in Nigeria (Dalziel, 1961; 7 Iwu, 1993). 8 Herbalists in Nigeria use leaves, seeds and stem backs of *P. nitida* in the treatment of fever, jaundice, hypertension, malaria, and gastro intestinal disorders (Dalziel, 1961;7 Iwu, 1993)8. In traditional medicine, the seeds of *P. nitida* are used as good substitutes for quinine in treatment of malaria (Francois, *et al*., 1996).9 Decoction of the crushed seed in traditional medicine in Ghana acts as Enema (Dalziel, 1961)7, while the crushed seeds are eaten as a cure for chest complaints and pneumonia. The seeds are widely used in West Africa especially in Nigeria, Cote-d'ivoire and Ghana as antipyretic, aphrodisiac, for treatment of malaria, pneumonia and other chest conditions (Nkere *et al,* 2005; 10Kouitcheu *et al*, 2008)11 .

The seeds are chewed as a tonic and stimulant also the seeds have strong sympathomimetic and local analgesic activity comparable to that of cocaine It acts selectively as a sympatholytic unaccompanied by Para sympatholytic effects, it inhibits the irritability of the sympathetic nervous system and opposes akuammine( Burkill 1985, 2004)12. Dry leaves are boiled in water and taken to treat guinea worm. In Cameroon fruit decoction is taken to cure cough or typhoid fever, the bitter bark is boiled with sugar and the decoction is drunk against food poisoning or venereal diseases. A hot water extract of the stem bark has a significant effect against *Trypanosoma brucei* (Wosu and Ibe, 1989)13 which was statistically comparable to that of diminazene aceturate (Berenil), commonly used in the treatment of sleeping sickness, In Gabon the Pahoin chew a little of the fruit and bark to allay hunger while on long marches in the bush (Burkill 1985, 2004).14 In Congo, leaf sap is dipped into the ears for otitis. In Southern Cameroon and Congo a bark decoction is drunk to cure sterility in men. The seeds are crushed or powdered and taken orally, and are mainly used for the treatment of malaria, and as a pain killer (Kapadia *et al*, 1993)15. *Picralima* has vast traditional roles in African folk medicine. It has been shown to possess anti-plasmodial, antimicrobial, anti-inflammatory, antipyretic, as well as anti-trypanosomiasis properties. Medicinally, the bark is used to treat malaria and male sexual impotence, while the fruits and seed are used for dysmenorrhea and gastrointestinal disorder (Fakoye *et al.*, 2000).16 A number of compounds have been isolated from the plant. Picraline, picraphyline, picracine, picralicine, picratidine, picratine, burnamine, akuammigine, pseudo-akuammigine, akuamimmenine, pseudo-akuammingine, Akuammine hydrate and pericine (Arens *et al*, 1982).17 Triterpenoid, Saponin, B-amyrin was isolated from the stem bark (Ezekwesili, 1983).18 Three coumestan glycosides and derivatives (3-hydroxy-9-methoxy-2-[2' (E)-3'-methyl-4'-0 - β- D – 3' –methyl -0 – β –D –glucopyranosylbutenyl] -8 –[ 2" (E)- 3"- methyl- 4"- oxobutenyl] coumestan, 3-hydroxy-9-methoxy-4-[2' (E)-3'-methyl-4'-0 - β- D – 3' –methyl -0 – β –D –glucopyranosylbutenyl] -8 –[ 2" (E)- 3"-methyl- 4"- oxobutenyl] coumestan, 3-hydroxy-9-methoxy-2-[2'(E)-4'-hydroxy-3'-methylbutenyl]-8-isoprenylcoumestan, 3-hydroxy-9-methoxy-2-[2'(E)-4'-hydroxy-3'-methylbutenyl]-8-[2"(E)-3"- methyl-4" oxobutenyl] coumestan and 3-hydroxy-9-methoxy-4-[2"(E)-3"-methyl-4"-oxobutenyl]coumestan) were isolated from the roots of *P. nitida* (Kouam *et al*, 2011) 19. Ajmaciline oxindole B: ((19∝)-19-methyl-2-oxoformosanan-16-carboxylic acid methyl ester) (Osuala *et al*., 2017).20

Other previous works on *Picralima nitida* include: The main alkaloid is akuammine which has local anaesthetic action. Its action can be compared to the anaesthetic action of cocaine (Ansa-Asamoah 1990)21. In a higher dose, it has strong inhibitory effect on intestinal peristaltic movement. It has hypertensive activity, which is weak and last longer in effect than Yohimbine (Menzies *et al.,* 1998)22. Akuammidine was established to have a sympatholitic and hypotensive actions and its local analgesic action is established to be three times that of cocaine hydrochloride (Menzies *et al*., 1998)22. Akuammidine also has hypotensive, local anesthetic and muscle relaxant effect. It opposes Akuammine. Akuamigine has sympatholitic effect and antagonizes the effect of adrenaline on the heart, vessels and the regulatory centres of circulatory system. Psuedo-Akuammigine is a reversible and competitive parasympathomimetic. In high doses, it has the capacity of inhibiting the central nervous system, respiration and also, contraction of skeletal and smooth muscles. It has local analgesic, anti

inflammatory effects (Dawiejua *et al*., 2002).23 It has hypotensive and cholinesterase-inhibiting activities. It increases the hexobarbitol-induced sleeping time. Picracine and Picralline have shown *in-vitro* opium antagonist activity (Menzies *et al.,* 1998)22. In experiments with mice, Alstonine has shown antipsychotic effects in the treatment of schizophrenia, without some commonly side effects as in Clozapine. The alkaloids akuammine, akuammidine, akuammicine and pseudo-akuammigine have varying degrees of agonist and antagonist activities at opioid receptors *in-vitro.* The extracts of the roots, the stem barks, and fruit-rind showed highly significant inhibitory effect *in-vitro* against *Plasmodium falciparum,* including chloroquine resistant strain, even in low concentrations W2 strain with $IC_{50}$ value of (10.9± 1.1) µ g/mL (Bickii *et al*., 2007)24. The root, stem bark nand fruit-rind extracts displayed significant inhibitory activities against asexual erythrocytic form of Plasmodium falciparum with $IC_{50}$ values of 0.188, 0.545 and 1.581 µ g/mL respectively (Francois *et al.,* 1996).9 An *in vitro* a,plasmodial activity of the ethanol seed extract of *P. nitida* was evaluated in chloroquine-sensitive *Plasmodium berghei berghei* infected mice. The result of this study showed that the ethanol seed extract of *P. nitida* exhibited significant *in vitro* antiplasmodial activity in both early (4-Day chemosuppressive test) and established infections (Curative test).Ethanol seed extract of *P. nitida* produced a dose dependent chemosupressive effect of 65.5 %, &0 .4 %, and 115 mg/kg/day doses (Okokon *et al*, 2007).25 Chloroform extract of the seed of *P.nitida* was evaluated for possible antileishmanial activity using a radiorespirometric microtest technique and the result of the study confirmed activity against Leishmania donovani at 50 µ g/mL (Iwu *et al.,* 1992) 26. The basic fraction of the methanol extract of the sterm back exhibited significant antimicrobial activity against a wide range of gram-positive bacteria and fungi, but limited activity against gram negative bacteria (Fakeye *et al*., 2000).16 The antimicrobial activity of the methanol extract of *P.nitida* fruit-rinds against 15 pathogenic strains of enteric bactaria and two yeast strains implicated in infective diarrhoea was investigated *in vitro* and the antidiarrhoea effect of the extract against *Shiglla dysenteriae* type 1 (sd 1)-induced diarrhoea was determined *in vivo* (Kouitcheu *et al*., 2013).27 The methanol fruit extract of *P. nitida* showed potent and dose-dependent anti-inflammatory activity. The extract when administered intraperitoneally inhibited carrageenan induced rat paw oedema with $IC_{50}$ value of 102 mg/kg, and with the highest dose tested (300 mg/kg) producing 72.2 % inhibition (Ezeamuzie *et al*,., 1994)28. The antipyretic activity of *P. nitida* fruit has been demonstrated. The result of the study showed that the methanol fruit extract at a dose of 50 mg/kg produced a mean percentage antipyrexia of 38.7 % on lopopolysaccharide-induced pyrexia in rabbits, which was comparable to aspirin (29.0 % at 200 mg/kg) (Ezeamuzie *et al.,* 1994).28 Aqueous and methanol extracts of the leaf and seed of *P. nitida* also have a concentration and time dependent larvicidal activity in the third and early fourth instar larve of *Anopheles gambiae* with 72 h $IC_{50}$ values of 0.164, 0.333 and 0.150 mg/mL for aqueous leaf extract, methanol leaf extract and methanol seed extract respectively (Dibua *et al*., 2013).29 In a study pseudo-akuammigine was found to exhibit analgesic effect *in vivo* The $ED_{50}$ value for this test was 10 m which was 3.5 and 1.6 times less potent than morphine and indomethacin respectively. In the study, naloxone inhibited the effect of pseudo-akuammigini suggesting that the analgesic actions are mediated via interaction with opioid receptors (Duwiejua *et al.,* 2002)23. Pulverized and encapsulated seeds of *P. nitida* are in sale in Ghana markets for medicinal purposes. *Picralima nitida* has mainly indole alkaloids and others in less quantity. The main alkaloid is akuammine which has local anaesthetic action. Its action can be compared to the anaesthetic action of cocaine and has strong inhibitory effect on intestinal peristaltic movement (Hamet *et al.,* 1940 30, Ansa-Asamoah 1990).21 It has hypertensive activity which is weak and last longer in effect than yohimbine. Akuammindine was established to have a sympatholytic and hypotensive action. Its local analgesic action is established to be three times that of cocaine hydrochloride (Menzies *et al.,* 1998)22. The effect of alkaloids and glycosides extracts of the seed of *P. nitida* on mean fasting blood sugar in alloxanized diabetic rats were evaluated by Okonta *et al*., (2007).31 Their findings showed that the glycosides extract have more potent hypoglycaemic effect than the alkaloids extract. Ouattara *et al* (2004)32 carried out the study on LC/MS/NMR analysis of isomeric divanilloylquinic acids from the root bark of *Fagara zanthoxyloides* Lam. Ouattara *et al*., (2009)33 screened the antisickling properties of divanilloylquinic acids isolated from *Fagara zanthoxyloides* Lam. The hypoglycaemic effect of the methanol extracts of the seed, pulp and rind of *P. nitida* were investigated by Inya-Agha *et al* the result showed a significant ($p < 0.01$) hypoglycaemic effect *of* all extracts at 300 and 900 mg/kg in alloxan-induced diabetic in rats (Inya-Agha *et al.,* 2006)34. Osayemwenre *et al.,* (2011)35 investigated the antipoliferative and apoptotic effects of fractionated extracts of *P. nitida* on humam breast cancer ceii line. The comparative study of the hypoglycaemic effects of water extract of *P. nitida* seeds (Apocynaceae) and Daonil in alloxan induced diabetic albino rats (Salihu *et al* 2009).36 Safety evaluation of ethanol leaf extract of *P. nitida* (Stapf) was screened (Ilodigwe *et al*, 2012)37.

## II. Materials And Methods

**Collection and Preparation of Plant Materials.**

Matured and riped fruits of *P. nitida* were collected in its fruit bearing season from Dim Anozie village in Isu Imo state in the month of May 2018. The plant was authenticated by a taxonomist at the Botany Herbarium of

the University of Nigeria Nsukka where Voucher specimen was maintained. The fresh matured fruits (8kg) were cut into transverse section to expose the seeds, the pulp and the rinds. The seeds were air-dried for three weeks and then pulverized with laboratory hammer mill into a homogenous powder, and stored inside an airtight container until used.

**Animals** Wister rats (135-140g) of both sexes were purchased from the animal unit, Department of Pharmacology/Toxicology Madonna University Elele. The animals were then housed in plastic cages.

**Reagents**

The following materials and reagents were used as procured from their supplies: Chloroform, methanol, ethanol (May & Baker, England), hexane dichloromethane and ethyl acetate (BDH, England), and other standard laboratory reagents were used.

Other materials: Rotary evaporator (Seneo, China), Hot air oven (Singifriend; England), Animal weighing balance (Attaus; Poland), water bath, TLC plate, desiccator, measuring cylinder, funnel, filter paper (No. 1 What man), Sieve, Miller (Thomas laboratory).

**Extraction of plant material**

The pulverized plant seed 2.5 kg was macerated in 1700ml methanol in a desiccator bottle at room temperature for 72 hours with a constant shaking. The mixture was filtered with Whatman (No 1) filter paper. The marc was rewashed to make sure the extractable constituents were completely washed out and then filtered. The whole filtrate after was concentrated in a rotary evaporator to a semi solid extract, which was transferred into a beaker and heated in a water bath below 45 $^{o}$C to driness. The weight of the dried extract was measured on a sensitive weighing balance and the percentage yield was also calculated. The concentrated extract was stored in a disposable bottle in a desiccators at room temperature until used.

**Determination of extractive yield**

**a. Water extractive value:** A 2 g quantity of the powdered material was weighed into 250 ml stoppered conical flask. Hundred mililitre of chloroform-water mixture was added and the flask firmly stoppered and was agitated mechanically for 6 hours. After that, the maceration continued for the next 18 hours. A 20 mL volume of the filtrate was evaporated to dryness in a 100 mL beaker over a hot water bath. The residue was dried to a constant weight at 105$^{0}$C. The water extractive was calculated.

**b. Alcohol extractive value:** A 2 g quantity of powdered material was weighed into a 250 ml stoppered flask and100 mL of 95 % ethanol was added. The stopper was firmly placed and the contents of the flask were agitated mechanically for six hours. The maceration continued in the flask for the next 18 hours. A 20 ml volume of the filtrate was evaporated to dryness in a boiling hot water bath. The residue was dried to a constant weight at 105 $^{o}$C and the alcohol extractive was calculated from

**Determination of proximate standards**

**Determination of moisture content**

A porcelain crucible was heated and weighed ($W_1$). A 2g powdered drug was measured into the crucible and reweighed ($W_2$). The sample was heated in the oven at temperature of 55$^{0}$C for hours until no sign of moisture is observed and a constant weight was obtained. Then the sample was cooled in the desiccator and reweighed ($W_3$) and the moisture content was calculated.

**Determination of ash values**

**Total ash values:** A tarred nickel crucible was heated dry, cooled and kept in a desiccators $W_1$. A 2 g quantity of the powdered drug was weighed into the crucible $W_2$ and heated in a muffle furnace at 650$^{0}$C until there was no change in weight. The heating continued until all the carbon content was evaporated to produce residue free of carbon. The residue was cooled in a desiccator and weighed $W_3$. The process of heating and cooling was repeated until constant weight was obtained. The total ash value was calculated

**Water insoluble ash value:** A nickel crucible was placed in muffle furnace and heated to a constant weight at the temperature of 450$^{0}$C, cooled and weighed ($W_1$). 2g of the powdered drug was placed in the crucible and reweighed (W2). The crucible containing the drug was incinerated at a low temperature initially to burn off the carbon content. The heat was gradually increased until all the carbon was burnt off. The crucible was cooled in desiccators, reweighed and the content was transferred into a small beaker ($W_3$). About 5ml of water was added to the content and boiled in the water bath for 5 minutes, filtered with an ashless filter paper and the filter paper containing the residue was dried in the oven, the filter paper thereafter containing the residue was compressed into the crucible and subjected to heat until the ashless filter paper was eliminated and the crucible is reweighed.

**Acid insoluble ash value:** The total ash obtained from the experiment above was transferred to a beaker containing 250 ml dilute hydrochloric acid and heated on a boiling water bath for five minutes. It was then filtered through ashless filter paper. The beaker and the crucible were washed repeatedly until they are free

from acid. The ashless filter paper was dried in the oven. It was later folded into a tarred crucible and heated completely to ash. The residual ash was then heated intensively and after cooling in desiccators, it was weighed.

**Phytochemical Screening**

The powdered seed sample and also the extract of *Picralima nitida* was screened for their phytochemical components. The tests were carried out according to the procedures and methods outlined by Harbourne (1973)38 and Trease and Evans (2008)39.

**Qualitative Phytochemical Analysis of powder and extract of *P.nitida*:** Test for Alkaloids, Saponins (Frothing test, Emulsion test), Flavonoids (Ammonium test, 1% Aluminium chloride solution test), Glycosides, Tannins (Ferric chloride test, Lead acetate test), Anthraquinones, Carbohydrates (Molish's test),Reducing sugar (Fehling's solution test), Test for oils, Test for protein (Millon's test), Xanthoproteic test,

**Quantitative Phytochemical Analysis of *P. nitida* seed extract**

**Alkaliods determination**

A method described by Harborne (1973) 38 was used. A portion (5 g) of sample was weighed into a 250 mL beaker and 200 ml of 10 % acetic acid in ethanol was added, covered and allowed to stand for 2 h. This was filtered and the extract was concentrated on a water bath to one – quarter of the original volume. Concentrated ammonium hydroxide was added drop-wise to the extract and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

**Flavonoids determination**

This was done following the method of Boham & Koupai-Abyazan (1994)40. A 10 g quantity of the sample was extracted repeatedly with 100 mL of 80 % aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

**Saponins determination**

The method of Obadoni and Ochuko (2001)41 was used. A portion (20 g) of the sample was put into a conical flask and 100 mL of 20 % aqueous ethanol was added. The sample was heated over a hot water bath for 4 h with continuous stirring. The mixture was filtred and the residue re-extracted with 200 ml of 20 % ethanol. The combined extracts were reduced to 40 mL over water bath at 90 $^{o}$C. The concentrate was transferred into a 250 mL separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqeous layer was recovered while the ether was discarded. The purification process was repeated.sixty mililitres of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5 % aqeous sodium chloride. The remaining solution was heated on a water bath. After evapouration the samples were dried in the oven to a constant weight. The saponins content was calculated in percentage of the 20 g extracted sample

**Tannins determination**

Tannin determination was done by Van-Burden and Robinson (1981)42 method. A portion (500 mg) of the sample was weighed into a 50 ml plastic bottle. A 50 mL volume of distilled water was added and shaken for 1 h on a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to mark. A 5 mL volume of the filtrate was pipette out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min.

**Tests for mineral constituents of the extract of *P. nitida*.**

The extract was tested for mineral ions. Heavy metals analysis was conduct using Varian AA240 Atomic Absorption Spectrophotometer according to the method conytained in American Public Health Association (APHA) (1995).43

**MACROSCOPICAL EVALUATION OF *Picralima nitida***

The following were used to characterize the seed of *Picralima nitida*

Condition, Colour ,Texture,Taste, Feel, and Odour

**Chemomicroscopy Test for Lignin**, **Starch ,Calcium oxalate, Cellulose**

Powdered leaf was mounted in a few drops of Sudan III solution and observed under microscope for a red colouration. (Evans, 2008).44

**Microscopical Evaluation**

**Epidermal Characteristics**

Epidermal cell(nature),Trichomes(type and distribution)

Cell inclusion (calcium oxalate)

**Microscopic Evaluation of Powdered sample of *Picralima nitida***

A small quantity of the powdered seed of *Picralima nitida* was put on a clean slide. Few drops of chloral hydrate was put and few drops of water was also put from one end and allowed to slip around it. The slide was carefully passed over a flame of bursen burner repeatedly until bubbles occurred and allowed to cool for proper clearing of the sample. Two drops of glycerine were added to the slide as mountant and then covered with cover

slip and viewed under the microscope. The following characters were noted: Cork cells, sclereids, fibres and calcium oxalate crystal.

**Test for minerals:** The Powder and extract were subjected to dry ash digestion and the resultant supernatants were used for macro- and micronutrients determination using the emission flame photometer and the absorption spectrophotometer.

**PHARMACOLOGICAL EVALUATION**

**Acute Toxicity Study ($LD_{50}$)**

The acute toxicity study was carried out using the method employed by Dietrich Lorke (1983).45 A total of 13 rats were used and the study was carried out in two stages. Different concentrations of the extract were prepared according to their groups. The weights of the rats were used to determine the dose for each rat. Stage one was done using 9 rats and they were grouped into 3 groups of animals per group. Group i, ii and iii received single oral dose of 10, 100 and 1000 mg/kg of the extract respectively. The animals were monitored intermittently over a period of 24 hours to record death.

The second stage employed 4 rats grouped into 4 groups of 1 animal each. The doses were given according to the weight of the animals. The first group received single oral dose of 1500 mg/kg, second group received single oral dose of 2500 mg/kg and third group received single oral dose of 3500 mg/kg. The forth group received single oral dose of 5000 mg/kg. The $LD_{50}$ was calculated using equation below:

$LD_{50} = \sqrt{a}$ x b

Where a, is the lowest dose that brought death and b is the highest dose that did not cause death.

**Castor oil-induced Diarrhea**

The method involves the use of male and female wister rats which were starved over night for 18 hours. They were housed in five groups containing three rats each, The drug doses were prepared as suspensions and were administered orally by gavage in different groups of animals labelled A, B and C with doses of 100, 200 and 400 mg/kg respectively. The group D was given the standard drug (loperamide) orally as suspension according to their weights. The group E, the negative control were not given any drug.

Thirty minutes after the treatment with the extract each animal recieves 1ml of castor oil orally by gavage and then were observed for defeaction up to the 3rd hour after the castor oil administration. The presence of characteristic diarrhoea droppings were noted in the filter paper placed beneath the transparent plastic plate. The effect of the different concentrations of the extract, the effect of the standard antidiarrhoea agent loperamide was calculated based on the frequency of their defeacations as compared to the negetive control (untreated rats) in group E.

**Gastrointestinal Motility Tests (Charcoal Transit)**

The gastrointestinal motility in Wister rats was tested to know the effect of the drug on them following the oral administration of the extract. Wistar rats were starved for 18 hours and placed in different cages containing three in each. Each animal was administered intraperitoneally with 1ml of charcoal meal (5 % w/v) charcoal in mucilage of carboxymethyl cellulose. Immediately after that, the 2nd and 3rd groups of the animals were treated orally, with the test drug at varying doses (200 amnd 400 mg/kg). Next group received atropine (0.5 mg/kg) intraperitoneally, the standard drug for comparison. 30 minutes later, each animal was sacrificed by spinal cord dislocation and the intestinal distance moved by the charcoal meal from the pylorus was cut and measured and expressed as a percentage of the distance the charcoal meal has moved from the pylorus to ceacum.

**Statistical Analysis**

Statical analysis of one way anova was carried out using a software Graph pad Prism5 version.

## III. Results

**Yield Of Extraction**

A 2500 g of the powdered seeds gave 345 g yield (13.80 % w/w)

**Result Of Phytochemical Analysis:** As in the table below, *Picralima nitida* contains: alkaloids, carbohydrates, reducing sugars, flavonoids, oils, glycosides, tannins, steroids.

**Table 1: Qualitative Phytochemical Analysis**

| S/N | TEST | OBSERVATION | INFERENCE |
|---|---|---|---|
| 1 | **Alkaloids** | | |
| a | Drangendoff's reagent | Brick red Precipitate | Present |
| b | Wagner's test | Reddish brown Precipitate | Present |
| c | Mayer's test | Milky Precipitate | Present |

| | | | |
|---|---|---|---|
| d | Hager's test | Yellowish Precipitate | |
| 2 | **Carbohydrate**<br>Molish's test | Brown ring interface | Present |
| 3<br>A | **Reducing Sugars**<br>Fehling's test | Blue to green | Present |
| 4 | **G lycosides** | Brick red Precipitate | Present |
| 5<br>a<br>b | **Flavonoids**<br>Ammonium test<br>Aluminium chloride test | Yellow colouration<br>Yellow colouration which disappears onstanding | Present<br>Present |
| 6 | **Oils** | Translucency of filter paper | Present |
| **7** | **Saponins**<br>Frothing test | Presence of froth (foam) | Present |
| 8<br>a<br>b | **Proteins**<br>Million's test<br>Xanthoproteic test | White Precipitate<br>Apperance of yellow colour which changes to orange colour in addition of sodium hydroxide | Present<br>Present |
| 9 | **Anthraquinones** | No colour change | Absent |
| 10<br>a<br><br>b | **Tannins**<br>Ferric chloride test<br><br>Lead acetate test | Green to blue-black Precipitate<br>Colour Precipitate | Present<br><br>Present |

## Proximate Analysis

**Table 2: Results of the proximate analysis for *Picralima nitida***

| **Constituent** | **Value (% w/w)** |
|---|---|
| Moisture content | 2.85 |
| Total ash value | 3.64 |
| Water extractive value | 23.35 |
| Alchol extractive value | 18.50 |
| Water soluble ash | 2.65 |
| Sulphated ash value | 8.10 |
| Acid insoluble ash | 6.10 |

## Mineral analysis

**Table 3: Results of the mineral analysis**

| **Parameter** | **Concentration** |
|---|---|
| Calcium | 12.686 ppm |
| Magnesium | 11.665 ppm |
| Potassium | 42.537 ppm |
| Chlorine | 159 mg/l |

## Chemomicroscopy tests

**Table 4: Result of the Chemomicroscopy tests**

| **Chemomicroscopy** | **Result** |
|---|---|
| **Lignin** | **Present** |
| **Starch** | **Present** |
| **Cellulose** | **Present** |
| **Calcium oxalate** | **Present** |

## Macroscopic analysis

**Table 5: Result of the macroscopic evaluation on *Picralima nitida* powder**

| **Parameter** | **Observation** |
|---|---|
| Condition | Dried |
| Colour | Brownish-yellow colour |
| Texture | Smooth |
| Taste | Bitter |
| Feel | Hard |
| Odour | Aromatic |

MICROSCOPY

.

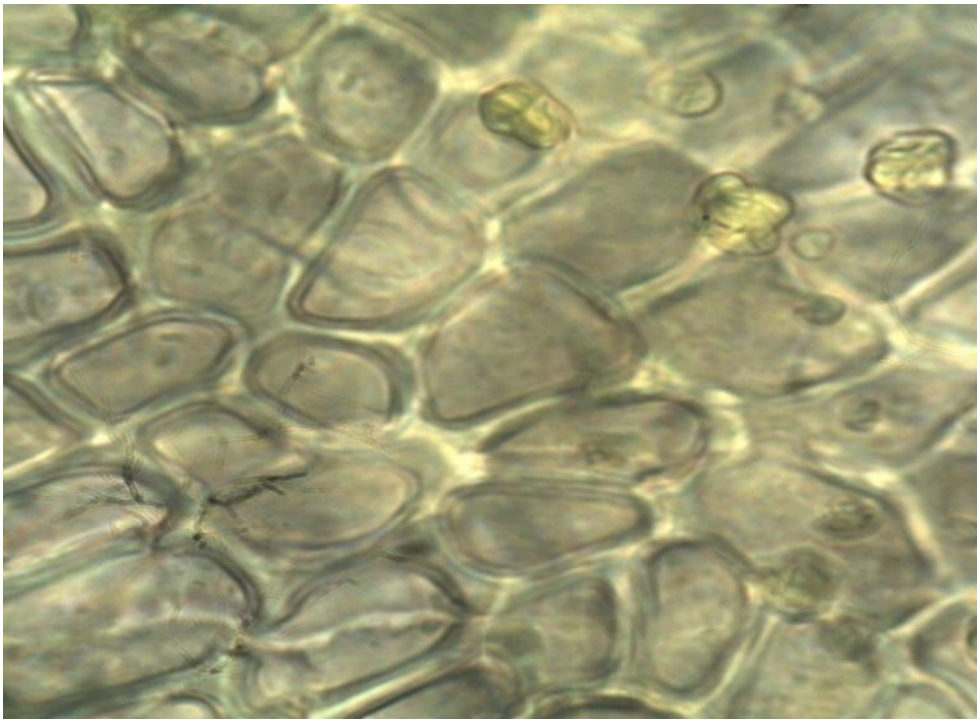

**Plate 1:** Powdered seed sample of *Picralima nitida* showing prism shaped calcium oxalate crystals and oil globules on the epidermal cells (x4).

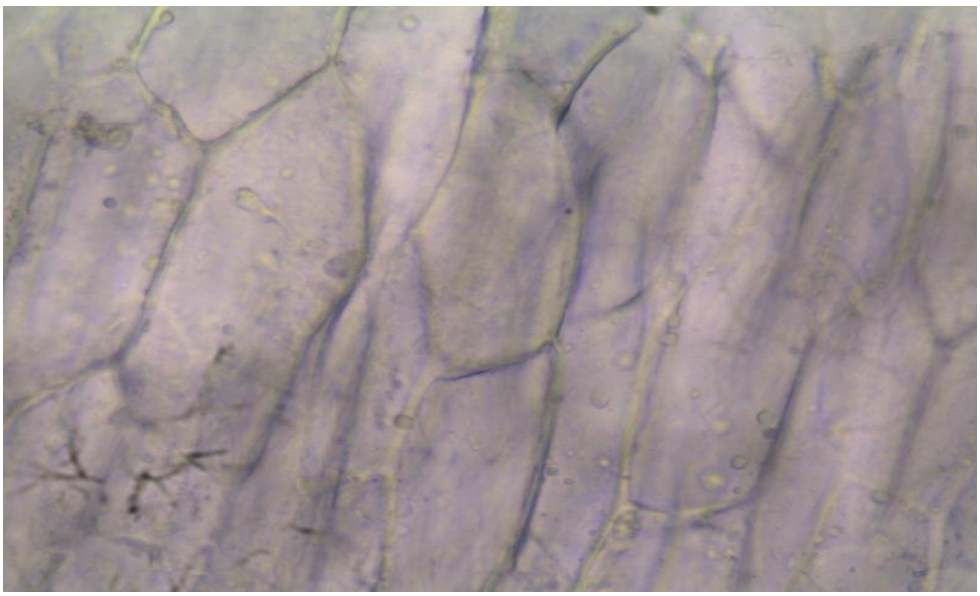

**Plate 2:** *Picralima nitida* powdered seed sample showing parenchyma cells (x4)

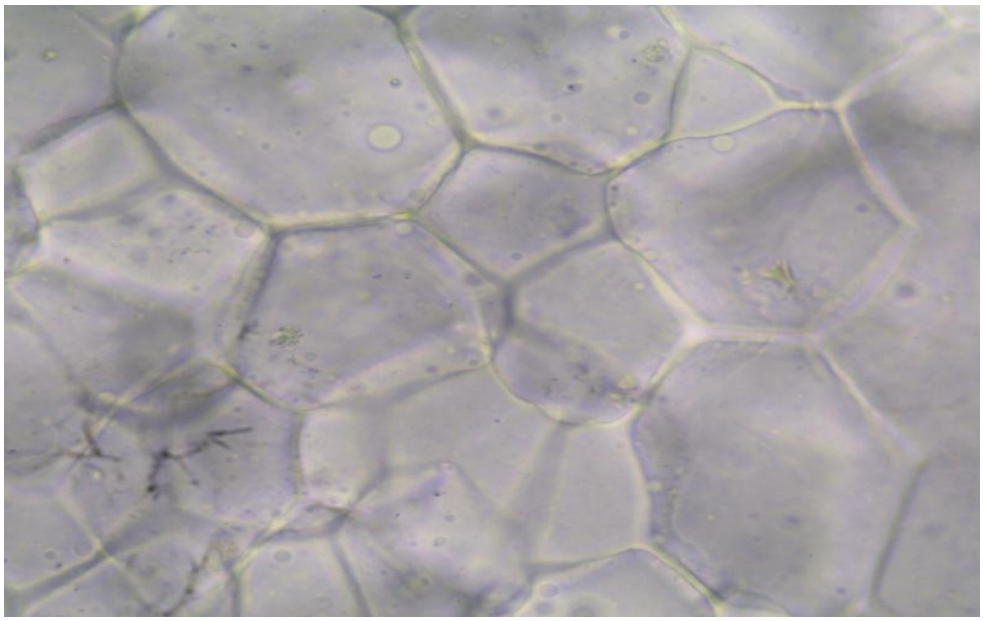

**Plate 3:** *Picralima nitida* powdered seed sample showing Collenchymateous cell (x4)

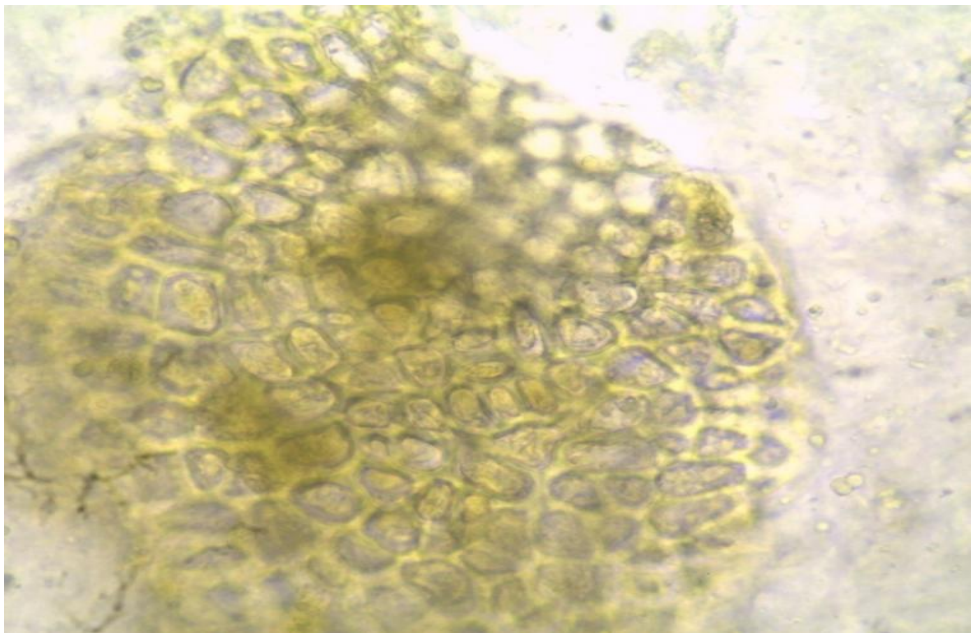

**Plate 4:** *Picralima nitida* powdered seed sample showing sclerids (stone cells) (x4)

**Acute Toxicity Test**

There was no death at 5000mg/kg which means that it has large safety margin.

**Anti-diarrheal Activity**

The result of prophylatic anti-diarrheal activity of the extract *Picralima nitida* on castor oil induced diarrhoeal in rats

**Table 6: Number of droppings for Dry feaces**

| Group | Treatment | Mean number of droppings | | | |
|---|---|---|---|---|---|
| | | 1 h | 2 h | 3 h | 4 h |
| A | Negative control | 0.8 ± 0.115 | 0.2 ± 0.029 | 0.0 ± 0.00 | 0.0 ± 0.00 |
| B | Positive control (Loperamide 2 mg/kg) | 0.3 ± 0.043 | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 |
| C | Extract 100 mg/kg | 0.5 ± 0.0720 | 1.8 ± 0.260 | 0.0 ± 0.00 | 0.0 ± 0.00 |
| D | Extract 200 mg/kg | 0.0 ± 0.00 | 4.6 ± 0.664 | 1.1 ± 0.159 | 0.0 ± 0.00 |
| E | Extract 400 mg/kg | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 |

*The mean difference is significant at the P> 0.05

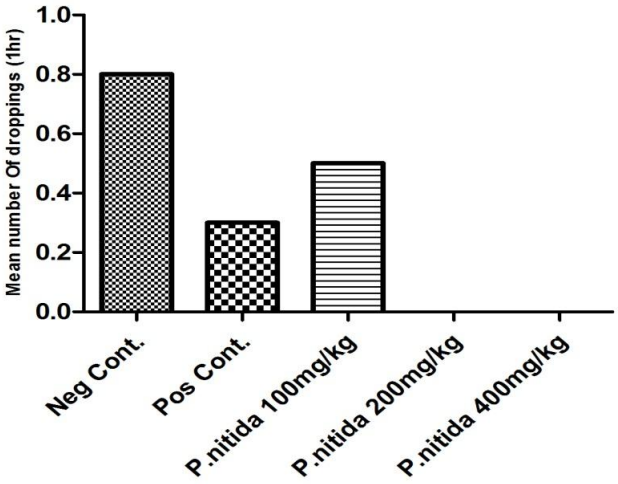


**Fig 1:** A bar chart of mean number of droppings(1 hr)

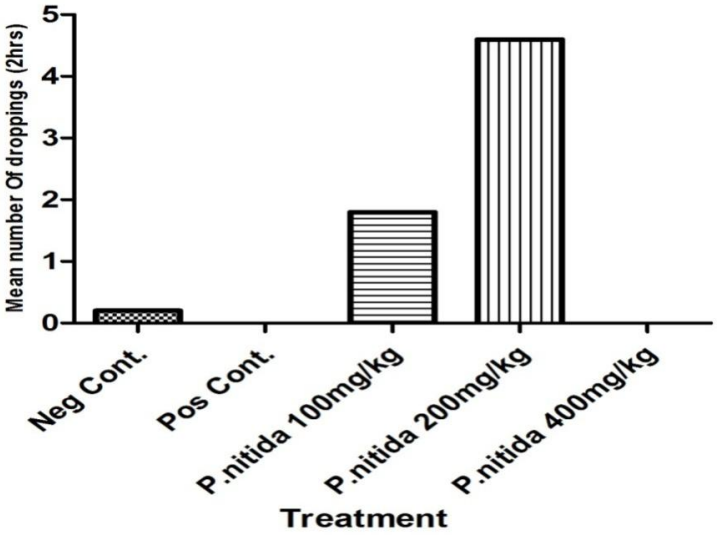


**Fig 2:** A bar chart of mean number of droppings (2 hr)

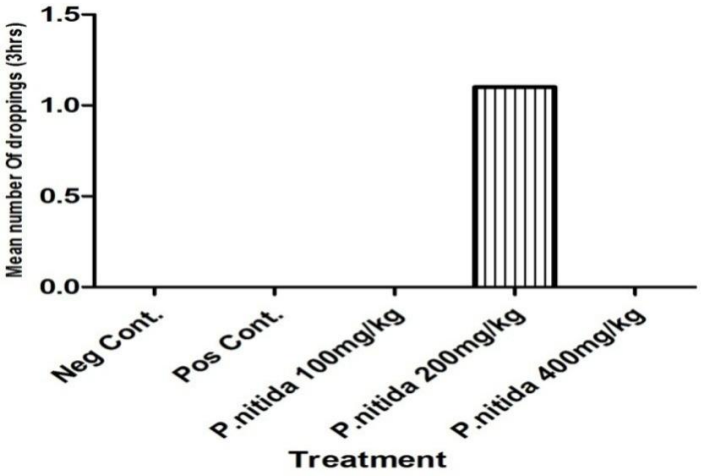


**Fig 3:** A bar chart of mean number of droppings (3 hr)

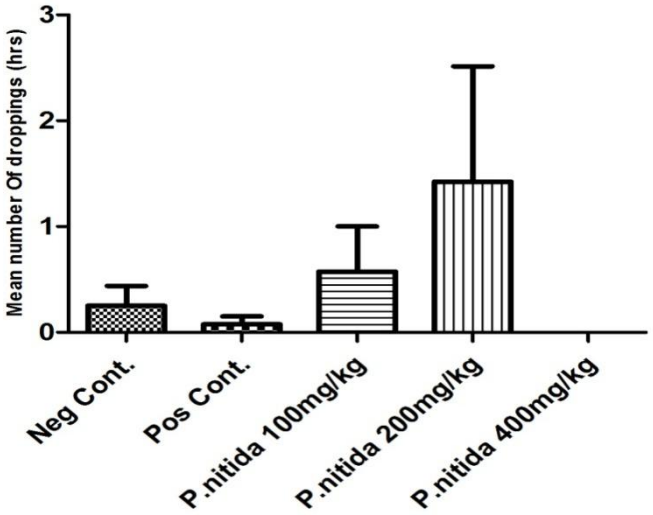


**Fig 4:** A cummulative chart for mean droppings for dry feaces

Results of prophylatic anti-diarrheal activity of the extract *Picralima nitida* on castor oil induced diarrhoeal in rats

**Table 8: Number of droppings for Wet feaces**

| **Group** | **Treatment** | **Mean number of droppings** | | | |
|---|---|---|---|---|---|
| | | 1 h | 2 h | 3 h | 4 h |
| A | Negative control | 1.6 ± 0.231 | 3.2 ± 0.462 | 14.0 ± 2.021 | 3.4 ± 0.491 |
| B | Positive control (Loperamide 2mg/kg) | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| C | Extract 100mg/kg | 0.0 ± 0.0 | 4.1 ± 0.592 | 4.8 ± 0.693 | 2.2 ± 0.318 |
| D | Extract 200mg/kg | 0.0 ± 0.0 | 2.0 ± 0.289 | 0.4 ± 0.058 | 2.6 ± 0.375 |
| E | Extract 400mg/kg | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |

*The mean difference is significant at < 0.05 level

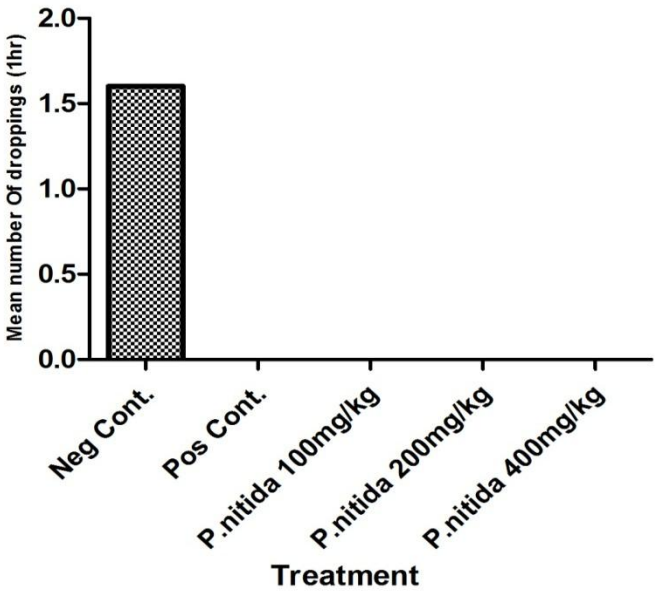


**Fig 5** A bar chart of mean number of droppings (1hr)

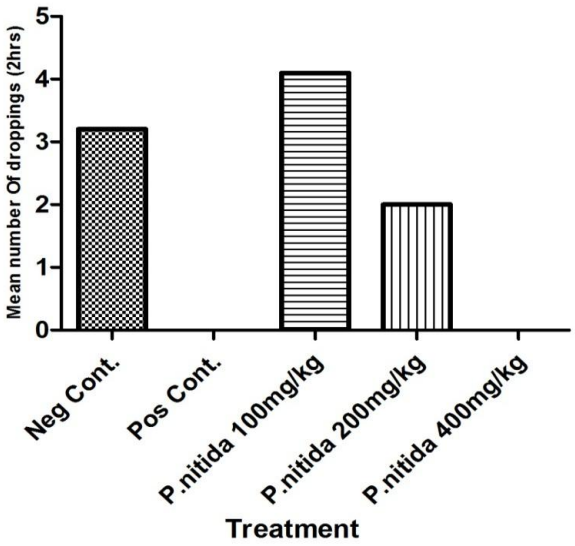


**Fig 6:** A bar chart for mean number of droppings (2hrs)

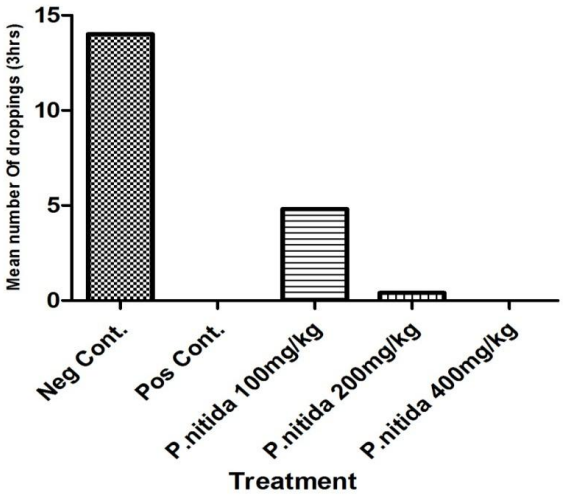


**Fig 7:** A bar chart of mean number of droppings (3hrs)

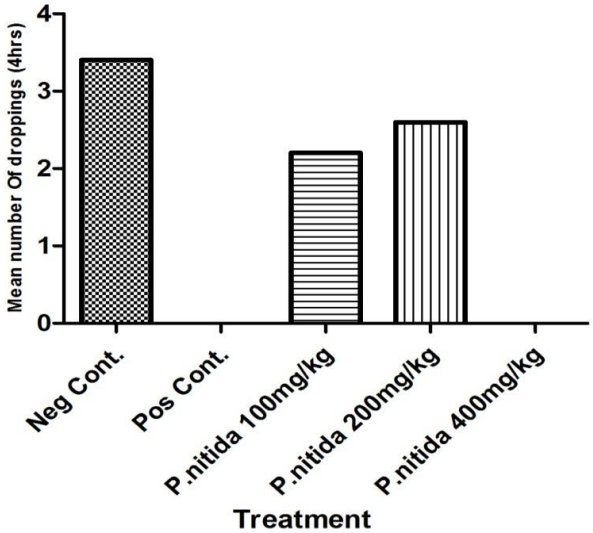


**Fig 8:** A bar chart of mean number of droppings (4hrs)

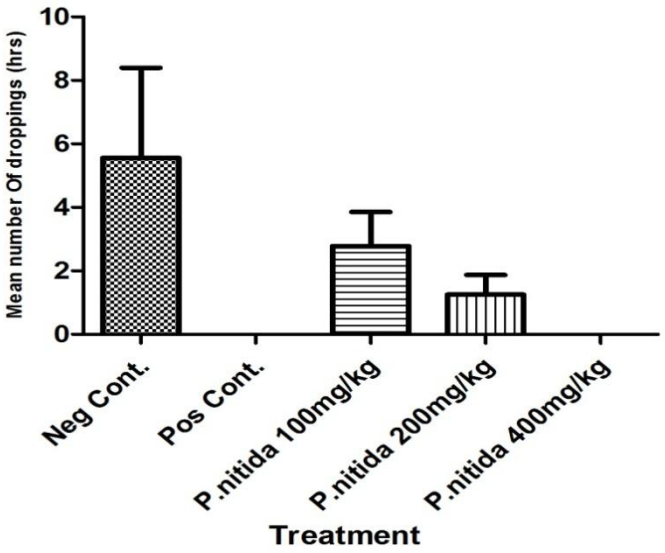


**Fig 9:** A cummulative chart of mean number of droppings wet feaces

**Result of the effect Of *Picralima nitida* on Gastrointestinal motility in rats**
The table below shows the results of the motility test on rats

**Table 9: Result of the Motility test**

| Group | Agent & dose | Mean length of small intestine (cm) | Mean distance travelled by charcoal meal (cm) | % motility |
|---|---|---|---|---|
| A | Negative control | 67.16 ± 10.26 | 38.0 ± 2.00 | 56.58 ± 5.774 |
| B | Atropine su;phate (1mg/ml) | 71.33 ± 10.78 | 11.83 ± 1.752 | 16.58 ± 3.175 |
| C | P. nitida 200mg/kg | 76.33 ± 11.27 | 26.0 ± 5.250 | 34.06 ± 0.00 |
| D | P. nitida 400mg/kg | 65.83 ± 10.41 | 6.06 ± 0.746 | 9.21 ± 0.00 |

*The mean difference is significant at the 0.05 level.

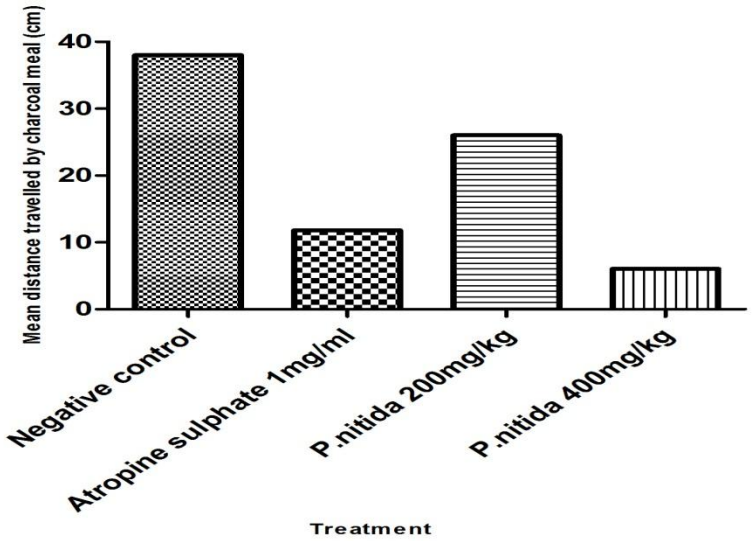


**Fig 10:** A bar chart of mean distance travelled by charcoal meal (cm)

## IV. Discussion And Conclusion

**DISCUSSION**

Studies have proved that in herbal medicines, the bio-active ingredients that have therapeutic activities in plants have holistic nature of treatment. Substances found in the medicinal plant containing the healing property of the plant are known as the active principles. It defers from plant to plant (Adebanjo *et al*., 1983)46

Studies have demonstrated the anti-diarrhoeal activity of tannins, flavonoids, alkaloids, saponins, reducing sugars, steroids and terpenes containing plant extracts (Kumar and Rajkumar, 2005)47. A preliminary phytochemical analysis of the extract revealed the presence of alkaloids, glycosides, reducing sugars, saponins, tannins, carbohydrate and steroids. These constituents may be responsible for the anti-diarrhoeal activity of the ethanol extract of P. nitida The **antimicrobial activity** of plant extracts is, possibly, due to their ability to complex with extra cellular and soluble proteins and to complex with bacterial cell walls disrupting microbial membranes (Neuwinger, 2000;48 Burkill, 1985). 12

The results of **phytochemical screening** revealed the presence of flavonoids, saponins, tannins and alkaloids and the aqueous extract had antimicrobial activities against ***Escherichia coli*** and *staphylococcus aureus* with varying degrees. The most potent inhibitory effect was observed with ***Escherichia coli***. These results have revealed that the peel and its extracts have pharmacological active compounds and antibacterial effects and as such could be used in ethno-medicine for the treatment of microbial infection and other ailments.

In chemomicroscopy, the plant was observed to have contained lignin, starch, calcium oxalate, tannin, cellulose.

The moisture content of 2.85 % indicates the less chance of microbial degradation of the crude drug. The total ash value of 3.26 % indicated the content of minerals i.e inorganic matters which may be one of the factors of the anti-diarrhoeal effect of the extractThe evaluation of the total ash is used for detection of foreign organic matter in the plant and a high total ash value indicates that the drug is adultrated and unsuitable for use. Acid insoluble ash value of the crude drug 6.1 % shows that a small amournt of the inorganic component is insoluble in acid. The alcohol and water extractive value 18.5 and 23.35 % respectively are important when the chemical nature of the medicinal components are not known and the value helps to detect alcohol and water exhausted drugs.

The methanol extract of *Picralima nitida* was found to have a very high safe margin even at the highest dose there was no death.

The Castor oil induced diarrhoea due to its active metabolite ricinoleic acid as a result of the action of lipases an enzyme which leads to stimulation of peristaltic activity in small intestine resulting to change in the electrolyte permeability of intestinal mucosa. Its action also leads to the release of endogenous prostaglandin. Castor oil is reported to induce diarrhoea by increasing the volume of intestinal content by preventing reabsorption of water. The liberation of ricinoleic acid results in irritation and inflammation of intestinal mucosa leading to release of prostaglandin (Harvay and Champe, 2009).49

Methanol seed extract of *Picralima nitida* was subjected to two different pharmacological models, Castor oil induced diarrhea and gastrointestinal motility test.The result obtained from the anti-diarrhoeal evaluation further proves that at a high concentration the methanol extract of *Picralima nitida* seed was observed to be an effective anti-diarrheal agent. This was evidenced by the significant reduction in the frequency of defecation or droppings. The result were comparable to that of the standard drug, loperamide. The gastrointestinal motility test showed a very significant inhibition with the 200 mg/kg, and at 400 mg/kg the extract showed more activity compared to the standard drug atropine (1mg/ml).

## V. Conclusion

In this study, the antidiarrheal effect of the extract from *Picralima nitida* may be due to the holistic action of its contents: glycosides, saponins, tannins and alkaloids. Extracts from the seeds of *Picralima nitida* can be recommended as an anti-diarrhoeal agent, hence it can serve as a lead to production of anti-diarrhoeal drugs.Further investigations should be carried out so as to isolate and characterize the pure active component(s) responsible for its anti-diarrheal activity.

## References

[1]. World Health Organization, (2013). "Diarrhoeal disease Fact Sheet". Archived from the original on 17 July 2014. Retrieved 9 July 2014.
[2]. Rang, H.P., Dale, M.M., Ritter, J.M., Flower, R.J. (2007). Rang and Dale's Pharmacology, 6th edition, Edsevier Ltd, Philadephia, pp 394-395
[3]. World Health Organization, (2008-2012). "Traditional Medicine Definitions". Retrieved 2014-04-20.
[4]. Kouitcheu mabeku L.B., Kouam J., penlapBeng V, Njadjui B.T., Fomum Z.T., Etou, F.X. (2005). Evaluation of Antimicrobial activity of the fruit rind of *Picralima nitida* (Apocynaceae) and stem bark of *Cycliodiscus gabumensis*. West African Journal of Pharmacology and Drug Research; 21:6-12.
[5]. Harris, D.J. (2002). The vascular plants of the Dzanga- Sangha Reserve, Central African Republic: 1-274. National Botanic Garden (Belgium), Meise.
[6]. Adjanohoun, E.J., Aboubakar, N., Dramane, K., Ebot, M.E., Ekpere, J.A., Enow-Orock, E.G., Focho, D., Gbile, Kamanyi, A., Kamsu, K.J., Keita, A., Mbenkum, Mbi, C.N., Mbiele, I.L., Mubiru, N.K., Nancy, W.L., Nkongmeneck, B., Satabie, B., Sofowora, A. and Tamze, (1996). Contribution to ethnobotanical and floristic studies in Cameroon. CSTR/QUA 641.
[7]. Dalziel, J.M (1961). The useful plants of west tropical Africa. The crown Agents, London Davies, S.C and A. Gilmore "The Role of Hydroxurea in the management of sickle cell Diseases" Blood Reviews 17 (June 2003); 99 -109
[8]. Iwu MM (1993) Handbook or African medicinal plants, CRC press, London pp (15),219-21

[9]. Francois, G Assist, L.A., Halonz, J and Bringmann G. (1996). Invitro inhibitory activities of *Picralima nitida* constituents against asexual erythrocytes form of *plasmodium falciparium*. Journal of Ethnopharmacol, (2-3): 113-17.

[10]. Nkere, C.k. and C.U. Iroegbu, 2005. Antibacterial screening of the root, seed and stembark extracts of *Picralima nitida*. Afr. J. Biotechnol., 4: 522-526.

[11]. Kouitcheu LB, Kouam J, Atangana P, Etoa FX (2008), Phytochemical screening and toxicological profile of methanolic extract of *Picralima nitida* fruit-rind (Apocynaceae) Toxicol Environ Chem, 90 pp. 815–28

[12]. Burkill HM (1985), The useful plants of west tropical Africa (2nd ed.), Royal Botanical Gardens, Kew pp. 456–596

[13]. Wosu, L.O. and Ibe, C.C., 1989. Use of extracts of Picralima nitida bark in the treatment of experimental trypanosomiasis: a preliminary study. Journal of Ethnopharmacology (3): 263-268.

[14]. Burkill. H.M, (1985). The useful plants of west Tropical Africa, 2nd Edition. Volume 1, Families A-D. Royal Botanical Gardens, Richmond, United Kingdom 960pp.

[15]. Kapadia, G.J., Angerhofer, C.K., Ansa-Asamoah, R. (1993). Akuammine: an antimalarial indolemonoterpene alkaloid of *Picralima nitida* seeds. *Planta medica*. (6):565-6).

[16]. Fakeye, T.O., Itiola, O.A. and Odelola, H.A. (2000). Evaluation of the antimicrobial property of the stem bark of *Picralima nitida*(Apocynaceae). Phytotherapy Research, 14: 368-70.

[17]. Arens H., Borbe, H.O., Uibrich, B. and Stoeckigt, J. (1982). Detection of pericine a new central nervous system active indole alkaloid from *Picralima nitida* cell suspension culture by opiate receptor binding studies. Planta Med., 46, pp. 210–214

[18]. Ezekwesili, J. O (1983) Biostudies of Picralima nitida (Stapf) Th & Hel M. Sc Dissertation, University of Nigeria, Nsukka, Nigeria.

[19]. Kouam J., Mabeku, L.B.K., Kuaite, J.R., Tiabou, A.T., Fomum, Z.T. (2011). Antimicrobial glycosides and derivatives from roots of *Picralima nitida.* Int J Chem, 3 pp 23-31

[20]. Osuala F.N. 2017, Anti-sickling screening of bioactive compounds from seeds of *Picralima nitida* Stapf (Apocynaceae). International Journal of Pharmacy. Photon 108, 516-523.

[21]. Ansa-Asamoah, R., Kapadia, G.J., Lloyd, H.A. and Sokoloski, E.A. (1990). Picratidine, a new indole alkaloid from *Picralima nitida* seeds J Nat Prod, 154 (4): 975-977.

[22]. Menzies, J.R.W., S.J. Paterson, M. Duwiejua and A.D. Corbett, (1998). Opiod activity of alkaloids extracted from Picralima nitida, (fam. Apocynaceae). Eur. J. Pharmacol.101-8.

[23]. Duwiejua M, Woode E, Obiri DD. (2002) Pseudo-akuammigine, an alkaloid from *Picralima nitida*seeds, has anti-inflammatory and analgesic actions in rats. Journal of Ethnopharmacology, 81: 73-79.

[24]. Bickii,J., Tchouya, G.R.F., Tchouankeu, J.C., Tsamo, E. (2007), Antimalarial activity in crude extracts of some Cameroonian medicinal plants. Afr. J. Tradit Complement Altern Med, 4 (1): 107-111.

[25]. Okokon J.E, Anita B.S, Igboasoiyi A.C, Essien E.E, Mbagwua HOC (2007), Evaluation of antiplasmodial activity of ethanolic seed extract of *Picralima nitida*. J Ethnopharmacol, 111:464-7.

[26]. Iwu M, Jackson J, Tally J, Klayman D (1992), Evaluation of plant extracts for antileishmanial activity using a mechanism-based radiorespirometric microtechnique (RAM) Planta Med, 58 pp. 436–41

[27]. Kouitcheu LBM, Tamesse JL, Kouam J (2013), The anti-shigellosis activity of the methanol extract of *Picralima nitida* on *Shigella dysenteriae* type I induced diarrhoea in rats BMC, 13 p. 211

[28]. Ezeamuzie, I.C., Ojinnaka, M.C., Uzogara, E.O. and Oji,S.E. (1994). Anti-inflammatory, antipyretic and antimalarial activities of a West African medicinal plant-Picralima nitida African Journal of Medicine and Medical Sciences, 23, 85-90.

[29]. Dibua UME, Odo GE, Nwabor OF, Ngwu GI Larvicidal activity of *Picralima nitida*, an environmental approach in malaria vector control, Am J Res Comm [Accessed on 20th Oct., 2013].

[30]. Hamet, Ansa-Asamoah, Amposo AA (1940). Analgesic effect of crude extract of *Picralima nitida* seeds. Afr. J. Pharmacol., 35-38.

[31]. Okonta JM, Aguwa CN (2007), Evaluation of hypoglycaemic activity of glycosides and alkaloids extracts of *Picralima nitida*Stapf (Apocynaceae) SeedIntern J Pharmacol, 3 (6):505-9.

[32]. Ouattara, B., Angenot, L., Guissou, P., Fondu, P., Dubois, J., Frédérich, M., Jansen, O., van Heugen, J., Wauters, J., Tits, M., 2004. LC/MS/NMR analysis of isomeric divanilloylquinic acids from the root bark of *Fagara zanthoxyloides* Lam. Phytochemistry 65(8):1145-51.

[33]. B Ouattara - 2009 - Cited by 39 - Related articles

[34]. *2009* Mar;16(2-3):125-9. doi: 10.1016/j.phymed.2008.10.013. Epub 2008 Dec 24. *Antisickling properties of divanilloylquinic acids* isolated from Fagara zanthoxyloides Lam. ... analysis in the root bark of F. zanthoxyloides [*Ouattara et al*., 2004.

[35]. Inya-Agha, S.I., Ezea S.C., Odukoya, A.O. (2006). Evaluation of *Picralima nitida:* Hypoglycaemic activity, toxicity and analytical standards. Intern. J. Pharmacol., 2(5), 576-580.

[36]. Osayemwenre E, Lutz NJ, Falodun A (2011), Antiproliferative and apoptotic effects of fractionated extracts of *Picralima nitida*on human breast cancer cell line Nig J life Sci, I (2): 75-82.

[37]. Salihu MA, Luqman AO, Oshiba OJ, Rabiu OJ, Sikiru AJ, Ayokunle O (2009), Comparative study of the hypoglyceamic effecs of coconut water extract of *Picralima nitida*seeds (Apocynaceae) and Daonil in alloxan induced diabetic albino rats Afri J Biotechnol, 8(4):574-6.

[38]. Ilodigwe, E.E., Okoye, G.O., Mbagwu, I.S., Agbata, C.A., Ajaghaku, D.L. (2012). Safety evaluation of ethanol leaf extract of *Picralima nitida* Stapf (Apocynaceae). Intern. J. Pharmacol. Therapeutics, 2 (4):6-17.

[39]. Harbourne, J. (1973). Phytochemical methods. A Guide to Modern Techniques of Analysis. Chapman and Hall, London, p 279.

[40]. Trease. G.I. & Evans, W.C (2008). Pharmacognosy, 15th ed. E.B Saunders, Edinburgh. London, 3-56.

[41]. 41. Obadoni BO, Ochuko PO: (2001) Phytochemical studies and comparative efficacy of the extracts of some haemostatic plants in Edo and Delta States of Nigeria. Glob J Pure Appl Sci. 8: 203–8.

[42]. Van Burden, J.P. and W.B. Robinson, (1981). scialert.net/fulltext/? Jul 2, 2011 ... Tannin determination. Formation of its complexes.

[43]. American Public Health Association (APHA) (1995) Standard Methods is a joint publication of the the American Water Works Association (AWWA).

[44]. W.C (2008). Pharmacognosy, 15th ed. E.B Saunders, Edinburgh. London, 3-56.

[45]. Lorke, D. (1983) A New Approach to pratical Acute Toxicity Testing Arch. Toxicol 54: 275-87.

[46]. Adebanjo, A.O., Adewunmi C.O.Essien E.E.(1983). Anti infective agents of higher plants. 5th International Symposium in Medical Plants,University of Ile ife (OAU), Nigeria, pp 152-158

[47]. Kumar and Rajkumar (2005). Anti-diarrheal activity of tannins". Phtyochemistry. 68:22-24.

[48]. Neuwinger, H.D., 2000. African Traditional Medicine: A Dictionary of Plant use and Applications. Medpharm Scientific, Stuttgart, Germany, pp: 589-612.

[49]. Harvey, R. A. and Champe, P. C. (2009). Pharmacology, 4ed., Wolters Kluwer Pvt. Ltd, New Delhi, p. 338.