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PHARMACOGNOSTIC, PHYTOCHEMICAL AND ANTIULCER PROPERTIES OF ETHANOL CRUDE EXTRACT AND FRACTIONS OF THE LEAVES OF *PICRALIMA NITIDA* DURAND AND HOOK (APOCYNACEAE)

Bruce S. O.[1]*, Okoye C. L.[1], Orji C. E.[2], Ezeonyi E. I.[3] and Ezewudo E. M.[4]

[1]Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

[2]Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences. Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

[3]Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Chukwuemeka Odumegwu Ojukwu University.

[4]Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

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***Corresponding Author**
Bruce S. O.
Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

ASTRACT

Picralima nitida leaf is a rich source of alkaloids, tannins, flavonoids, and saponins. The leaf of *Picralima nitida* has been used ethanomedicinally used in the treatment of ulcers, diabetes, sexual impotence and hypertension. To evaluate the pharmacognostic properties and anti-ulcer activity of the ethanol crude extract and fractions of *Picralima nitida* leaves. The Pharmacognistic properties of the dry leaves were determined using standard method. The leaves were extracted using ethanol, the acute toxicity was determined. The crude ethanol extract was then fractionated using solvents of increasing polarity (n-Hexane, ethyl acetate, butanol and water) fraction. The qualitative and quantitative phytochemical constituents and the antiulcer evaluation were performed on ethanol induced in wister rats using standard method. *Picralima nitida* leaf shows the presence of saponins, terpenoids, Alkaloids, glycosides, flavonoids. The crude ethanol extract has high safety margin. A significant reduction in antiulcer activity was found in 500mg ethanol crude extract and butanol fraction when compared to the standard drug (Famotidine). The

Pharmacognostic properties can act as a reliable tool for standardization and quality evaluation of the plant. The ethanol extract and butanol fraction produce a significant reduction in the effect of ulcer in the rats. This claim confirms the ethnomedicinal use of the plant.

KEYWORDS: *Picralima nitida*, Pharmacognostic, Phytochemical, Acute toxicity (LD_{50}), Antiulcer.

INTRODUCTION

Peptic ulcers (PU) are sores or lesions in the gastrointestinal mucosa extending throughout the muscular mucosae, typically characterized by different stages of necrosis, neutrophil infiltration, blood flow reduction, increased oxidative stress and inflammation.[1] Peptic ulcers (PU) manifest as a non-fatal disease, majorly represented by periodic symptoms of epigastric pain, which are often relieved by food or alkali, besides to trigger much discomfort to patients, disrupting their daily routines and also causing mental agony.[2] The disease is mostly categorized based on its anatomical origins, such as gastric (found along the lesser curvature of the stomach) and duodenal (occurring in the duodenal bulb—the most exposed area to gastric acid) ulcers.[3] Studies have shown that peptic ulcer disease (PUD) occurs because of an imbalance between aggressive injurious (e.g., pepsin, HCl) and defensive mucosa-protective factors (e.g., prostaglandins, mucus and bicarbonate barrier and adequate blood flow).[4] All ulcers of the upper gastrointestinal tract were originally thought to be caused by the aggressive action of pepsin and gastric acid on mucosa. However, the denomination “peptic ulcer” has lately pointed to *Helicobacter pylori* infection, where the chronic use of non-steroidal anti-inflammatory drugs (NSAIDs) and acetylsalicylic acid (ASA) are some of the disease-causing factors. The most common symptom of a peptic ulcer is burning abdominal pain that extends from the navel to the chest, which can range from mild to severe. Other common signs of a peptic ulcer include: changes in appetite, nausea, bloody or dark stools (melena), unexplained weight loss, indigestion, and vomiting.[5] Maintaining a healthy lifestyle through a balanced diet rich in fruits, vegetables, and whole grains, quitting smoking and other tobacco use will also help in the prevention of peptic ulcer disease.[6]

Figure 1: Photo of *picralima nitida.*

Picralima nitida is the only species of the genus Picralima. It is related to Hunteria and Pleiocarpa. It belongs to the hunterieae tribe of the Apocynaceae family, and is commonly called Ose Igwe in Igbo, agege or obere in Yoruba and asewa or otun in Ibibio. In other parts of West Africa, the plant is called Gbe-Fon dangne in Benin Republic, Adangme in Ghana, Abure ebissi in Ivory Coast and Susu balunyi in Sierra Leone (Burkill, 1985). It is widely distributed in high deciduous forest of West-Central Africa, from Ivory Coast to West Cameroons, extending across the Congo basin and Uganda.[7,8] It grows to a height of 3.5m, up to 60cm in diameter with white latex in all parts. It belongs to the Apocynaceae family and is a single species of *Picralima.* It is restricted to Africa and widely distributed in Nigeria. The fruit is yellow to orange in colour, smooth, apex rounded, 11-20cm long and many seeded. The seeds are obliquely ovate, flattened 2.54.5cm long, smooth, brown to orange in colour and embedded in soft white to orange pulp.[9] Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments.[10] Previous work on the leaves, stem bark, fruits, seeds and pods of *Picralima nitida* revealed polyphenols, peptide, amide, ester, terpenoids, and indole, alkaloids; akuammine, akuammicine, akuammidine and akuammiline as major compounds. The seeds are rich in alkaloids (3.5-4.8%). The antimalarial activity of the plant was attributed to its content of alkaloids.[11] Extracts of various parts of the plant showed trypanocidal, hepatoprotective, antioxidant, antileishmanial, antiulcer, analgesic, antipyretic, anti-inflammatory,

antiplasmodial, and antimicrobial activities.[12] This study has led us to evaluate the Pharmacognostic, Phytochemical and Anti-Ulcer Properties of Ethanol Crude Extract and Fractions of the Leaves of *Picralima nitida* (Durand and Hook) (Apocynaceae).

MATERIALS AND METHODS

Apparatus and Equipment

The following Apparatus and equipment were used in the course of the study: Glass column, flasks, beakers, test tubes, measuring cylinders, rotary evaporator, Analytical Weighing Balance (Metler H30, Switzerland), Spectrophotometer (B. Bran Scientific &Instrument Company, England), Water Bath (Techmel & Techmel, Texas, USA), Appendoff tube, plain bottle, and Micropippete (Finnipipette® Labsystems, Finland).

Chemicals, Reagents and Drugs

The following chemicals, reagents and drugs were used: Ethanol (HD England), Famotidine (CP Pharmaceuticals, UK)

Plant materials

The plant part was collect in April, 2021 from the Herbarium, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University Awka. Anambra State, and authenticated by a taxonomist in the Department of botany by name Dr. Okafor Okonta.

Methods

Extraction and Fractionation Procedures

The fresh leaves of were washed in a running tap to remove dust and other debris, and air dried for two weeks. Dried part of *the plant* were pulverized with grinding machine and kept in clean air tight amber bottle. The powdered material (1000g) was cold macerated in 80% ethanol for 48 hours. The filtrate was recovered and concentrated to dryness using ‘water bath at 40^0C. The extract was stored in a refrigerator before use. The crude ethanol extract of *P. nitida* (15 g) was fractionated by adsorbing the crude extract on silica gel 60 g. Organic solvents of increasing polarity such as n-Hexane, ethyl acetate, butanol and aqueous were used as the mobile phase, to obtain the different fractions.[13]

Pharmacognostic studies

Microscopic examination

Microscopic studies were carried out by preparing thin sections of leaf. The thin sections were further washed with water, staining was done by clearing in chloral hydrate solution then heat fixed and allowed to cool, then mounted using glycerine. The specimen was gently covered with a cover slip and placed on the stage of the microscope for observation (40x).[14]

Chemomicroscopic examination

Examination of the powder for lignin, starch, mucilage, calcium oxalate crystals, cellulose, fatty oil and protein were carried out using standard techniques.[15]

Physicochemical analysis

The parameters which were studied are moisture content, ash values and extractive values.[16,17]

Phytochemical analysis

Qualitative phytochemical analysis

The plant crude extracts were tested for the presence Reducing sugar, Hydrogen cyanide, carbohydrate, Tannins, Alkaloids, Steroids, Terpenoids, Phenol, Flavonoids, Saponins and Glycosides using standard methods.[15]

Acute toxicity

The acute toxicity study of *Picralima nitida* was carried out according to the method employed by Lorke's method[18] but modified, using a total of 21 rats.[19,20]

Animal husbandry

Thirty Wistar Rats (120–150 g) were obtained from the animal house, Department of Pharmacology and Toxicology, Nnamdi Azikiwe University Awka. They were fed with grower mash (vital feed, grand cereal) and water and kept for 2 weeks to acclimatize with the animal house conditions (a cross-ventilated room with temperature between 25°C and 32°C, 12 h light/12 h dark cycle) before the commencement of the study. The research was conducted in accordance with the Nnamdi Azikiwe University Research and Ethical Committee guidelines, the ARRIVE guidelines (reporting of in vivo experiment), and the National Institutes of Health (NIH) guide for the CARE and use of laboratory animals (NIH Publications No. 8023, revised 1978).

Experimental design

The sixty (60) Wistar rats were randomly divided into twelve groups of five rats each and fasted for 18 h before administration of extract. The rats in Group 1 were pretreated with (Negative control) 1 ml/kg Ethanol, Group 2 were pretreated with (Positive control) 30 mg/kg Famotidine, Groups 3 and 4 were pretreated with Ethanol crude extract of *Picralima nitida* at 250 mg/kg and 500 mg/kg. Group 5 and 6 were pretreated with n-Hexane fraction of *Picralima nitida* at 250 mg/kg and 500 mg/kg, Group 7 and 8 were pretreated with Ethylacetate fraction of *Picralima nitida* at 250 mg/kg and 500 mg/kg, Group 9 and 10 were pretreated with Butanol fraction of *Picralima nitida* at 250 mg/kg and 500 mg/kg, lastly, Group 11 and 12 were pretreated with Aqueous fraction of *Picralima nitida* at 250 mg/kg and 500 mg/kg. All the rats were sacrificed 1 h after 80% ethanol administration using ketamine injection, and the stomachs were cut open, the stomach content were deposited in a beaker, the ulcer index was determined. The gastric content was collected in test tube and centrifuged at 3000 rpm for 10 min. The pH of the supernatant was measured using digital pH meter. The volume of supernatant was measured and expressed as ml/100 g body weight.[21,22,23]

Histopathology procedure

The stomachs were fixed in 10% neutral buffered formalin, dehydrated in graded series of alcohol, cleared in xylene, and embedded in paraffin wax. The tissues were sectioned at 5 µm with a rotary microtome and stained with hematoxylin and eosin (H and E) and cresyl violet stain.[24]

Statistical analysis

Data obtained from the study were analyzed using Statistical Package for Social Sciences (SPSS-21). Results were presented as mean ± Standard error of mean (SEM) of sample replicates. Raw data were subjected to one way analyses of variance (ANOVA) followed by post hoc turkey's test. $p<0.05$ were considered to be statistically significant.

RESULTS

Powder microscopy

Table 1: Powder microscopy of the leaves of *picralima nitida.*

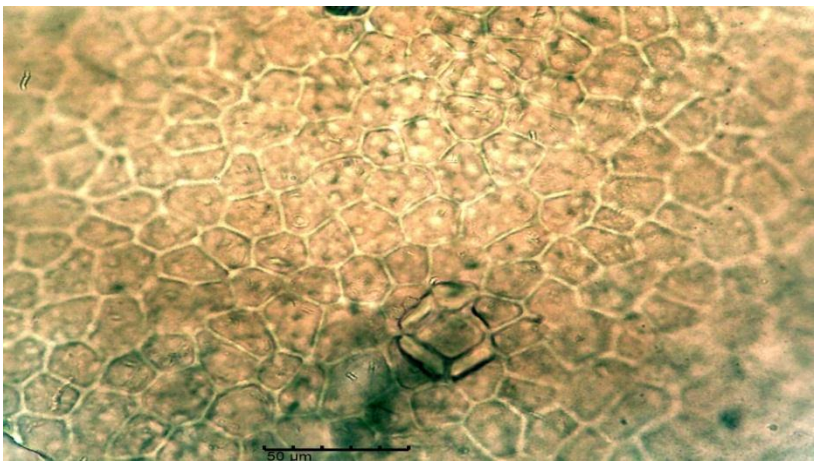
Parameter	leaf
Starch grains	Present
Lignified tissues	Present
Calcium oxalates	Present; Prism shaped
Cystolith	Absent

Tannin	Present
Cellulose	Present
Gum/Mucilage	Absent
Protein	Present
Oil globules	Not prominent

Table 2: Chemomicroscopic examination.

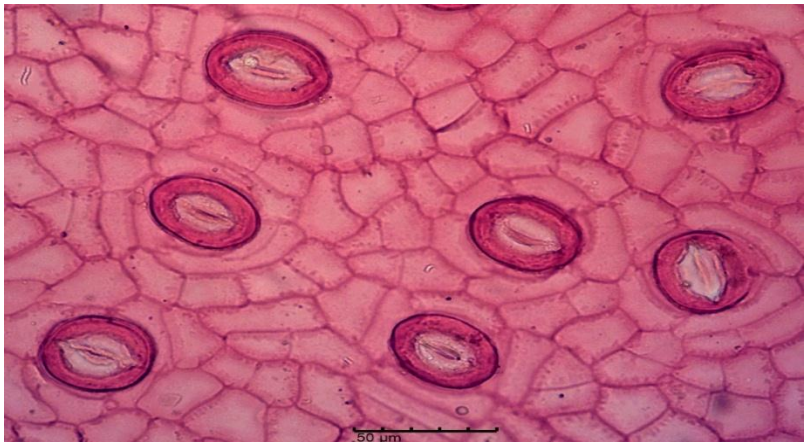
Test reagent	Observation	Inference
Sample + phloroglucinol + conc hcl	Red colour observed	Lignin present
Sample + iodine	Blue colour observed	Starch granules present
Sample + hydrochloric acid	Crystals dissolved	Calcium oxalate crystals present
Sample + ruthenium red	No colour change	Mucillage absent
Sample + clor-zinc iodine or n/50 idoine + 66% h2so4	Blue colour observed	Cellulose present
Sample + sudan iv reagent	Pink colour observed	Fatty acid present
Sample + 1% picric acid and millions reagent	Red colour observed	Protein present

Picralima nitida



x40

Figure 2: Adaxial (upper) surface of the leaf of *Picralima* showing polygonally-shaped epidermal cells with straight anticlinal walls. Both stomata and trichomes are absent.



x40

Figure 3: Abaxial (lower) surface of the leaf of *Picralima* showing polygonally-shaped epidermal cells with straight anticlinal walls. Stomata are present and are of the paracytic type (a pair of subsidiary cells lies parallel to the guard cells). Trichomes are absent.

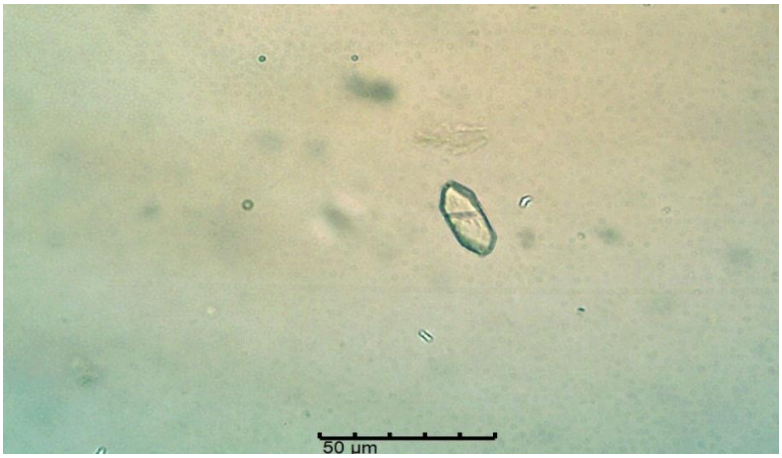


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Figure 4: Prism-shaped calcium oxalate crystal as seen in the powder microscopy.

x40

Figure 5: Chemomicroscopy of the powder showing reticulate type of vessel elements aligned with fibre and parenchyma cells.

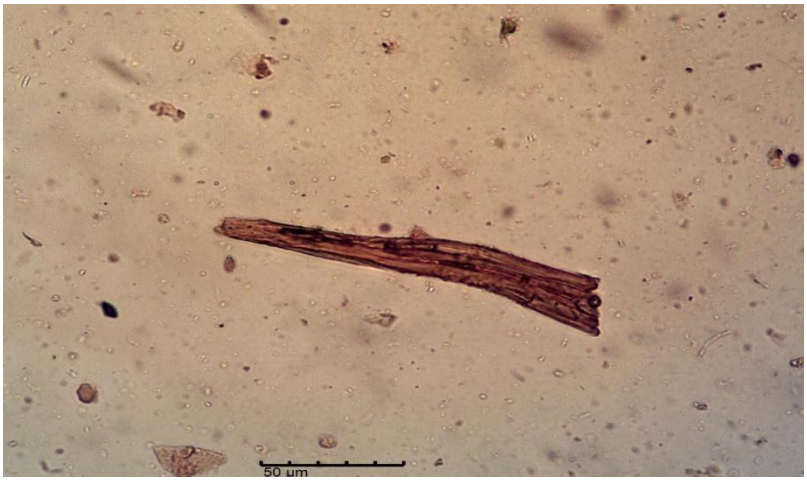

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Figure 6: Chemomicroscopy of the leaf powder showing lignified Tissue and Scattered starch granules.

Proximate analysis

Table 3: Proximate analysis of the leaves of *picralima nitida.*

Parameter	Mean ±SEM (% w/w)
Moisture content	5.34 ±0.010
Total ash	2.235± 0.025
Acid – insoluble ash	1.34 ± 0.00
Water – soluble ash	0.575 ± 0.01
Alcohol – soluble extractive	10.01 ± 0.01
Water – soluble extractive	8.48 ± 0.04

Values of % composition shown are mean ± SEM

Phytochemical analysis

Table 4: Phytochemical analysis of the leaves of *picralima nitida.*

	Phyto-constituents	Ethanol Crude extract	n-Hexane fraction	Ethyl acetate fraction	Butanol fraction	Aqueous fraction
1	Saponins	+++	+++	+++	+	++
2	Tannins	+++	++	+++	++	+
3	Carbohydrates	++	+	++	+	+
4	Reducing Sugars	+	+	+	+	+
5	Flavonoids	+++	+++	+++	+	+
7	Alkaloids	++	++	++	+	+
8	Glycosides	++	++	++	-	-
9	Steroids	+	+	+	+	+
10	Fats and oils	++	+	+	+	-
11	Proteins	++	+	-	-	-
12	Hydrogen cyanide	-	-	-	-	-

(-) => Not Present, (+) => Present in small concentration, (++) => Present in moderately high concentration, (+++) => Present in high concentration.

Acute toxicity

Table 5: Acute toxicity study of the leaves of *Picralima nitida* (LORKES, 1983).

Phases	Dose (mg/ kg)	Mortality
Phase 1	10	0/3
	100	0/3
	1000	0/3
Phase 2	2000	0/1
	3000	0/1
	4000	0/1
	5000	0/1

Antiulcer activity

Table 6: Dose dependent studies of *Picralima nitida* crude Extract and Fractions using ethanol induced ulcer model rat model.

Treatment	Dose	Ulcer Index	Total Acidity (m Eq/L)	Acid Volume (ml)	pH
(Control) Distilled water	(1ml)	11.05±0.10	117.1±1.15	7.57±0.34	2.2±0.20
Famotidine	30 mg/kg	4.70±0.72**	57.5±0.52**	4.13±0.40**	4.9±0.16**
Ethanol extract	250mg/kg	8.55±0.28*	87.5±0.22*	6.02±0.31*	3.44±3.05*
Ethanol extract	500mg/kg	4.65±0.1*	63.3±0.21*	4.99±0.04**	4.02±3.33**
n-Hexane fraction	250mg/kg	8.91±0.04*	83.2±0.51*	6.09±0.09*	3.27±4.77**
n-Hexane fraction	500mg/kg	4.92±0.13*	70.3±0.21*	5.05±0.08**	3.99±4.22**
Ethyl acetate fraction	250mg/kg	9.56±0.13*	85.2±0.31*	6.11±0.01**	3.20±3.14**
Ethyl acetate fraction	500mg/kg	5.62±0.31*	64.6±0.44*	6.00±0.71**	3.09±3.13**
Butanol fraction	250mg/kg	8.31±0.45*	81.7±0.44*	5.01±0.02**	3.64±2.70**
Butanol fraction	500mg/kg	4.00±0.1*	50.1±0.51*	4.02±0.31**	4.60±3.11**
Aqueous fraction	250mg/kg	10.11±0.30*	89.5±0.22*	7.02±0.04**	2.86±4.97**
Aqueous fraction	500mg/kg	9.00±0.9*	86.0±0.00*	6.86±0.09**	3.02±3.41**

Values are presented as mean ± Standard error of mean (SEM), n =5.

Histology of the stomach of the ethanol - induced ulcer in wistar rats

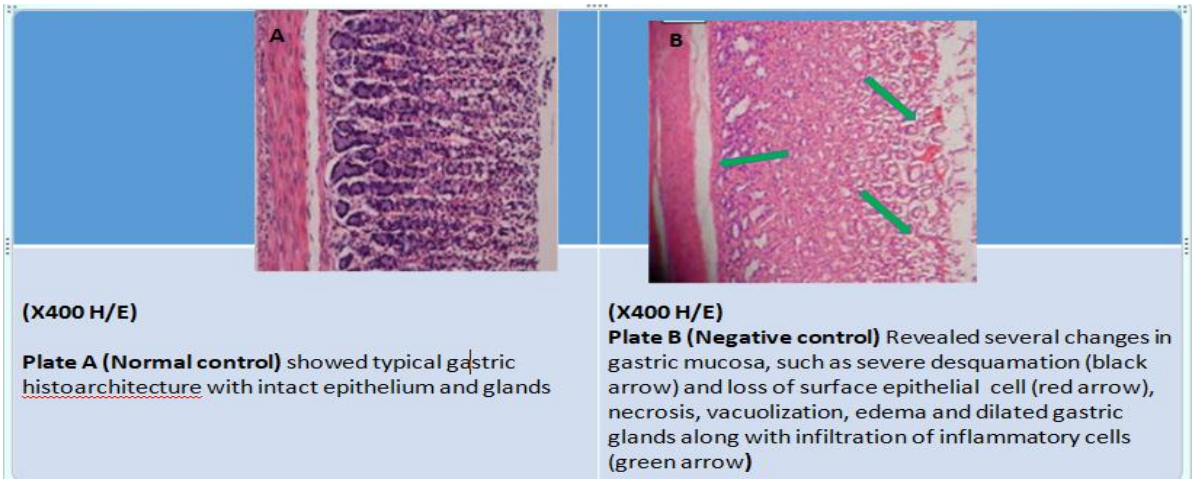

Figure 7: Plate A and B.

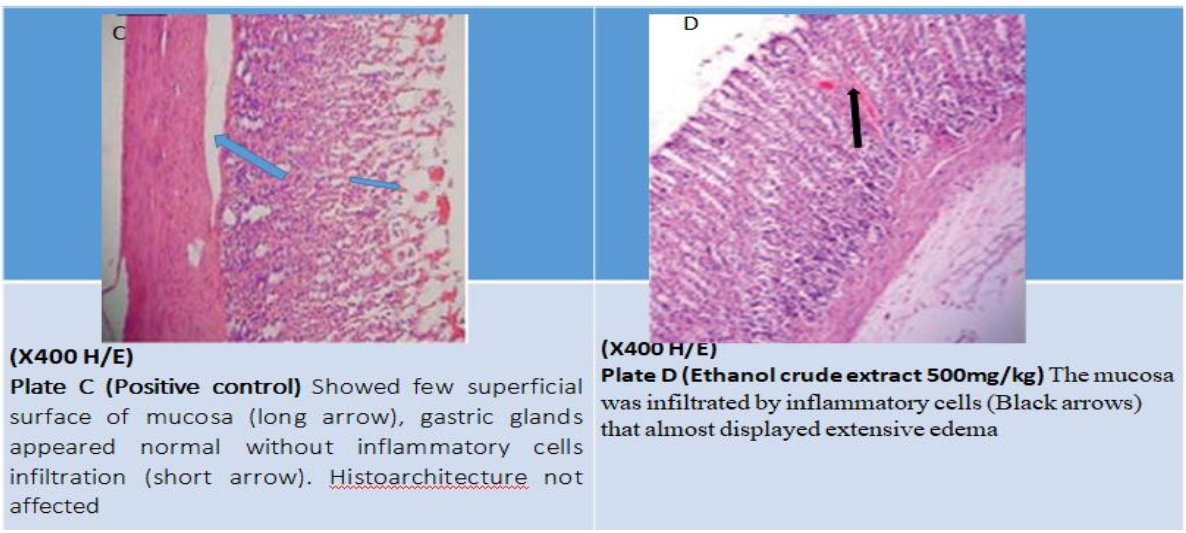

Figure 8: Plate C and D.

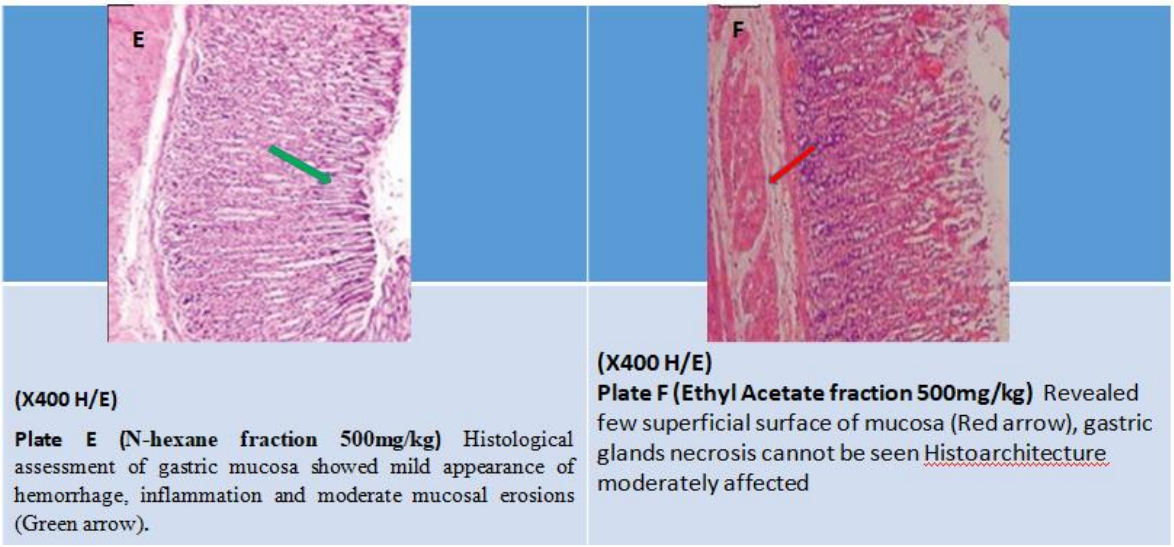

Figure 9: Plate E and F.

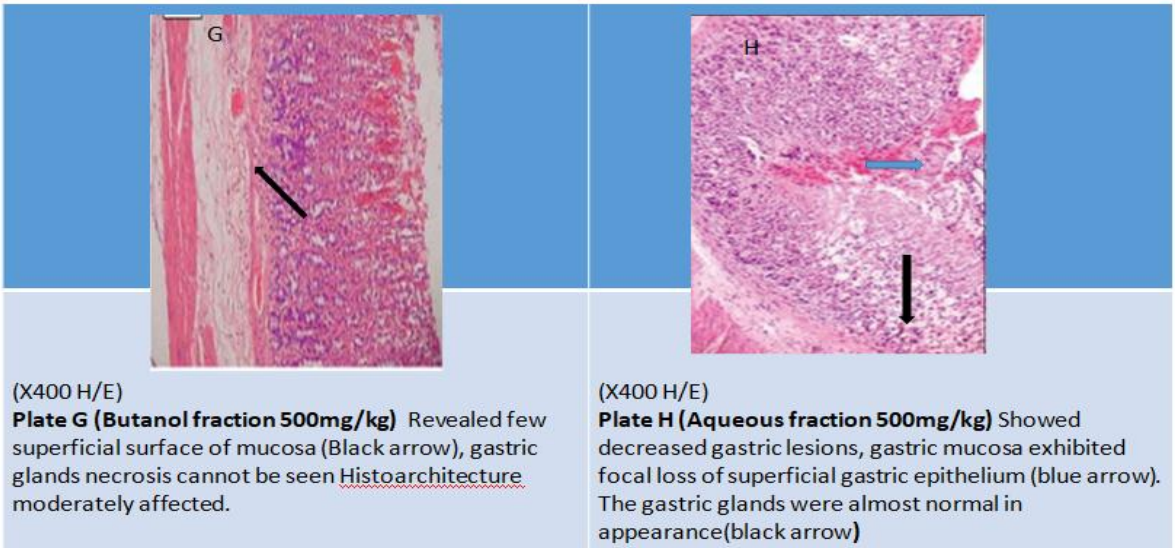

Figure 10: Plate G and H.

DISCUSSION

Peptic ulcer is one of the fastest growing disease in the world, despite many advances in the therapeutic management of gastric ulcer, the prevalence of this disease is still high (Sharath *et al,* 2015). Microscopic and pharmacognostic standardization helps in the identification and authentication of the genuine plant materials, the presence of starch grains, lignified tissues, cellulose, proteins, and oil globules, as shown in tables 1. The microscopy was carried out on the leaf and was found to possess stomata but with absence of trichomes. The pulverized form was found to show the presence of calcium oxalate crystals, and also lignified tissue with scattered starch granules. The adaxial (upper) surface of the leaf of *Picralima nitida* showed polygonally-shaped epidermal cells with straight anticlinal walls and both stomata and trichomes were absent. The abaxial (lower) surface of the leaf *Picralima nitida* showed polygonally shaped epidermal cells with straight anticlinal walls. Stomata are present and are of the paracytic type (a pair of subsidiary cells lies parallel to the guard cells). Trichomes were absent.

The chemomicroscopy of the powder showed reticulate type of vessel elements aligned with fibre and parenchyma cells. The presence of lignin, starch granules, calcium oxalate crystals, cellulose, fatty acid and proteins, the absence of mucilage and trichomes as shown in table 2. The leaf epidermal features which include types of stomata, epidermal cells, and hairs are significant tools in delimiting the taxonomy in many plants.[8,25,26,27] Ahmad et al.[28] reported variations in the pattern of the epidermal cells that can be used as an important microscopic tool to identify many closely related species.

The proximate composition of *Picralima nitida* leaf extract contains total ash value of 2.2%, acid-insoluble ash of 1.34% water-soluble ash of 0.5%, moisture content value of 5.37%, alcohol soluble extractive value of 10.01% and water soluble extractive value of 8.48% as shown in table 3. The proximate analysis is used to determine the purity of the plant specimen in relation to the standard values. Low moisture content tends to obstruct or prevent microbial contamination and chemical degradation.[25] The growth of microorganism (yeast and fungi) is enhanced by high moisture content of crude drugs, causing the breakdown of crucial bioactive compounds. The high value of ash is due to contamination and the presence of impurities.[29] The extractive values are useful to evaluate the chemical constituents present in the crude drugs and also help in estimation of specific constituents soluble in estimation of specific constituents soluble in a particular solvent.[12] As compared with previous studies of the proximate composition of *Picralima nitida* seed extract contains moisture content 1.2 %, total ash 4 %, acid insoluble ash 1%, water soluble ash 3.5%, water soluble extractive value 2.8 % and crude fibre 3.6 %, while the *Picralima nitida* pod extract contains moisture content 3.8 %, total ash 5 %, acid insoluble ash 1%, water soluble ash 3.5 %, water soluble extractive value 3.8 % and crude fibre 5.2 %.[8] Estimation of extractive values determines the amount of the active constituents in a given amount of the active constituents in a given amount of plant material when extracted with a particular solvent yield a solution containing different phytoconstituents. Pharmacognostic and physicochemical studies of the plant parts act as a reliable tool for detecting adulteration and plant identification.[30,31,32]

Phytochemical test was carried out on the ethanol crude extract as well as the individual solvent fractions, as shown in table 4. The phytochemical screening of ethanol crude extract of the leaf of *Picralima nitida* showed the presence of various chemical constitutions mostly saponins, tannins, carbohydrates, flavanoids, alkaloids, glycosides, reducing sugar, proteins, fats and oils are conspicuously present in large amount and hydrogen cyanide was absent. The n-Hexane fraction contains saponins, tannins, flavonoids, alkaloids and glycosides in large quantity but hydrogen cyanide was absent. The ethyl acetate fraction possesses saponins, tannins, carbohydrate, flavonoids, alkaloids and glycosides in large amount, protein and hydrogen cyanide were absent. The butanol fraction possesses steroids and tannins in large quantity but glycosides, protein and hydrogen cyanide were absent. Lastly, the aqueous fraction possesses steroids in large quantity but glycosides, fats and oils, protein and hydrogen cyanide were absent. Therefore, the phytochemical analysis of the ethanol crude extract and the fractions revealed the presence of glycosides, saponins, terpenoids, alkaloids,

tannins. These secondary metabolites have been reported to have anti-ulcer activity.[33] Many photochemical studies have shown that plant compounds posses an important role in the prevention of gastric ulcer.[34] The composition of these phytoconstituents depends upon the nature of the drugs and the solvent used. It also gives an induction whether the crude drug is extracted or not.[35]

Despite many advances in the therapeutic management of gastric ulcers, the prevalence of this disease is still high. Many phytochemical studies have shown that plant compounds possess an important role in the prevention of gastric ulcer.[36]

The acute toxicity test being carried out showed that the ethanol leaf extract of *Picralima nitida* was highly safe having a high safety margin when compared to the standard range when given orally and this can be seen in the table 5. No deaths were recorded after 24 hours of administration of the various doses (10, 100 and 1000 mg/kg body weight) of the ethanol extract of *Picralima nitida*. In the second stage, three dose ranges were also used 2000, 3000, 4000 and 5000 mg/kg body weight and there was no death after 24 hours. The LD_{50} was determined as 5000mg/kg.[25]

Ethanol – induced gastric ulcer is a common animal model to investigate the anti-ulcer drugs. Administration of ethanol causes gastric necrotic damage and subsequent inflammatory cell infiltration and reduces the secretion of bicarbonate, gastric mucus and nitric oxide. In addition, ethanol reduces the gastric blood flow and induces the oxidative stress by increasing the production of malondialdehyde and reducing glutathione production (Sharath *et al,* 2015).

Histological examination of the stomach of rats pretreated with negative control revealed several changes in gastric mucosa, such as severe desquamation (black arrow) and loss of surface epithelial cell (red arrow), necrosis, vacuolization, edema and dilated gastric glands along with infiltration of inflammatory cells (green arrow**)**. The stomach of rats pretreated with the positive control (Famotide) revealed few superficial surface of mucosa (long arrow), gastric glands appeared normal without inflammatory cells infiltration (short arrow). Histoarchitecture not affected. The stomach of rats pretreated with *P. nitida* ethanol crude extract (500 mg/kg) showed the mucosa was infiltrated by inflammatory cells (Black arrows) that almost displayed extensive edema. The stomach of rats pretreated with *P. nitida* n-Hexane fraction (500 mg/kg) showed mild appearance of hemorrhage, inflammation and moderate mucosal erosions (Green arrow). The stomach of rats pretreated with *P. nitida*

ethylacetate fraction (500 mg/kg) revealed few superficial surfaces of mucosa (Red arrow), gastric glands necrosis cannot be seen, histoarchitecture moderately affected. The stomach of rats pretreated with *P. nitida* butanol fraction (500 mg/kg) revealed few superficial surfaces of mucosa (black arrow), gastric glands necrosis cannot be seen, histoarchitecture moderately affected. The stomach of rats pretreated with *P. nitida* aqueous fraction (500 mg/kg) showed decreased gastric lesions, gastric mucosa exhibited focal loss of superficial gastric epithelium (blue arrow). The gastric glands were almost normal in appearance (black arrow**).** The healing process of gastric ulcer including several processes in gastric mucous e.g. congestive, hemorrhagic, edema, necrosis, inflammation, erosion, ulceration and dysplastic change.[56] Evaluation for the healing process in the clinical setting was based on visual endoscopy, but this study was based on microscopic evaluation and gastric ulcer determination. Microscopic evaluation showed gastric glands dilatation, increase of connective tissue, increase of micro vascularization, and recovery of a sensory nerve. It could be the basis for evaluating the quality of the healing process of gastric ulcers.[57,58]

High concentrations of ethanol induce vascular endothelium injury of the gastric mucosa, which become edematous, and congestive, present point and scattered bleeding lesions, focal hemorrhage, necrosis, and giant deep ulcers were visible. The *Picralima nitida* crude extract and fractions showed a significant reduction ($p < 0.05$) in ulcer index, total acidity, Gastric acid volume, and pH of gastric secretion, when compared with the negative control Distilled water (1 ml/kg) as shown in Table 6. The *Picralima nitida* crude extract and fractions also decreases the severity and incidence of gastric erosions in ethanol treated rats. For ulcer index, the rats pretreated with Famotidine (4.70±0.72) and *P. nitida* ethanol extract, n-Hexane, Ethyl acetate, butanol and aqueous fractions at 500mg/kg, showed a significantly reduction in ulcer index (4.65±0.1, 4.92±0.13, 5.62±0.31, 4.00±0.1, 9.00±0.9) than the rats pretreated with the negative control Distilled water (1 ml/kg) (11.05±0.10) at $p < 0.05$. For total acidity, the rats pretreated with Famotidine (57.5±0.52) and *P. nitida* ethanol extract, n-Hexane, Ethyl acetate, butanol and aqueous fractions at 500mg/kg, showed a significantly reduction in total acidity (87.5±0.22, 83.2±0.51, 85.2±0.31, , 81.7±0.44, 89.5±0.22) than the rats pretreated with the negative control Distilled water (1 ml/kg) (117.1±1.15) at $p < 0.05$. For acid volume, the rats pretreated with Famotidine (4.13±0.40) and *P. nitida* ethanol extract, n-Hexane, Ethyl acetate, butanol and aqueous fractions at 500mg/kg, showed a significantly reduction in acid volume (4.99±0.04, 5.05±0.08, 6.00±0.71, 4.02±0.31, 6.86±0.09) than the rats pretreated with the negative control Distilled water (1 ml/kg)

(7.57±0.34) at $p < 0.05$. For pH of gastric secretion, the rats pretreated with Famotidine (4.9±0.16) and *P. nitida* ethanol extract, n-Hexane, Ethyl acetate, butanol and aqueous fractions at 500mg/kg, showed a significantly reduction in acid volume (4.02±3.33, 3.99±4.22,3. 64±2.70, 4.60±3.11, 3.09±3.13) than the rats pretreated with the negative control Distilled water (1 ml/kg) (2.2±0.20) at $p < 0.05$, as shown in table 6.

Ethanol-induced gastric ulcer is a common animal model to investigate the new anti-ulcer drugs. Administration of ethanol causes gastric necrotic damage and subsequent inflammatory cell infiltration and reduces the secretion of bicarbonate, gastric mucus, and nitric oxide. In addition, ethanol reduces the gastric blood flow and induces the oxidative stress by increasing the production of malondialdehyde and reducing glutathione production.[36] The etiology of peptic ulcer is unknown in most of the cases, yet it is generally accepted that it results from an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defense mechanisms.[37] To regain the balance, different therapeutic agents are used to inhibit the gastric acid secretion or to boost the mucosal defense mechanisms by increasing mucosal production, stabilizing the surface epithelial cells, or interfering with the prostaglandin synthesis.[38] The ability of the gastric mucosa to resist injury by endogenous secretions (acid, pepsin, and bile) and ingested irritants (eg, alcohol, non -steroidal anti-inflammatory drugs [NSAIDs]) can be attributed to a number of factors that have been collectively referred to as “mucosal defense.[39] The concept of gastric cytoprotection against various necrotizing agents has been routinely used to assess the anti-ulcer potential of different compounds. The ethanol-induced acute gastric mucosal injury model is considered to be one of the widely used experimental models of ulcer disease.[40] Ethanol easily penetrates the gastric mucosa and causes gastric ulcer 1 hour after administration. Ulceration is due to a decrease in gastric mucus, prostaglandin levels, glutathione, mucosal blood flow, and bicarbonate secretion as well as an increase in lipid peroxidation, oxidative stress, leukotriene production, and generation of free radicals leading to cell and membrane damage. It has been reported that leukotriene antagonist and 5-lipoxygenase inhibitors are capable of inhibiting alcohol and NSAID-induced gastric ulceration in rats.[40] In the present study, the ethanol-induced model was employed to confirm the gastric cytoprotective effect of the plant extract. This finding signifies that the extract possesses a gastroprotective effect, which is as good as the standard drug.

CONCLUSION

Microscopic and pharmacognostic properties help in the identification and authentication as a reliable tool for the standardization and quality evaluation of the plant part. This study indicates that the plant *Picralima nitida* is a safe plant having carried out the acute toxicity and found to have a high safety margin. The anti-ulcerogenic properties exhibited by the plant extract and fractions can be ascertained that at 500mg/kg it showed a good anti-ulcer activity as compared with the standard drug 30mg/kg Famotidine statistically. Thus, the present work validates the use of *Picralima nitida* for gastric ulcer in the Nigeria folk medicine.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

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