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Pharmacognostic, Antimicrobial and hepatoprotective activities of the sub-fractions of *Picralima nitida* (Durand and Hook) (APOCYNACEAE) seeds

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ABSTRACT

Picralima nitida seed is used ethno medicinally for treating malaria, diarrhea, liver disease and inflammation. The purpose of this study is to evaluate the antimicrobial and hepatoprotective activities of the sub-fractions of the ethanol extract of *Picralima nitida* seeds. The ethyl acetate fraction was subjected to vacuum liquid chromatography (VLC) using nHexane/ ethyl acetate and ethyl acetate/ methanol. The sub-fractions were subjected to *in vitro* antimicrobial screening using Agar-well diffusion assay and hepatoprotective activity against paracetamol induced hepatotoxicity in albino rats. The antimicrobial screening showed that the most effective VLC fractions at 50mg/ml are ethyl acetate: methanol (6:4) and ethyl acetate: methanol (9:1). Ethyl acetate: methanol (6:4) inhibited both bacterial and fungal isolates *S. aureus* 3mm, *B. subtilis*2mm, *E. coli* 6.5mm, *P. aeruginosa* 4.5mm, *A. niger* 2mm, *C. albican* 3mm; while ethyl acetate: methanol (9:1) inhibited the growth of only the bacterial (*S. aureus* 2.5mm, *B. subtilis* 12mm, *E. coli* 6mm, *P. aeruginosa* 4mm). The most effective VLC sub-fractions with hepatoprotective activity are ethyl acetate: methanol (6:4) and ethyl acetate: methanol (9:1), which showed percentage inhibition very similar to that of the positive control (silymarin). This study showed that *P. nitida* seeds have antimicrobial activity which may be useful against opportunistic infections. The plant seeds also have hepatoprotective activity which is useful in managing liver disease and hepatic injury. Thus, this study supports the ethno medicinal uses of *Picralima nitida* seeds in treatment of infections and liver disease.

Key words: *Picralima nitida*; Phytochemical; Antimicrobial; Antibacterial; Antifungal; Hepatoprotective

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INTRODUCTION

Medicinal plants have been acknowledged and are extremely valued all over the world as a prosperous source of bioactive for the prevention and treatment of ailments. Herbal medicines are being used by about 80% of the world population primarily in the developing countries for primary health care and they have stood the test of time for their safety, efficacy, cultural acceptability and minimal side effects (Klaassen *et al*., 1991).*Picralima nitida* is one of the herbs used as medicine in Nigeria.

P. nitida is a topical small bushy tree with White latex in all part, hard bark, brittle, pale to dark grayish black or brown. It is a tree of about 15 m high and with circumference of about 50 cm, crown dense, trunk 560 m diameter; cylindrical, the wood is pale yellow, hard, elastic, fine- grained and taking a high polish. *P. nitida* bears white flowers about 3cm long with ovoid fruits which at maturity are yellowish in colour. The leaves are broad (3 - 10 cm) and oblong (6 - 20 cm long) with tough tiny lateral nerves of about 14 to 24 pairs (Meyer *et al*., 2006).

In Nigeria, *P. nitida* is known as Akuamma plant (local name), Ose-Igwe (Igbo), Abere (Yoruba), Asewa (Ibibio dialect). Elsewhere in West Africa, the plant is called Gbe-Fon dangne (Benin Republic), Adangme (Ghana), Abure ebissi (Ivory Coast) and Susu balunyi (Sierra Leone) (Burkill, 1985; Bruce *et al*., 2016)

Throughout its distribution area the seeds, bark and roots of *Picralima nitida* have a reputation as a febrifuge and remedy for malaria. They are also extensively used for pain relief and to treat chest and stomach problems, pneumonia and intestinal worms. Usually, the seeds or bark are crushed or chewed and eaten for this purpose or a decoction from the roots, seeds or bark is drunk.

In Ghana and DR Congo, immature fruits are pounded and thrown in the water as a fish poison. The wood, called ebam in trade, is used to make a variety of small utensils, e.g. paddles, shuttles for weaving, dolls, combs, walking sticks, pestles and mortars, incense holders, bows and arrows, spade handles or spoons. Spoons or dippers are also made of the hard shell of the fruit (Neuwinger, 1996).

The pharmacognostic standards of *Picralima nitida* could act as a reference point or baseline data on this plant for possible inclusion in the Pharmacopoeia. Microbial infection is a global health issue with a high mortality rate when not properly treated, therefore antimicrobial treatment has posed resistant against microbial infections. In the treatment and management of liver diseases many conventional drugs used, have serious adverse effects and are not easily accessible, and available, this has led to the search for herbal antimicrobial and hepatoprotective treatments. Therefore, this study focuses on the pharmacognostic, antimicrobial and hepatoprotective activity of the VLC sub-fraction of *P. nitida* seed extract.

MATERIALS AND METHODS

Hot air oven (Genlab, UK), Electronic weighing balance (Ohaus Corp, USA), water bath (Serological, England), beakers (Pyrex;10 ml, 50 ml, 100 ml, 1000 ml), measuring cylinders, Hand grinding machine (Ohaus Corp, USA), Syringes and needles (1 ml, 2 ml and 10 ml capacity), Refrigerator (Thermocool, England), Cotton wool (Pyrex), Microscope, UV-Vis Spectrophotometer (Shanghai, China), Sephadex LH-20, Autoclave, Hot Air Oven, Syringes, Incubator, Electronic weighing balance, thermostatic water bath, micropipette, Cork borer, Petri plates, Freezer, Distilled water (NBC, Nigeria), Ethanol (JHD, China), Concentrated sulphuric acid, naphthol solution in ethanol (Molisch reagents), Ammonium solution, Aluminium chloride, Fehling solution A and B, Hager's reagent (saturated solution of picric acid), Wagner's reagent (iodine and potassium iodide), nHexane, Ethyl acetate, Butanol, Ferric chloride, Chloroform, epinephrine, carbonate buffer, thiobarbituric acid, sodium hydroxide, glacial acetic acid (JHD, China), hydrogen peroxide (Fine Chemicals Industries, Nigeria), phosphate buffer, dichromate acetic reagent (JHD, China).

Collection and authentication of plants materials: The plant material *P. nitida* seeds were collected from Eke Awka market in Anambra State and authenticated with the assistance of a plant taxonomist. Voucher specimens of the plant material were deposited at the Department of Pharmacognosy and Traditional Medicine, Nnamdi Azikiwe University Awka, Nigeria with Herbarium number PCG 474/A/025

Preparation of the plant materials, extraction and activity screening: Powdered plant seeds (3.2kg) were extracted with 5L of ethanol by cold maceration at room temperature for 48hr. The extract was concentrated to dryness using a rotary evaporator at reduced pressure. The concentrated extract was weighed and stored in an air-tight container and kept in the refrigerator at 4^{o}C.

Phytochemical analysis

Qualitative Phytochemical Screening: The crude extract of *P. nitida* seeds was screened for the presence of alkaloids, flavonoids, tannins,

saponins, cardiac glycosides, terpenoids, steroids, protein and carbohydrate using standard phytochemical methods (Sofowara,1993, Onyegbule *et al*., 2019, Onyegbule *et a*l., 2020).

Test for alkaloids: About 20ml of 30% sulphuric acid in 50% ethanol was added to 2g of the extract and heated on boiling water for 10 minis, cooled and filtered. 2 ml of the filtrate was tested with a few drops of Mayer's reagent (potassium mercuric iodide solution (1%). The remaining filtrate was placed in 100ml separator funnel and made alkaline with dilute ammonia solution. The aqueous alkaline solution was separated and the extract was tested with few drops of Mayer's, Wagner's, Dragendoff's reagent and picric acid solution. Alkaloids give milky precipitate with Mayer's reagent and reddish-brown precipitate with the other indicator.

Test for Saponins: About 20 ml of distilled water was added to 0.25g of the extract and boiled on a hot water bath for two minutes. The mixture was filtered while hot and allowed to cool and filtrate was used for the following test.

Emulsion test: About 5 ml of the filtrate was diluted with 15 ml distilled water and was shaken vigorously. The formation of emulsion indicates the presence of saponin.

Test for tannins

About 1 g of the powdered material was boiled with 20 ml of water, filtered and used for the following test.

Ferric chloride Test: To 3 ml of the filtrate few drops of Ferric chloride were added. A greenish black precipitate indicated the presence of tannins.

Test for flavonoids: About 10 ml of ethyl acetate was added to 0.2g of the extract and heated on water bath for 3 minutes. The mixture was cooled, filtered and used for the following test.

Test for steroids and triterpenoids: About 9 ml of ethanol was added to 1 g of the extract it was refluxed for a few minutes and filtered. The filtrate was concentrated on a boiling water bath. 5 ml of hot distilled water was added to the concentrated solution, the mixture was allowed to stand for 1 hour and the waxy matter was filtered off. The filtrate was extracted with 2.5 ml of chloroform using separating funnel. To 0.5 ml of the chloroform extract in a test tube was carefully added 1 ml of concentrated H_2SO_4 to form a lower layer. A reddish-brown interface shows the presence of steroids. 0.5 ml of the chloroform was evaporated to dryness on a water bath and heated with 3 ml of the concentrated sulphuric acid for 10 minutes on a water bath. A grey colour indicates the presence of terpenoids.

Tests for carbohydrate

Molisch's Test: Two drops of α-naphthol solution were added to 2 ml of the sample. A drop wise concentration of H_2SO_4 was added to the inclined test tube containing the mixture. The appearance of violet colour indicates the presence of carbohydrate.

Test for protein: Xanthoproteic reaction test: 5 ml volume of the filtrate obtained from boiling few grams of powdered plant was heated with few drops of concentrated nitric acid; Yellow colour that changes to orange on addition of alkali indicates the presence of protein.

Quantitative Phytochemical Analysis: The coarse powder of the plant material was subjected to quantitative estimation to determine the quantity of alkaloids, flavonoids, saponins and tannins present.

Alkaloids Determination: Five grams of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 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 until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Flavonoids Determination: Ten grams of the plant sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper. 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 samples were ground and 20 g of each were put into a conical flask and 100 cm^3 of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55^o**c**. the mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40ml over water bath at both 90^o**c**. the concentrate was transferred into 250 ml separated funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice

with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight; the saponin content was calculated as percentage.

Tannins Determination: Weigh 500 mg of powdered sample into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtrate was pipette out into a test tube and mixed with 2 ml of 0.1 M FeCl in 0.1 N HCL and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10min.

Fractionation of the crude extracts: The crude ethanol extracts were absorbed in silica gel and sequentially fractionated using nHexane, ethyl acetate, butanol, and water in increasing order of polarity. The fractions so obtained were filtered twice using Whatman filter paper. A rotary evaporator was used to concentrate the fractions at 45^0**c**. The fractions obtained were stored at 4^0**c** (Jean *et al*., 2001, Onyegbule *et al*., 2019, Bruce *et al*., 2021).

Vacuum Liquid Chromatography (VLC): VLC was carried out according to standard method (AOAC, 2005). The adsorbent is applied dry into a sintered glass funnel. The sample is applied by dry method; 5g of ethyl acetate fraction was thoroughly mixed with a silica gel 60F254 using mortar and pestle, till the whole fraction was properly absorbed by the gel. A 10.24g of the fraction mixed with silica gel were then packed onto the top of the column, then the mobile phase is added portion by portion and vacuum is applied after each portion to collect each fraction. The lower part of the vertical column is connected to the vacuum pump to generate a vacuum condition. The column was ran using n- hexane: ethyl acetate and ethyl acetate: methanol at varying ratios of 10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9, 0:10 (500:0, 450:50, 400:100, 350:150, 300:200, 250: 250, 200:300, 150:350, 100:400, 50:450 and 0:500), which was quickly passed through the column by vacuum condition and the successive fractions were collected.

Thin Layer Chromatography (TLC): TLC plates were first activated by placing the plates in the oven at 1200c for 30 minutes. With the aid of a pencil, a faint line (starting line) was drawn 1cm from the edge of one end of the TLC plate. The solvent line was also drawn on the other end of the plate, 7cm from the starting line. Using a capillary tube, a spot of the different solvent ratios of nHexane and ethyl acetate and ethyl acetate and methanol (10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9, 0:10) were made on the starting line. The solvent system nHexane and ethyl acetate (6:4) were measured into a solvent tank and the plate carefully placed in the solvent tank with the edge of the spotted end touching the solvent. The solvent tank covered with a glass and monitored (Touchstone, 1983). When the mobile phase got to the solvent line, the plate was removed from the tank and allowed to dry. After drying, the spots observed under UV lamp at 254 nm and were circled with a pencil. The plate was then place in the fume cupboard; where it was sprayed with 12% H_2SO_4 was noted (Onyemailu et al., 2021).

Pharmacognostic Properties of *P. nitida* Seeds

Macroscopic Examination: The macroscopic features of the seed of *P. nitida* were examined using physical examination which showed the shape, taste, colour and texture (Evans, 1996, Ihekwereme *et al*., 2020, Bruce *et al*., 2016).

Microscopic Examination: The powdered seed of *P. nitida* were prepared for microscopic studies. The staining was done using standard laboratory method, by clearing in chloral hydrate solution, heat and allowed to cool, then mounted using glycerin. The specimen was gently covered with a cover slip and placed on the stage of the microscope for observation (Jackson and Snowdon, 1974, Okoye *et al*., 2020, Bruce *et al*., 2021).

Microbiological Evaluation

Anti-microbial screening (*in vitro*): The agar plate diffusion assay method described by Subbulakshmi, 2012 (with modification) was used to evaluate the antibacterial and antifungal activity against the test microorganisms.

Antibacterial assay: Broth cultures of test four bacterial isolates, which includes two Gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram negative bacteria (*Pseudomonas aeruginosa and Escherichia coli*) were diluted to a concentration of $1x10^8$ CFU/mL, and in an exponential growth phase, were spread onto the surface of Mueller Hinton agar plates by using sterile cotton swabs. All the culture plates were allowed to dry for about five min. Agar well was prepared by using sterile cork-borer (6 mm in diameter) Stock concentrations of the extracts were made using DMSO as the diluents were, 800 mg of Pod and Seed extracts of *P. nitida* were dissolved in 2 ml of DMSO (400 mg/ml respectively as stock concentration) thereafter serial dilutions were made using the same diluents DMSO to get (200, 100, 50, 25, 12.5, 6.25 mg/ml for Pod and Seed extracts respectively) then, Aliquots (60 µl) of the extracts at concentrations of the different dilutions were applied in each of the wells in the culture plates previously seeded with the test organism. The

plates were then incubated at 35-37°C for 24 h and the zone of inhibition measured. Ciprofloxacin (10µg) was used as the positive control and DMSO, the negative control (Bruce *et al*., 2016).

Antifungal assay: Liquid cultures of test fungal cultures (*Aspergillus niger* and *Candida albican*) were spread out onto the surface of Sabouraud Dextrose agar medium. All the culture plates were allowed to dry for about five minutes. Agar well was prepared by using sterile cork-borer (6 mm in diameter) Stock concentrations of the extracts were made using DMSO as the diluents were, 800 mg of Pod and Seed extracts of *P. nitida* were dissolved in 2ml of DMSO (400 mg/ml respectively as stock concentration) thereafter serial dilutions were made using the same diluents DMSO to get 200, 100, 50, 25, 12.5, 6.25 mg/ml for Pod and Seed extracts respectively then, aliquots (60 µl) of the extracts at concentrations of the different dilutions were applied in each of the wells in the culture plates previously seeded with the test organism.
The fungal culture was incubated at 25-27oC for 48-72 h and the zone of inhibition were recorded. Miconazole (50 µg) was used as the positive control and DMSO as the negative control (Bruce *et al*., 2016).

Hepatoprotective evaluation: The hepatoprotective activity of Vaccum Liquid Chromatography (VLC) fractions of P. *nitida* seeds described by Baheti *et al.,* 2006. The albino mice were grouped into twelve groups of five mice per group. The animals were allowed to acclimatized for two weeks with proper feeding and water, then it was treated with the (VLC) fractions for two more weeks before induction of hepatotoxicity and administration of the standard to the positive control while the negative control treatment standard and paracetamol respectively, all drug administration was done orally using cannula (Bruce *et al*., 2021).

Experimental design for treatment.
The animals were treated as follows:
Group1: Treatment group with 0.29ml/0.019kg of EA-M extract of P. *niida*
Group 2: Treatment group with 0.27ml/0.018kg of EA-M extract of *P. nitida*
Group 3: Treatment group with 0.27ml/0.017kg of EA-M extract of *P. nitida*
Group 4: Treatment group with 0.28ml/0.019kg of EA-M extract of *P. nitida*
Group 5: Treatment group with 0.25ml/0.017kg of EA-M extract of *P. nitida*
Group 6: Treatment group with 0.22ml/0.015kg of EA-M extract of *P. nitida*
Group7: Treatment group with 0.27ml/0.018kg of EA-M extract of *P. nitida*
Group 8: Treatment group with 0.28ml/0.019kg of N-H: EA extract of *P. nitida*
Group 9: Treatment group with 0.24ml/0.016kg of N-H: EA extract of *P. nitida*
Group 10: Treatment group with 0.27ml/0.018kg of N-H: EA extract of *P. nitida*
Group11: Treatment group with 0.27ml/0.017kg of EA-M extract of *P. nitida*
Group12: Positive control treated with 100mg/0.019kg of Silymarin
Group13: Negative control treated with paracetamol only

These treatments were given once daily for two-week days, then on the 14th day of treatment, paracetamol (mg/kg) was administered to the animals in treatment groups according to their respective individual weights 24hours after the paracetamol administration the mice was sacrificed for serum enzyme ALT (Alanine aminotransferase), AST, ALP and Bilirubin (Bruce *et al*., 2021).

RESULTS

Qualitative phytochemical analysis of seed extracts of *P. nitida:* The phytochemical screening of the seed extract of *P. nitida* revealed the presence of high concentrations of bioactive compounds such as alkaloids, saponins, flavonoids, terpenoids, cardiac glycosides, protein and carbohydrate; and absence of tannins and steroids. In the fractions of the seed extract, alkaloids were present in n-Hexane and aqueous fractions but absent in ethylacetate and butanol fractions, flavonoids and saponins were present.

Table 1: Qualitative phytochemical analysis of seed extract of *P. nitida*

Phytochemicals	Test
Alkaloids	+
Flavonoids	+
Tannins	-
Saponins	+
Cardiac glycosides	+
Terpenoids	+
Steroids	+

Protein	+
Carbohydrate	+

Quantitative phytochemical analysis of the seed extract of *P. nitida:* The Quantitative phytochemical analysis of the seed extract of *P. nitida* revealed that Flavonoids (10.2%) and Saponins (9.8%) are the highest phytochemical constituents while Tannins (0.42%) and Alkaloids (6%) have the lowest constituents.

Macroscopical features of the Seeds of *P. nitida:* The macroscopic features of the seed and pod of *P. nitida* were examined using physical examination which showed Ovate or obovate shape, bitter taste, brown colour and smooth in texture.

Chemomicroscopical features of the seeds of *P. nitida:* The chemomicroscopical features of the seed of *P. nitida* revealed the presence of the specific characteristic of the plant such as epidermal cell, parenchyma layer, starch granules, calcium oxalate and pigment layer in the seed extract (Jackson and Snowdon, 1974) as represented below.

Table 2: Quantitative phytochemical analysis of the seed extract of *P. nitida*

PHYTOCHEMISTRY	SEED EXTRACT
Tannins	0.0%
Alkaloids	10.2%
Saponins	9.5%
Flavonoids	10.2%

Table 3: Chemomicroscopic result of *P. nitida* seeds

Chemical	Reagent	*P. nitida* seeds
Starch	Iodine solution	Present
Lignins	Phloroglucinol conc. HCl	Present
Cellulose	Zinc chloride; conc. Sulphuric acid	Present
Calcium oxalate	Iodine solution; conc. Sulphuric acid	Present
Protein bodies	Biuret reagent; Nihydrin	Present
Tannins	Ferric chloride	Absent

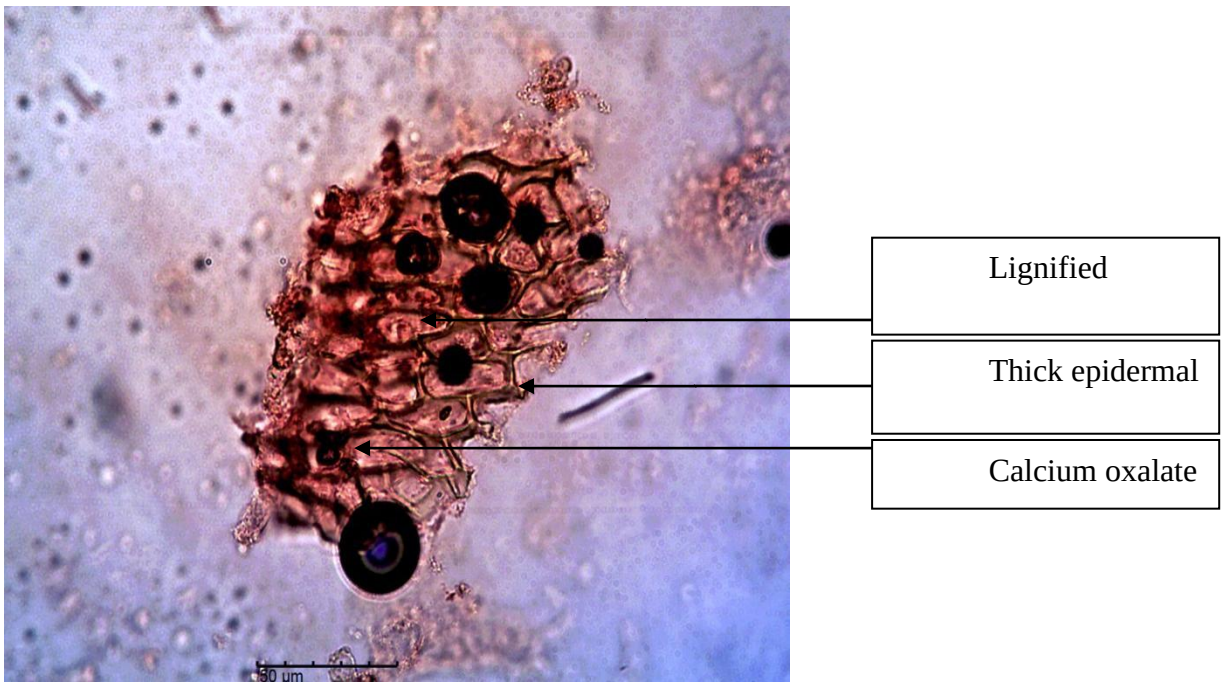

Figure 1: Chemomicroscopy of *P. nitida* seed showing lignified parenchyma tissue with thick epidermal cell walls and calcium oxalate crystal. They are polygonal in shape

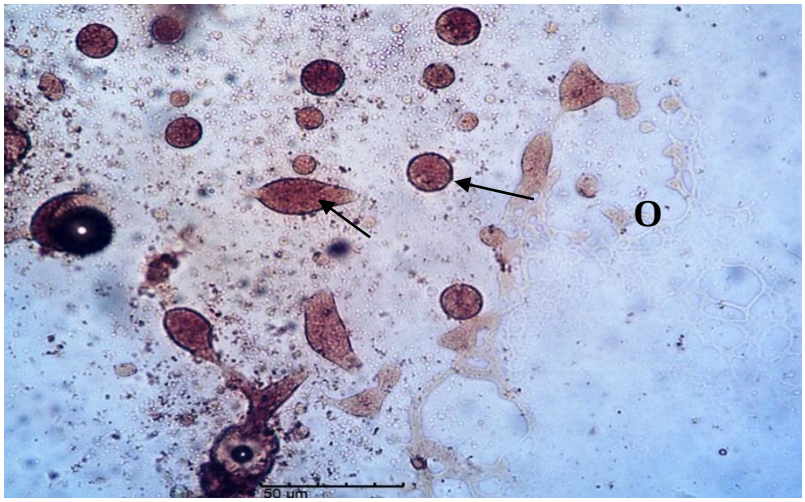

Figure 2: Chemomicroscopy of *P. nitida* seed showing fiber elements (Fe) and oil globules (Og)

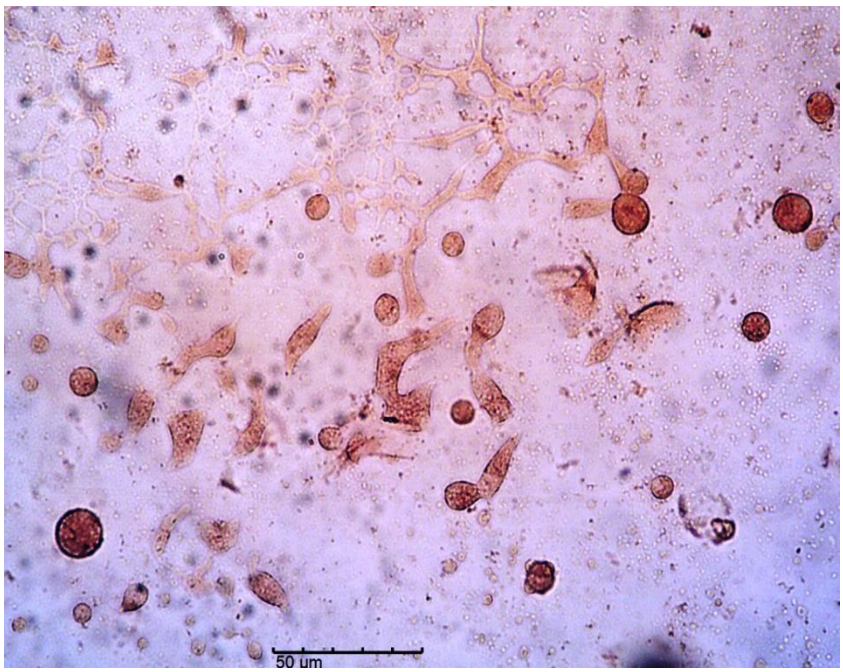

Figure 3: Chemomicroscopy of *P. nitida* seed showing oil globules (Og)

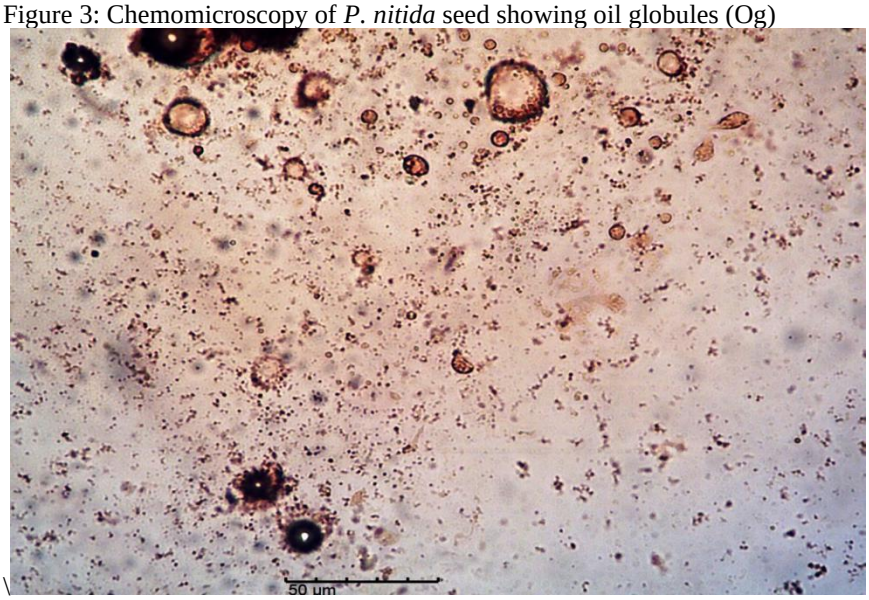

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Figure 4.Chemomicroscopy of *P. nitida* seed showing starch granules

Table 4: Antimicrobial activity (in IZD) of nHexane – Ethyl Acetate (0: 10) Seed Extract of *Picralimanitida* on the test organisms

Test organisms	Inhibition zone diameter (mm)					
	Concentration of the Extract (mg/mL)					Ciprofloxacin
	5.0	2.5	1.25	0.625	0.313	10 µg/ml
S. aureus	9.0 ± 0.0	6.0 ± 0.0	4.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	10.0 ± 0.0
B. subtilis	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	9.0 ± 0.0
E. coli	9.5 ± 0.7	7.0 ± 1.4	5.5 ± 2.1	4.0 ± 1.4	2.0 ± 0.0	2.0 ± 0.0
P. aeruginosa	6.5 ± 0.7	4.0 ± 0.0	4.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
						Miconazole50 µg/ml
A. niger	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.0
C. albicans	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3.0 ± 0.0

Results are expressed as mean ± standard deviation,

Table 5: Antimicrobial activity (in IZD) of nHexane – Ethyl Acetate (1: 9) Seed Extract of *Picralima nitida* on the test organisms

Test organism	Inhibition zone diameter (mm)					
	Concentration of the Extract (mg/mL)					Ciprofloxacin
	5.0	2.5	1.25	0.625	0.313	10 µg/ml
S. aureus	6.0 ± 0.0	4.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	0.0 ± 0.0	10.0 ± 0.0
B. subtilis	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	9.0 ± 0.0
E. coli	8.5 ± 0.7	7.5 ± 0.7	6.0 ± 1.4	4 ± 1.4	2.5 ± 0.7	2.0 ± 0.0
P. aeruginosa	4.0 ± 0.0	3.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.0
						Miconazole50 µg/ml
A. niger	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.0
C. albicans	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3.0 ± 0.0

Results are expressed as mean ± standard deviation

Table 6: Antimicrobial activity (in IZD) of nHexane – Ethyl Acetate (2: 8) Seed Extract of *Picralima nitida* on the test organisms

Test organism	Inhibition zone diameter (mm)					
	Concentration of the Extract (mg/mL)					Ciprofloxacin
	5.0	2.5	1.25	0.625	0.313	10 µg/ml
S. aureus	3.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	10.0 ± 0.0
B. subtilis	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	9.0 ± 0.0
E. coli	4.5 ± 0.7	3.5 ± 0.7	2.5 ± 0.7	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.0
P. aeruginosa	2.5 ± 0.7	2.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.0
						Miconazole 50 µg/ml
A. niger	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.0
C. albicans	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3.0 ± 0.0

Results are expressed as mean ± standard deviation

Table 7: Antimicrobial activity (in IZD) of nHexane – Ethyl Acetate (3: 7) Seed Extract of *Picralima nitida* on the test organisms

Test organisms	Inhibition zone diameter (mm)					
	Concentration of the Extract (mg/mL)					Ciprofloxacin
	5.0	2.5	1.25	0.625	0.313	10 µg/ml
S. aureus	4.0 ± 0.0	3.0 ± 0.0	2.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	10.0 ± 0.0
B. subtilis	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	9.0 ± 0.0
E. coli	6.0 ± 0.0	5.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0	3.0 ± 0.0	2.0 ± 0.0
P. aeruginosa	4.0 ± 0.0	3.0 ± 0.0	2.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.0
						Miconazole50 µg/ml
A. niger	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.0
C. albicans	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3.0 ± 0.0

Results are expressed as mean ± standard deviation

Table 8: A table of the Antimicrobial activity (in IZD) of nHexane -Ethyl Acetate (4: 6) Seed Extract of *Picralima nitida* on the test organisms

Test organism	Inhibition zone diameter (mm)					
	Concentration of the Extract (mg/mL)					Ciprofloxacin
	5.0	2.5	1.25	0.625	0.313	10 µg/ml
S. aureus	4.0 ± 0.0	3.0 ± 0.0	2.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	10.0 ± 0.0
B. subtilis	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	9.0 ± 0.0
E. coli	12.0 ± 0.0	7.5 ± 0.7	9.5 ± 0.7	12.0 ± 0.0	12.0 ± 0.0	2.0 ± 0.0
P. aeruginosa	6.0 ± 0.0	4.0 ± 0.0	2.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.0
						Miconazole 50 µg/ml
A. niger	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.0
C. albicans	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3.0 ± 0.0

Results are expressed as mean ± standard deviation

Table 9: Antimicrobial activity (in IZD) of nHexane – Ethyl Acetate (5: 5) Seed Extract of *Picralima nitida* on the test organisms

Test organisms	Inhibition zone diameter (mm)					
	Concentration of the Extract (mg/mL)					Ciprofloxacin
	5.0	2.5	1.25	0.625	0.313	10 µg/ml
S. aureus	6.0 ± 0.0	4.0 ± 0.0	2.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	10.0 ± 0.0
B. subtilis	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	9.0 ± 0.0
E. coli	13 ± 1.4	10.5 ± 0.7	7.0 ± 0.0	5.0 ± 0.0	3.5 ± 0.7	2.0 ± 0.0
P. aeruginosa	4.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.0
						Miconazole50 µg/ml
A. niger	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.0
C. albicans	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3.0 ± 0.0

Results are expressed as mean ± standard deviation

Table 10: A table of the Antimicrobial activity (in IZD) of Ethyl Acetate – Methanol (1: 9) Seed Extract of *Picralima nitida* on the test organisms

Test organisms	Inhibition zone diameter (mm)					
	Concentration of the Extract (mg/mL)					Ciprofloxacin
	5.0	2.5	1.25	0.625	0.313	10 µg/ml
S. aureus	6.0 ± 0.0	4.0 ± 0.0	3.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	10.0 ± 0.0
B. subtilis	5.5 ± 0.7	4.5 ± 0.0	3.5 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	9.0 ± 0.0
E. coli	2.5 ± 0.7	3.0 ± 0,0	4.0 ± 0.0	3.5 ± 0.7	3.0 ± 0.0	2.0 ± 0.0
P. aeruginosa	3.0 ± 0.0	2.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.0
						Miconazole50 µg/ml
A. niger	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.0
C. albicans	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3.0 ± 0.0

Results are expressed as mean ± standard deviation

Table 11: Antimicrobial activity (in IZD) of Ethyl Acetate – Methanol (2:8) Seed Extract of *Picralima nitida* on the test organisms

Test organisms	Inhibition zone diameter (mm)					
	Concentration of the Extract (mg/mL)					Ciprofloxacin
	5.0	2.5	1.25	0.625	0.313	10 µg/ml
S. aureus	9.5 ± 0.0	8 0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	10.0 ± 0.0
B. subtilis	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	9.0 ± 0.0
E. coli	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.0
P. aeruginosa	3.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.0
						Miconazole 50 µg/ml
A. niger	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.0
C. albicans	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3.0 ± 0.0

Results are expressed as mean ± standard deviation

Table 12: Antimicrobial activity (in IZD) of Ethyl Acetate – Methanol (4: 6) Seed Extract of *Picralima nitida* on the test organisms

Test organisms	Inhibition zone diameter (mm)					
	Concentration of the Extract (mg/mL)					Ciprofloxacin 10 µg/ml
	5.0	2.5	1.25	0.625	0.313	
S. aureus	3.5 ± 0.7	2.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	10.0 ± 0.0
B. subtilis	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	9.0 ± 0.0
E. coli	6.5 ± 0.7	5.5 ± 0.7	5.0 ± 0.0	4.5 ± 0.0	2.5 ± 0.7	2.0 ± 0.0
P. aeruginosa	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.0
						Miconazole 50 µg/ml
A. niger	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.0
C. albicans	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3.0 ± 0.0

Results are expressed as mean ± standard deviation

Table 13: Antimicrobial activity (in IZD) of Ethyl Acetate – Methanol (6: 4) Seed Extract of *Picralima nitida* on the test organisms

Test organisms	Inhibition zone diameter (mm)					
	Concentration of the Extract (mg/mL)					Ciprofloxacin 10 µg/ml
	5.0	2.5	1.25	0.625	0.313	
S. aureus	3.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	10.0 ± 0.0
B. subtilis	2.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	9.0 ± 0.0
E. coli	6.5 ± 0.7	5.0 ± 0.0	4.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.0
P. aeruginosa	4.5 ± 0.7	2.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.0
						Miconazole50 µg/ml
A. niger	2.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.0
C. albicans	3.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3.0 ± 0.0

Results are expressed as mean ± standard deviation

Table 14: Antimicrobial activity (in IZD) of Ethyl Acetate – Methanol (7: 3) Extract Seed Extract of *Picralima nitida* on the test organisms

Test organisms	Inhibition zone diameter (mm)					
	Concentration of the Extract (mg/mL)					Ciprofloxacin 10 µg/ml
	5.0	2.5	1.25	0.625	0.313	
S. aureus	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	10.0 ± 0.0
B. subtilis	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	9.0 ± 0.0
E. coli	5.0 ± 1.4	3.5 ± 0.7	3.5 ± 0.7	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.0
P. aeruginosa	3.5 ± 0.7	2.5 ± 0.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.0
						Miconazole 50 µg/ml
A. niger	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.0
C. albicans	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3.0 ± 0.0

Results are expressed as mean ± standard deviation

Table 15: Antimicrobial activity (in IZD) of Ethyl Acetate – Methanol (9: 1) Seed Extract of *Picralima nitida* on the test organisms

Test organisms	Inhibition zone diameter (mm)					
	Concentration of the Extract (mg/mL)					Ciprofloxacin 10 µg/ml
	5.0	2.5	1.25	0.625	0.313	
S. aureus	2.5 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	10.0 ± 0.0
B. subtilis	12.0 ± 0.0	7.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	9.0 ± 0.0
E. coli	6.0 ± 0.0	5.0 ± 0.0	4.0 ± 0.0	3.0 ± 0.0	2.5 ± 0.7	2.0 ± 0.0
P. aeruginosa	4.0 ±0.0	3.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.0
						Miconazole 50 µg/ml
A. niger	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.0
C. albicans	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3.0 ± 0.0

Results are expressed as mean ± standard deviation

Table 16: Antimicrobial activity (in IZD) of Ethyl Acetate – Methanol (10: 1) Seed Extract of *Picralima nitida* on the test organisms

Test organisms	Inhibition zone diameter (mm)					
	Concentration of the Extract (mg/mL)					Ciprofloxacin 10 µg/ml
	5.0	2.5	1.25	0.625	0.313	
S. aureus	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	10.0 ± 0.0
B. subtilis	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	9.0 ± 0.0
E. coli	4.0 ± 0.0	3.0 ± 0.0	2.5 ± 0.0	2.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.0
P. aeruginosa	2.5 ± 0.7	4.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.0
						Miconazole 50 µg/ml
A. niger	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.0
C. albicans	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3.0 ± 0.0

Results are expressed as mean ± standard deviation

Hepatoprotective results

Table 17: Liver enzymes test results for the chromatography fractions of the ethyl acetate fraction from the *P. nitida* seed of ethanol extract

	ALT	AST	ALP	DIRECT BILIRUBIN	TOTAL BILIRUBIN
EA-M(0.29ml)	173±1.73	105± 1.73	56.4± 1.96	28.2± 12.62	28.6±12.45
EA-M(0.27ml)	173± 3.5	109.3± 1.7	90 ± 3.5	45.1± 20.1	45.5±19.9
EA-M(0.27ml)	173±2.9	108.7± 1.4	89 ± 2.3	44.6± 19.8	45 ± 19.7
EA-M(0.28ml)	173± 2.6	99.5± 0.3	100.3± 0.5	50.2± 22.4	50.6± 22.2
EA-M(0.25ml)	173± 2.7	100.1± 2.1	91.5± 1.2	45.8± 20.4	46.2± 20.3
EA-M(0.22ml)	173± 1.6	101.4± 1.7	49.6± 0.9	24.8± 11.1	25.0± 11
EA-M(0.27ml)	173± 0.6	96.1± 1.4	90.6± 1.6	47.4± 20.2	45.8± 20
EA-M(0.28ml)	173±1.1	91.8± 1.1	45.0± 1.1	22.5± 10.1	23.2± 9.8
NH-EA(0.24ml)	173±2.1	104± 3.8	98.2± 1.4	49.2± 21.9	49.6± 21.7
NH-EA(0.27ml)	173± 3.4	102.8± 3.2	99.2± 1.4	49.6± 22.2	50.1± 21.9
EA-M(0.27ml)	173± 2.2	101.1± 1.5	99.4± 0.5	49.8± 22.2	50.2± 21.9
STANDARD(0.3ml)	173± 0.2	95.5± 0.9	46.8± 4.8	23.5± 10.7	23.7± 10.5
NEGATIVE CONTROL	227.3 ± 3.4	134 ± 2.7	101.1± 1.5	50.6± 22.7	51.1± 21.3

Values are: Mean± SEM, n=3 observations
Key: EA-M =Ethyl acetate/Methanol
NH-EA=nHexane/ethyl acetate

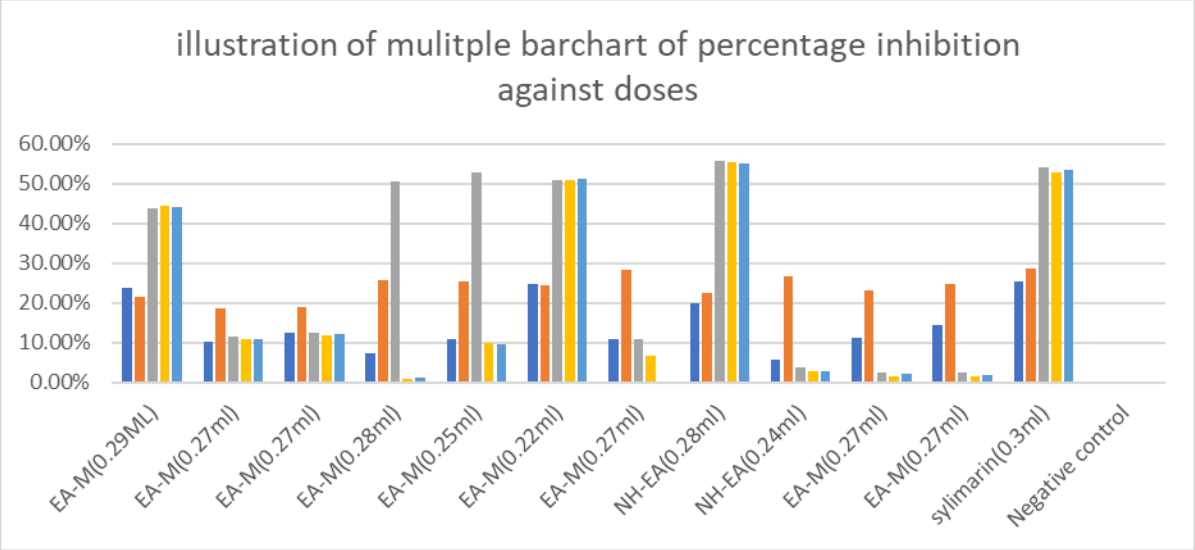

Figure 5: Barchart illustrating percentage inhibition of liver enzymes against doses.

DISCUSSIONS

Phytochemical screening of the seed extracts of *P. nitida* indicated the presence of alkaloids, saponins, flavonoids, cardiac glycoside, terpenoids, protein and carbohydrate. Some of the phytochemical compounds detected such as glycoside, saponins, tannins, flavonoids, terpenoids and alkaloids have been reported to have antimicrobial activity (Okeke *et al*., 2001, Bruce *et al*., 2019). It is significant to note that alkaloids contribute to plant species fitness for survival;

The saponins composition of the seed extract ranged from 9.8% to 9.5% respectively. Some alkaloids and saponins have been found to possess antimicrobial activity (Osborn, 2003) and hence the activities being exhibited by the extracts may be as a result of presence of the alkaloids and saponins in the plant under study. Flavonoids have been shown to have antibacterial, anti- inflammatory, anti-allergic, antiviral antineoplastic activity (Alan and Miller, 1996). Therefore, the inhibition of bacterial and fungal growth is of great importance to the health care system, thus it can be used as an alternative to orthodox antibiotics in the treatment of infection due to these isolates especially as they are becoming resistant to known antibiotic (El-shemy *et al.*, 2007, Bruce *et al*., 2016).

The Inhibition zone diameter of the gram positive and gram negative bacterial and also the fungi (*A. niger* and *C. albibans)* of the VLC fractions of *P. nitida* seed extract, as well as the antibiotic and antifungal power of these extracts were considered in Tables 4-16. From reading these tables, we noted that the different extracts hold relative antibacterial activities against bacterial strains such as *S. aureus, B. subtilis*, *E. coli* and *P. aeruginosa.* In this study, the VLC sub-fractions were obtained with nHexane/ethyl acetate and ethyl acetate/methanol solvent systems. The ethyl acetate/methanol sub-fractions demonstrated higher activity on the test organisms than the nHexane/ethyl acetate sub-fractions; this suggests that the active principle(s) were more soluble in ethyl acetate/ methanol solvent system than in other solvent systems. Also, it was deduced the *P. nitida* VLC sub-fractions of both nHexane/ethyl acetate and ethyl acetate/ methanol were more susceptible to both gram positive and gram negative when compare to the standard drug (ciprofloxacin) at higher concentrations. Hence, the higher the concentration, the higher the susceptibility. The present investigation also confirmed, the antimicrobial activity of the ethyl acetate: methanol (6:4) sub-fractions, showed a broad spectrum of activity on both bacterial (Gram positive and Gram negative) and fungi (*Aspergillus spp* and *Candida albicans*). The nHexane – ethyl acetate (0: 10, 1:9, 2:8, 3:7, 4:6 and 5:5) sub-fractions showed activity against only *Staphylococcus aureus* (Gram positive) and *P. aeroginosa* and *E. coli* (Gram negative) and resistant to *Bacillus subtilis*. While ethyl acetate: methanol (9: 1) showed activity against both Gram positive and Gram-negative bacterial isolates.

The most effective VLC fractions are ethyl acetate: methanol (6: 4) and ethyl acetate: Methanol (9: 1). Ethyl acetate: methanol (6: 4) inhibited both bacterial and fungal, while ethyl acetate: Methanol (9: 1) inhibited the growth of only the bacterial (Gram positive and Gram negative). Bruce *et al.*, 2016 compared that the ethyl acetate fraction demonstrated higher activity on the test organisms than the n-hexane, butanol and aqueous fractions of *P. nitida* seed extract; this suggests that the active principle(s) were more soluble in ethyl acetate than in other solvents. The factors responsible for the high antibiotic susceptibility of the test organisms, which shows the mean IZD produced by various antibiotics, may be attributed to differences in the organism's physiology and anatomy and the presence of secondary metabolites (Omogbai and Eze, 2011, Koudoro *et al*., 2014). The antibacterial activities noted with VLC fractions from the seed of *P. nitida* could probably be related to the chemical profile of the seed of the same plant either to the action of a secondary metabolites or to a synergy effect between secondary metabolites (polyphenols, tannins, saponins, flavonoids, alkaloids) (Mbaveng *et al*., 2008, Bruce *et al*., 2021).

The isolation and characterization of the active principle(s) for further investigations would greatly improve both the intensity and spectrum of activity. This observed difference in susceptibility could be attributed to the inherent resistance factors of the test organisms among other factors (Ekpo and Etim, 2009, Onyemailu *et al*., 2021). Hence, it is therefore seen that the constituent(s) or the principle(s) responsible for the antimicrobial activity is largely in the ethyl acetate/ methanol fractions from the *in vitro* study.

In the assessment of liver injury or damage by Paracetamol, the determination of liver enzyme levels such as AST, ALT, ALP is widely used. Necrosis or liver damage releases these enzymes into circulation, which can be measured in the serum. A high level of AST indicates liver damage, such as that caused by viral hepatitis as well as cardiac infarction and muscle injury. AST catalyses the conversion of alanine to pyruvate and glutamate and is released in a similar manner. Therefore, ALT is more specific to the liver, and is thus a better parameter for detecting liver injury. Elevated levels of serum enzymes are indicative of cellular leakage

and loss of functional integrity of cell membrane in liver (Drotman and lawhan. 1978, Bruce *et al*., 2021). Serum ALP and total protein levels on other hand are related to the function of hepatic cell. Increase in serum level of ALP is due to increased synthesis; in presence of increasing biliary pressure (Muriel and Garcipiana. 1992). Administration of Paracetamol caused elevated liver enzyme levels, AST, ALT and ALP. There was a restoration of these enzyme levels on administration of the herb extract of VLC fractions of *P. nitida* seed in a dose dependent manner and also by silymarin at a dose of 100 mg/kg.

The VLC fractions of *P. nitida* seed were investigated for their hepatoprotective effects of Paracetamol induced liver cell damage model. The effect of the VLC fractions of the seeds of *P. nitida* at different doses, the fractions of nHexane/ethyl acetate, and ethyl acetate/methanol(group 1, group 2, group 3, group 4, group 5, group 6, group 7, group 8, group 9, group 10, and group 11) were used at different concentration, and silymarin at 100 mg/kg (positive control) on hepatotoxicity induced by Paracetamol led to the significant reduction ($P \leq 0.01$) in the liver enzymes (AST, ALT, ALP) and bilirubin showed no significant ($P<0.05$) VLC fractions. The % inhibition of the VLC fractions of the seed of *P. nitida* at different doses, Group 6 and Group 8 has the highest percentage inhibition of the liver enzymes Group 6(EA/M) has ALT (24.7 %), AST (24.3 %), ALP (51 %) and direct bilirubin level (50.8 %),and total bilirubin (51.1 %), and Group 8 (NH/EA) has ALT (19.9 %), AST (22.4 %), ALP (55.8 %) and direct bilirubin level (55.5 %), and total bilirubin (55 %) compared with the Positive control (Silymarin), ALT (25.5 %), AST (28.7 %), ALP(54.1 %), direct Bilirubin (53 %),total Bilirubin (53.6 %) has the highest percentage inhibition of the liver enzymes, therefore, its protective effect remained lower than the silymarin effect. The seed extract of *P. nitida* fractions such as ethyl acetate fraction at 400 mg/kg produced a significant ($P \leq 0.05$) inhibition in liver enzymes (ALT (82.97%), AST (71.95 %), ALP (43.04 %) and bilirubin level (67.27 %) (Ibrahim*et al*., 2014, Bruce *et al*., 2016 and Onyegbule *et al*., 2019). The seed extract of *P. nitida* (Apocynaceae) produce a significant ($p \leq 0.01$) reduction in serum liver enzymes, when administered at different dose daily for 14 days which has inhibitory activity against the Paracetamol induced hepatotoxicity. All mentioned data indicated that silymarin was more efficient as hepatoprotective agent followed by Group 6 (EA/M) VLC fraction of *P. nitida*. Therefore, the plant seeds have hepatoprotective activity which is useful in managing liver disease and hepatic injury.

CONCLUSION

The standardization of this plant is an integral part of establishing the correct identity of the crude drug before any drug can be included in the pharmacopoeia. Therefore, the crude ethanol extract of the seeds of *P. nitida* contains alkaloids, amide, peptide, terpenoids and alkaloids, flavaniods. This study shows that *P nitida* seeds have antimicrobial principles which may be useful against opportunistic infections caused by *E. coli and P. aeruginosa*. It also has hepatoprotective effect using nHexane/ Ethyl acetate and Ethyl acetate/methanol fractions in paracetamol induced hepatotoxicity, it showed no significant ($P<0.05$) VLC fractions (groups 6 and 8) showed a promising activity compared with the standard drug Silymarin 100mg statistically.

RECOMMENDATION

More work should be done such as purification using Gas Chromatography Mass Spectroscopy (GC-MS) and characterisation by structural elucidation using NMR (Nuclear magnetic resonance)

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