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EVALUATION OF THE HEPATO-PROTECTIVE AND ANTI-BACTERIAL ACTIVITIES OF ETHANOL EXTRACT OF *Picralima nitida* SEED AND POD

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ABSTRACT

Picralima nitida seed and pod extracts and fractions were screened for antimicrobial activity, using Agar-well diffusion assay and hepatoprotective activity against CCl_4induced hepato-toxicity in albino rats. Proximate, phytochemical and HPLC analyses were carried out to ascertain the constituents of *Picralima nitida* seed and pod. The result of the antimicrobial screening showed that at 400 mg/ml of extract, the test organisms were sensitive (IZD) to the seed and pod extracts with *S. aureus* (21 and 18 mm) and *B. subtilis* (16 and 16 mm) respectively. The test organisms were also sensitive (IZD) to ethyl acetate fraction of the seed extract, at 50 mg/ml, with *B. subtilis* (19 mm), *E. coli* (22 mm), *P. aeruginosa* (21mm) respectively. The extracts of the seed and pod; and the ethyl acetate fraction of the seed extract produced a significant ($p \leq 0.001$) reduction in liver enzymes. The proximate analysis of seed and pod respectively, indicated moisture content (1.2% and3.8%), total ash (4% and 5%), acid insoluble ash (1% and 1%), water soluble extractive value (2.8% and 3.8%) and crude fibre (3.6% and 5.2%). The phyto-evaluation of the seed and pod extracts showed respectively, tannin (0.42% and 0.33%), alkaloid (6% and 7.6%), saponin (9.8% and 9.5%) and flavonoid (10.2% and 1.2%).The HPLC analysis of the ethyl acetate fraction of the seed extract and the pod crude extract revealed phyto-compounds with reported antibacterial and hepatoprotective activities. This study showed that *P. nitida* seed and pod extracts exhibited antibacterial and hepatoprotective activities; confirming its ethnomedicinal use for treating infections and liver problems.

Keywords: *P. nitida*, antibacterial, hepatoprotective, phyto-constituents, anti-proliferative.
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INTRODUCTION

The use of medicinal plants for treatment of various infections in traditional communities has been an age-long global practice. It has been estimated that 80% of African population use herbal regimen for treatment and control of diseases (Hugo and Russell, 2003). Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found *in vitro* to have antimicrobial properties, this provides a rational for studying medicinal plant extracts as possible sources of alternative therapy against infections. Apart from the high cost of some antibiotics, most of the clinically important antibiotics have major setbacks. Conventional antibiotics have been found to have different side effects including being: neuro-toxic, nephro-toxic and hypertensive while few others cause severe damage to the liver and bone marrow depression (Chong and Pagano, 1997).

The liver is one of the largest organs in the human body and the major site of intense metabolism and excretion. The major

functions of the liver are carbohydrate, protein and fat metabolism, detoxification, secretion of bile and storage of vitamin. The main causes of liver damage are drugs, toxic compounds, excess consumption of alcohol, biological factors (bacteria, virus, and parasites) and autoimmune diseases (immune hepatitis, primary biliary cirrhosis), (Deshwal, 2011). Hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and oxidative damages, which results in hepatitis and cirrhosis (Nabavi, 2009). Herbal formulations used in liver disorder are Silymarin (*Silybum marianum*), Liv-52 (*Capparis spinosa, Cichoriumintybus, Solanum nigrum, Terminalia arjuna, Cassia occidentalis, Achillea millefolium, Tamarix gallica*), Cirrhitin, Sriliv (capsules). Some other polyherbal preparations such as Livex, HD-03, Hepatomed, Live 100 and Hepatoguard with proven efficacy are also used in different types of liver disorders (Paula, 2009).

It has been reported that about 170 phyto-constituents isolated from 110 plants belonging to 55 families possess hepatoprotective activity (Handa, 1991). Considering the vast potentiality of plants as sources for antimicrobial and hepatoprotective drugs, this research was undertaken to evaluate the antimicrobial and hepatoprotective activities of *P. nitida* seed and pod extracts. Earlier report by Okenwa and Yusuf (2017) reviews that *Picralima nitida* fruit-rind extract contains several phytochemical constituents similar to this study. *P. nitida* (Fam. Apocynaceae) occurs in tropical rain forest of Africa. Crushed or powdered seed of the plant is taken to treat malaria, diarrhea and inflammation (Ezeamuzie *et al*., 1994). The fruit is used in West Africa for the treatment of gastrointestinal disorder and dysmenorrhoea (Duwiejua *et al.*, 2002). This study shows *P. nitida* seed and pod have hepatoprotective activity at 400 mg/kg once daily for 14 days in CCl_4 induced liver damage or injury. This justifies its use in traditional medicine in Southern Nigeria to treat liver problem.

MATERIALS AND METHODS

Plant Material

The seed and pod of *Picralima nitida* were purchased from Ogbaru in Onitsha, Anambra State, Nigeria. The plant was identified and authenticated at the Pharmacognosy

Herbarium in Faculty of Pharmaceutical Sciences, NnamdiAzikiwe University, Awka, Nigeria and assigned Herbarium number PCG 474/A/025.

Preparation of plant material

Fresh Seed and Pod of the plant were properly washed and rinsed with distilled water. The extracts were concentrated to dryness using a rotary evaporator at reduced pressure. The materials were dried, pulverized to fine particles and were weighed using weighing scale. These were stored in air tight containers.

Extraction of plant material

The extracts of seed and pod of the plant were prepared by cold maceration in 95 % Ethanol. A 1 kg amount of each of the plant parts was separately soaked in 4.5 L of 95 % Ethanol for 48 h with occasional stirring and the filtrate was filtered through Whatman® filter paper No.1 and evaporated to dryness using a rotary evaporator (RE300 Model, United Kingdom).

Fractionation of *Picralima nitida* seed extract and determination of the most active fraction

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 water were used as the mobile phase, to obtain the different fractions. The fractions were screened for anti-microbial activity. The most active fraction was subjected to vacuum liquid chromatography (VLC). About 3 g of the ethylacetate fraction were subjected to VLC using a 5 L sintered column packed with Silica gel to a 10 cm bed size. The column were eluted with 500 ml each of hexane:ethyl acetate (100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90, 0:100) and bulked for subsequent HPLC screening, 2 mg of the extract and fractions were reconstituted with 2 ml of HPLC grade ethanol, using Dionex P580 HPLC system coupled to photodiode array detector (UVD340S, DionexSofttron GmbH, Germany) were used in the isolation process to

determine the active constituents (Jean *et al*., 2001).

Phytochemical screening:
Qualitative Phytochemical Analysis
The plant crude extracts were screened for the presence alkaloid, flavonoid, tannin, saponin, cardiac glycosides, terpenoid, steroid, protein and carbohydrate using standard methods (Sofowora, 1993).

Quantitative Phytochemical Analysis
The coarse powder of the plant material were tested to determine the quantity of alkaloids, flavonoids, saponins and tannins present (Edeogha *et al*., 2000)
Proximate analysis: The plant crude extract was evaluated for moisture content, ash values and extractive values (AOAC, 2005).
Acute Toxicity: Determination of median lethal dose (LD_{50}) was done with a total of 21 albino rats using the Lorke's method (Lorke, 1983).

Antibiotic Sensitivity Test: Broth cultures of test bacterial (*Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa and Escherichia coli*) were isolated. The organisms were spread on the surface of Muller Hinton agar plate using sterile cotton swabs. Antibiotic discs (Abtek Biologicals Ltd., England) containing Gentamicin (10 µg/mL), Cefixime (5 µg/mL), Ofloxacin (5 µg/mL), Augmentin (30 µg/mL), Nitrofuraton (30 µg/mL), Ciprofloxacin (5 µg/mL), Ceftazidime (30 µg/mL), Cefuroxime (30 µg/mL) were placed on Muller-Hinton agar plates. The plates were inverted and left on the work bench for 30 min to allow for diffusion of antibiotics into the agar incubated at 37°C for 48 h and zone of inhibition measure (Hudzicki, 2009).

Antimicrobial assay
Bacterial isolates: The microorganisms used were all clinical isolates obtained from the Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences Nnamdi Azikiwe University Awka. They include *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, Aspergillus niger* and *Candida albican.*

Determination of antimicrobial activity (Agar-Well Diffusion Assay) of crude extracts and fractions of *Picralima nitida* Seed and Pod (Subbulakshmi *et al.*, 2012**)**

Antibacterial and antifungal assay
Broth cultures of test bacterial isolates (*Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa and Escherichia coli and fungi* isolates *Aspergillus niger and Candida albican*) were made and adjusted to 0.5 MacFarland turbidity standard and inoculated onto Mueller Hinton Agar (MHA, Oxoid) plates (diameter: 15 cm) using sterile cotton swabs. All the culture plates were allowed to dry for about five minutes; wells were made using a sterile cork-borer (6 mm in diameter). Aliquots of 60 µl of extract dilutions, reconstituted in 50% DMSO (for organic extracts) and distilled water (for aqueous extracts) at concentrations of 400, 200, 100, 50, 25, 12.5, 6.25 mg/ml for Pod and Seed extracts respectively) and 50, 25, 12.5, 6.25, 3.13 and 1.56mg/ml, were applied in each of the wells in the culture plates previously seeded with the test organisms. The plates were incubated at 35-37°C for 24 h and 25-27°C for 48 h.10 µg/ml of gentamicin and 50 µg Nystatin served as positive controls. Antimicrobial activity was determined by measuring the zone of inhibition around each well. For each extract, three replicate trials were conducted against each organism.

***In vivo* hepatoprotective screening of seed and pod extracts**
The hepatoprotective activity of *P. nitida* seed and pod extracts were studied according to the method employed by Baheti *et al* (2006). A total of 40 albino rats were used. The rats were grouped into 8 groups of 5 per group, blood samples were collected from animals in each group and ALT (Alanine transaminase), AST (Aspartate transaminase), ALP (Alkaline phosphate) and Bilirubin were analysed for basal values, subsequently acute experimental liver injury was induced in each animals by single intraperitoneal administration of 1ml/kg Carbon tetrachloride (CCL_4) diluted with olive oil (1:1 vol/vol). The animals received treatment as follows: Groups 1, 2, 3, 4, 5, 6, 7 and 8 received 5ml/kg distilled water,

100mg/kg silymarin, 100mg/kg of seed extract, 100mg/kg of pod extract, 200mg/kg of seed extract, 200mg/kg of pod extract, 400mg/kg of seed extract and 400mg/kg of pod extract respectively daily for 2 weeks and on the 7th and 14th day post treatment, their ALT (Alanine transaminase), AST (aspartate transaminase), ALP (Alkaline phosphate) and Bilirubin levels assessed. The results were compared with the basal values and also the control

$$\%Reversibility = \frac{Injury\ Time\ - Treatment\ Time}{Injury\ Time}\ X\ \frac{100}{1}$$

This method was substituted or self-innovated from % inhibition.

***In vivo* Hepatoprotective screening for the fractions of the most active extract (Seed extract)**

The hepatoprotective activity of *P. nitida* seed fractions of n-hexane, ethyl acetate, aqueous and butanol were studied according to the method employed by. A total of 20 albino rats were used for this study. The animals were grouped into 4 groups of 5 rats each. Blood samples were collected from animals in each group and were analysed for basal ALT (Alanine transaminase), AST (Aspartate transaminase), ALP (Alkaline phosphate) and Bilirubin levels. Subsequently, acute experimental liver injury was induced in each animal by single intraperitoneal administration of 1ml/kg Carbon tetrachloride (CCL_4) diluted with olive oil (1:1 vol/vol). Then the animals were treated randomly as follows: Groups 1, 2, 3 and 4 received 400 mg/kg daily of n-hexane fraction, ethyl acetate fraction, butanol fraction and aqueous fraction of *P. nitida* seed extract respectively. These treatments lasted for 2 weeks and their ALT (Alanine transaminase), AST (aspartate transaminase), ALP (Alkaline phosphate) and Bilirubin levels assessed on the 7th and 14th day post treatment. The results were compared with the basal values and also the control group (Baheti, 2006).

Statistical analysis

The statistical significance of hepatoprotective activity was determined using one way analysis of variance (ANOVA) followed by Tukey-Kramer Multiple Comparisons Test. The values were expressed as mean ± standard mean error (SEM). $P \leq 0.05$was considered statistically significant. The mean of replicate measurements and standard deviation were determined for antimicrobial activity.

RESULTSAND DISCUSSION

Percentage yield of extracts: The percentage yield of the seed and pod extracts were 15% and 10% respectively. This compares well with the reported yield of aqueous and ethanol extracts of *P. nitida* seed of 11.08 % and 8.76 % respectively (Ubulom *et al*., 2011).The percentage yield of the seed extract fractions are shown in Table I with the butanol fraction having the highest yield. Although maceration has been reported to result in low extract yield compared to soxhlet and other methods of extraction (Ibrahim *et al*., 1997), it was used in this study as it does not require heating, thus preserving thermo-labile components.

Table 1: Yield from fractionation of 15 g of the ethanol crude extract of *P. nitida* seed

Extract/Fractions	Yield (%)
N-hexane fraction	11.43
Ethyl acetate fraction	12.38
Butanol fraction	12.95
Aqueous fraction	12.72

Acute toxicity study of *P. nitida* seed and pod extract

The amount of *P. nitida* seed and pod extract required to kill 50% of the test population LD_{50} is 4472.13 mg/kg. The seed extract up to 4000 mg/kg gave no mortality, but 5000 mg/kg produce mortality; while the pod extract up to 3000 mg/kg and 5000 mg/kg gave no mortality but 4000 mg/kg gave mortality.

Qualitative phytochemical analysis of seed and pod extracts of *P. nitida*.

Alkaloids, saponins, flavonoids, cardiac glycosides and terpenoids, have been reported to have antimicrobial activity (Okeke *et al*., 2001).

Table 2: Qualitative phytochemical analysis of seed, pod extracts and fractions of *P. nitida*

Phyto-chemical	*P. nitida* Seed	*P. nitida* Pod	n-Hexane fraction of seed extract	Ethyl acetate fraction of seed extract	Butanol fraction of seed extract	Aqueous fraction of seed extract
Alkaloid	++	++	+	+	+	++
Flavonoid	++	++	+	+	-	+
Tannin	-	-	-	+	-	-
Saponin	+	+	-	+	-	+
Cardiac glycoside	++	+	+	+	-	-
Terpenoid	++	+	++	++	+	++
Steroid	-	-	-	-	-	-
Protein	++	++	-	-	-	-
Carbohydrate	+	+	-	-	-	-

KEY: + =mildly present, ++ =moderately present, +++ = abundantly present, - = absent

Quantitative Phytochemical Analysis of *Picralima nitida* Seed and Pod extract

The quantity of alkaloids, flavonoids, saponins and tannins present (Edeogha *et al*., 2000)

The result of the quantitative analysis of *P. nitida* seed is shown in Table 3. The alkaloid content of the seed and pod extracts ranged from 6% to 7.6 % respectively, with a higher level in the pod. Pure isolated alkaloids and the synthetic derivatives have been reported to have analgesic, antispasmodic and bacterial properties (Stray, 1998). Saponins have been reported to show tumour inhibiting activity on experimental animals (*Rattusnovergicus*) (Akindaunsi and Salawu, 2005). The saponin composition of the seed and pod extract ranged from 9.8 % to 9.5 % respectively, saponins are more in the seed. 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). The flavonoid composition of the seed and pod extract ranged from 10.2 % to 1.2 % respectively; flavonoids have been reported with hepatoprotective effects (Yoshikawa *et al*., 2003). The cardiac glycosides therapeutically have the ability to increase the efficiency of the heart and at the same time steady excess heart beats without strain to the organ.

Table 3: Quantitative Phytochemical Analysis of *Picralima nitida* Seed and Pod extracts

PhytochemistrySeed	Pod	
Tannin	0.42 %	0.33 %
Alkaloid	6.0 %	7.6 %
Saponin	9.8 %	9.5 %
Flavonoid	10.2 %	1.2 %

Proximate analysis of *Picralima nitida* Seed and Pod extract

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 %

presented in Table 4. Low moisture content tends to obstruct or prevent microbial contamination and chemical degradation (Hussain *et al.,* 2009). The moisture content of the seed and pod extract ranged from 1.2 % to 3.8 % respectively. The results are within considerable limits and are comparable with other reported data (Horax *et al.,* 2010). The water soluble extractive value indicates the quality and safety of the crude drug.

Table 4: Proximate Analysis of *Picralima nitida* Seed and Pod extract

Proximate Analysis	Seed Extract	Pod Extract
Water soluble extractive value	2.8 %	3.8 %
Moisture content	1.2 %	3.8 %
Total ash	4 %	5 %
Acid insoluble ash	1 %	1 %
Water soluble ash	3.5 %	3.5 %
Crude fibre	3.6 %	5.2 %

Antimicrobial screening

The susceptibility pattern of the test organisms or isolates to the commonly used antibiotics is shown in Table 5 using Hudzicki, 2009.

According to the Clinical and Laboratory Standard Institute, 2015; which states that inhibitory zone diameter of antimicrobial agents to the nearest whole mm, ≥ 20 is sensitive, 15-19 is intermediate and ≤ 14 is resistant. *Staphylococcus aureus* was sensitive to the seed extract (at 400 mg/ml) and *Bacillus subtilis* was intermediate (both Gram positive bacteria). Amongst the Gram negative bacteria, *E. coli* was resistant to the extract and also *Pseudomonas aeruginosa* was resistant. The test fungi, *Aspergillus niger* and *Candida albican* were resistant to the extract at concentration of 400 mg/ml (Table 6).

Staphylococcus aureus and *Bacillus subtilis* were intermediate to the pod extract (at 400 mg/ml) (both Gram positive bacteria).

Amongst the Gram negative bacteria, *E. coli* and *Pseudomonas aeruginosa* were resistant to the pod extract. The test fungi; *Aspergillus niger* and *Candida albican* were resistant at400 mg/ml of pod extract as shown in Table 7.

The results indicated that the seed and pod extracts of *P. nitida* inhibited the growth of both Gram positive and Gram negative bacteria test isolates but the test fungi isolates were resistant. The result also indicates that Gram positive organisms were more susceptible to the crude extracts than Gram negative organisms, with *S. aureus* being the most susceptible test organism and *P. aeruginosa* the most resistant.

The ethyl acetate fraction of *P. nitida* seed extract exhibited more antibacterial activity against all the test organisms compared to the butanol, aqueous and n-hexane fractions. *E.*

coli was sensitive to the ethyl acetate fraction at a concentration of 50 mg/ml and *Pseudomonas aeruginosa* (both Gram negative bacterial). Amongst the Gram positive bacteria, *Bacillus subtilis* had intermediate sensitivity to the ethyl acetate fraction at a concentration of 50 mg/ml while *Staphylococcus aureus* was resistant.It would therefore seem that the constituent(s) or the principle(s) responsible for the antimicrobial activity is largely in the ethyl acetate fraction (Tables 8-11).

From the *in-vitro* study, it can be deduced that *P. nitida* may be useful in the treatment of opportunistic infections caused by *Escherichia coli and Pseudomonas aeruginosa.* This is also compared with the seed extract of *P. nitida* (aqueous and ethanol), which did not inhibit the growth of *M. canis*, but did inhibit the growth of *A. flavus* and *C. albican*, with differing values of inhibition zone diameter (Ubulom *et al*., 2011). The isolation and characterization of the active principle(s) for further investigations would greatly improve both the intensity and spectrum of activity.

Table 5: Antibiotic Susceptibility Profile of Test Organisms Showing the Mean IZD

ANTIBIOTICS	**CONC.**	**IZD (mm)**			
		S. aureus	***B. subtilis***	***P. aeruginosa***	***E. coli***
Gentamicin	10 µg	22 ± 0.7	25 ± 1.4	14 ± 1.4	34 ± 0.7
Cefixime	5 µg	12 ± 0.7	0 ± 0	0 ± 0	0 ± 0
Ofloxacin	5 µg	12 ± 0.7	23 ± 0.7	18 ± 0.7	26 ± 0.7
Augmentin	30 µg	26 ± 0.7	12 ± 2.8	0 ± 0	34 ± 0.7
Nitrofuraton	30 µg	30 ± 1.4	26 ± 2.8	0 ± 0	30 ± 0.7
Ciprofloxacin	5 µg	13 ± 0.7	28 ± 2.8	27 ± 4.2	30 ± 0.7
Ceftazidime	30 µg	13 ± 0.7	0 ± 0	22 ± 0.7	10 ± 2.1
Cefuroxime	30µg	24 ± 0.7	10 ± 0.7	0 ± 0	24 ± 0.7

Table 6: Antimicrobial activity of Seed extracts of *Picralima nitida on the test organisms*

Test organisms	Seed extract/ concentration (mg/mL) / IZD (mm)						
	400	**200**	**100**	**50**	**25**	**12.5**	**Gentamicin (10 µg/mL)**
Staphylococcus aureus	21 ± 4.2	14 ± 1.4	10 ± 1.4	6 ± 1.4	3 ± 1.4	0 ± 0	17
Bacillus subtilis	16 ± 0.7	13 ± 0.7	9 ± 0.7	5 ± 0.7	0 ± 0	0 ± 0	17
Pseudomonas aeruginosa	6 ± 1.4	6 ± 0.7	5 ± 0.7	4 ± 0.7	4 ± 0.7	0 ± 0	17
Escherichia coli	14 ± 0	10 ± 1.4	8 ± 0.7	4 ± 0.7	3 ± 0.7	0 ± 0	19
							Nystatin (50 µg/mL)
Candida albicans	2 ± 0	0 ± 0	0 ± 0	0 ± 0	0±0	0 ± 0	17
Aspergillus niger	0±0	0±0	0±0	0±0	0±0	0±0	13

The results are expressed as mean ± standard deviation

Table 7: Antimicrobial activity of pod extracts of *Picralima nitida* on the test organisms

Test organisms	**Pod extract/ concentration (mg/mL) / IZD (mm)**						
	400	**200**	**100**	**50**	**25**	**12.5**	**Gentamicin (10 µg /mL)**
Staphylococcus aureus	18 ± 9.2	10 ± 1.4	8 ± 1.4	5 ± 0.7	3 ± 0.7	0 ± 0	17
Bacillus subtilis	16 ± 0.7	11 ± 1.4	10 ± 1.4	10 ± 0 .7	7 ± 0.7	0 ± 0	17
Pseudomonas aeruginosa	4 ± 0.7	4 ± 0.7	4 ± 0.7	4 ± 1.4	4 ± 0.7	0 ± 0	17
Escherichia coli	11 ± 0.7	7 ± 1.4	6 ± 0.7	6 ± 0.7	4 ± 0.7	0 ± 0	19
							Nystatin (50 µg/mL)
Candida albican	9 ± 0.7	3 ± 0.7	0 ± 0	0 ± 0	0 ± 0	0 ± 0	17
Aspergillus niger	8 ± 0.7	2 ± 0.7	0 ± 0	0 ± 0	0 ± 0	0 ± 0	**13**

The results are expressed as mean ± standard deviation

Table 8: Antimicrobial activity of n-hexane fraction of *Picralima nitida* seed extract

Concentration	***S. aureus***	***B. subtilis***	***P. aeruginosa***	***E. coli***	***C. albican***	***A. niger***
50	0 ± 0	4 ± 0.7	10 ± 2.1	6 ± 0.7	0 ± 0	0 ± 0
25	0 ± 0	0 ± 0	6 ± 0.7	0 ± 0	0 ± 0	0 ± 0
12.5	0 ± 0	0 ± 0	5 ± 0.7	0 ± 0	0 ± 0	0 ± 0
6.25	0 ± 0	0 ± 0	4 ± 0.7	0 ± 0	0 ± 0	0 ± 0
3.13	0 ± 0	0 ± 0	3 ± 0.7	0 ± 0	0 ± 0	0 ± 0
1.56	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Gentamicin (10 mg/mL)	17	17	17	19	Nd	Nd
Nystatin (50 µg/mL)	Nd	Nd	Nd	Nd	17	13

The results are expressed as mean ± standard deviation

Table 9: Antimicrobial activity of Ethyl acetate fraction of *Picralima nitida* Seed extract

Concentration	***S. aureus***	***B. subtilis***	***P. aeruginosa***	***E. coli***	***C. albican***	***A. niger***
50	14 ± 0.7	19 ± 0.7	21 ± 0.7	22 ± 0.7	0 ± 0	0 ± 0
25	11 ± 0.7	15 ± 0.7	19 ± 0.7	19 ± 0.7	0 ± 0	0 ± 0
12.5	6 ± 0.7	14 ± 0.7	18 ± 0.7	16 ± 0.7	0 ± 0	0 ± 0
6.25	4 ± 0.7	12 ± 0.7	15 ± 0.7	13 ± 0.7	0 ± 0	0 ± 0
3.13	0 ± 0	8 ± 3.5	13 ± 0.7	6 ± 0.7	0 ± 0	0 ± 0
1.56	0 ± 0	5 ± 2.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Gentamicin (10 mg/mL)	17	17	17	19	Nd	Nd
Nystatin (50 µg/mL)	Nd	Nd	Nd	Nd	17	13

The results are expressed as mean ± standard deviation

Table 10: Antimicrobial activity of Butanol fraction of *Picralima nitida* Seed extract

Concentration	***S. aureus***	***B. subtilis***	***P. aeruginosa***	***E. coli***	***C. albican***	***A. niger***
50	11 ± 0.7	9 ± 0.7	4 ± 0.7	10 ± 0.7	0 ± 0	0 ± 0
25	5 ± 0.7	7 ± 0.7	2 ± 0.7	6 ± 0.7	0 ± 0	0 ± 0
12.5	2 ± 2.1	6 ± 0.7	0 ± 0	3 ± 2.8	0 ± 0	0 ± 0
6.25	0 ± 0	5 ± 0.7	0 ± 0	2 ± 2.8	0 ± 0	0 ± 0
3.13	0 ± 0	4 ± 0.7	0 ± 0	0 ± 0	0 ± 0	0 ± 0
1.56	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Gentamicin (10 mg/mL)	17	17	17	19	Nd	Nd
Nystatin (50 µg/mL)	Nd	Nd	Nd	Nd	17	13

The results are expressed as mean ± standard deviation

Table 11: Antimicrobial activity of aqueous fraction of *Picralima nitida* seed extract

Concentration	*S. aureus*	*B. subtilis*	*P. aeruginosa*	*E. coli*	*C. albican*	*A. niger*
50	7 ± 0.7	9 ± 0.7	0 ± 0	14 ± 0.7	0 ± 0	0 ± 0
25	4 ± 0.7	6 ± 0.7	0 ± 0	12 ± 0.7	0 ± 0	0 ± 0
12.5	0 ± 0	5 ± 0.7	0 ± 0	9 ± 0.7	0 ± 0	0 ± 0
6.25	0 ± 0	3 ± 0.7	0 ± 0	5 ± 0.7	0 ± 0	0 ± 0
3.13	0 ± 0	2 ± 0.7	0 ± 0	2 ± 0.7	0 ± 0	0 ± 0
1.56	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Gentamicin (10 mg/mL)	17	17	17	19	Nd	Nd
Nystatin (50 µg/mL)	Nd	Nd	Nd	Nd	17	13

The results are expressed as mean ± standard deviation. Gentamicin **(10 mg/mL)** served as the positive control for bacterial isolates while Nystatin **(50 µg/mL)** served as positive control for the fungi isolates.

Hepatoprotective Screening

The result of the hepatotoxic effect in albino rats demonstrated that the ethanol extract of *P. nitida* seed possess significant hepatoprotective activity compared with *P. nitida* pod extract and the positive control. 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 %) with the highest percentage reversibility of 82 %, n-hexane fraction (69 %), aqueous fraction (75 %) and butanol fraction (74 %) shown in Table 16. When compared with the different fractions (n-hexane, ethyl acetate, butanol and water), the ethyl acetate fraction has a significant percentage reversibility effect when administered for 7days and 14 days respectively. The results of this study, show that seed and pod extracts of *P. nitida* produced a reduction in liver enzymes (ALT, AST, and ALP) and bilirubin. When injury time was compared to *P. nitida* seed extract at 400 mg/kg once daily for 7 and 14 day treatment, ethyl acetate fraction had the highest percentage reversibility at 72 % and 84 %, which reversed significantly at $P \leq 0.05$, therefore the crude ethanol extract and the ethyl acetate fraction of the seed of *P. nitida* has greater reversibility effect on the liver damage than the pod extract, when compared with the seed of *Garcinia kola* (Heckel) when administered at a dose of 1.2 g/kg, three times a week for two weeks (14 days) consecutively, inhibited the CCl_4 mediated decrease in the activities of these enzymes by 60, 65, 55 and 63 % respectively (Farombi, 2000).

Table 12: Effect of *P. nitida* Seed and Pod extract (AST test)

Treatment	% Reversibility Day 7	% Reversibility Day 14
5ml/kg Distilled water	16.35 ± 3.5^{ns}	26.44 ± 2^{ns}
100mg/kg Silymarin	46.89 ± 0.9^{ns}	$69.86 \pm 1.3^{***}$
100mg/kg *P. nitida* pod	26.7 ± 0.5^{ns}	40.78 ± 0.9^{ns}
200mg/kg *P. nitida* pod	34.27 ± 1.0^{ns}	$52.58 \pm 1.1^{**}$
400mg/kg *P. nitida* pod	$54.76 \pm 0.9^{**}$	$64.29 \pm 0.6^{***}$
100mg/kg *P. nitida* seed	29.25 ± 0.6^{ns}	41.98 ± 0.9^{ns}
200mg/kg *P. nitida* seed	37.96 ± 1.1^{ns}	$57.87 \pm 0.8^{**}$
400mg/kg *P. nitida* seed	$59.52 \pm 0.6^{**}$	$76.19 \pm 0.7^{***}$

Table 13: Effect of *P. nitida* Seed and Pod extract (ALT test)

Treatment	% Reversibility Day 7	% Reversibility Day 14
5ml/kg Distilled water	22.73 ± 1.6^{ns}	34.09±1.0^{ns}
100mg/kg Silymarin	58.62 ± 0.4**	68.76 ± 0.9***
100mg/kg *P. nitida* pod	34.48 ± 0.4^{ns}	41.53 ± 0.9^{ns}
200mg/kg *P. nitida* pod	42.42 ± 0.2^{ns}	51.52 ± 1.1**
400mg/kg *P. nitida* pod	54.64 ± 0.4**	64.71 ± 0.6***
100mg/kg *P. nitida* seed	39.15 ± 0.2^{ns}	44.22 ± 0.9^{ns}
200mg/kg *P. nitida* seed	43.29 ± 0.4^{ns}	54.36 ± 0.8**
400mg/kg *P. nitida* seed	56.57 ± 0.7**	76.77 ± 0.7***

Table 14: Effect of *P. nitida* Seed and Pod extract (ALP test)

Treatment	% Reversibility Day 7	% Reversibility Day 14
5ml/kg Distilled water	16.19 ± 0.7^{ns}	17.96 ± 0.7^{ns}
100mg/kg Silymarin	41.36 ± 0.7^{ns}	53.8 ± 0.7**
100mg/kg *P. nitida* pod	22.74 ± 0.8^{ns}	30.2 ± 0.5^{ns}
200mg/kg *P. nitida* pod	28.98 ± 0.7^{ns}	34.29 ± 0.7^{ns}
400mg/kg *P. nitida* pod	47.1 ± 0.7^{ns}	57.68 ± 0.7**
100mg/kg *P. nitida* seed	25.97 ± 0.8^{ns}	36.55 ± 0.5^{ns}
200mg/kg *P. nitida* seed	32.52 ± 0.7^{ns}	55.60 ± 0.7**
400mg/kg *P. nitida* seed	57.68 ± 0.7**	68.2 ± 0.7***

Table 15: Effect of *P. nitida* Seed and Pod extract (Bilirubin test)

Treatment	% Reversibility Day 7	% Reversibility Day 14
5ml/kg Distilled water	4.35 ± 0.8^{ns}	10.87 ± 0.6^{ns}
100mg/kg Silymarin	33.15 ± 0.9^{ns}	58.7 ± 0.9**
100mg/kg *P. nitida* pod	27.72 ± 0.9^{ns}	30.98 ± 0.9^{ns}
200mg/kg *P. nitida* pod	39.13 ± 0.7^{ns}	45.11 ± 0.7^{ns}
400mg/kg *P. nitida* pod	50.54 ± 0.9**	67.39 ± 0.9***
100mg/kg *P. nitida* seed	29.35 ± 0.7^{ns}	32.61 ± 0.7^{ns}
200mg/kg *P. nitida* seed	45.11 ± 0.8^{ns}	58.7 ± 0.8**
400mg/kg *P. nitida* seed	55.43 ± 0.7**	74.46 ± 0.7***

Table 16: Effect of the fractions of *P. nitida* Seed extract (AST test)

Treatment	% Reversibility Day 7	% Reversibility Day 14
N-hexane Fraction 400 mg/kg	62.65 ± 0.6***	69.76 ± 0.59***
Ethyl acetate Fraction 400 mg/kg	74.46 ± 0.93***	82.97 ± 0.55***
Aqueous Fraction 400 mg/kg	71.53 ± 0.78***	75.8 ± 0.72***
Butanol Fraction 400 mg/kg	58.44 ± 0.64***	74.77 ± 0.6***

Table 17: Effect of the fractions of *P. nitida* Seed extract (ALT test)

Treatment	% Reversibility Day 7	% Reversibility Day 14
N-hexane Fraction 400 mg/kg	67.53 ± 4.89***	69.13 ± 4.47***
Ethyl acetate Fraction 400 mg/kg	66.97 ± 0.51***	71.95 ± 0.79***
Aqueous Fraction 400 mg/kg	48.49 ± 0.78^{ns}	49.28 ± 0.78^{ns}
Butanol Fraction 400 mg/kg	55.98 ± 0.78***	65.94 ± 0.76***

Table 18: Effect of the fractions of *P. nitida* Seed extract (ALP test)

Treatment	% Reversibility Day 7	% Reversibility Day 14
N-hexane Fraction 400 mg/kg	21.44 ± 0.78*	32.37 ± 0.59**
Ethyl acetate Fraction 400 mg/kg	30.77 ± 0.57**	43.04 ± 0.78***
Aqueous Fraction 400 mg/kg	8.85 ± 0.93^{ns}	11.4 ± 0.61^{ns}
Butanol Fraction 400 mg/kg	3.14 ± 0.75^{ns}	9.43 ± 0.75^{ns}

Table 19: Effect of the fractions of *P. nitida* Seed extract (Bilirubin test)

Treatment	% Reversibility Day 7	% Reversibility Day 14
N-hexane Fraction 400 mg/kg	19.64 ± 0.07^{ns}	27.98 ± 0.04^{ns}
Ethyl acetate Fraction 400 mg/kg	60.61 ± 0.07***	67.27 ± 0.07***
Aqueous Fraction 400 mg/kg	1.21 ± 0.07^{ns}	18.18 ± 0.07^{ns}
Butanol Fraction 400 mg/kg	4.85 ± 0.007^{ns}	11.52 ± 0.007^{ns}

All values are expressed as mean ± standard error of mean. N = 5 in each group. $P < 0.05$
^{Ns} indicates not significant; $P > 0.05$. *indicates Significant; $P \leq 0.05$. **indicates significant; $P \leq 0.01$ and ***indicates extremely significant; $P \leq 0.001$

HPLC Chromatogram:

The HPLC chromatogram of *P. nitida* ethyl acetate VLC fractions of seed extract and pod extract, showed the presence of many compounds; these compounds were identified based on comparison of UV scan and similarity of UV scan data in the inbuilt library. The chromatograms of *P. nitida* ethyl acetate VLC fractions of seed extract showed the presence of Cerebroside, Pestalamide, Scorzodihydrostilbene A, Septicine, Cycloananyl tryptophan, Enniatin B and Shanzin methyl ester, while that of *P. nitida*pod extract showed the presence of Neurolenin B, Papuamine, Nigricinol and Fettsaure. Figures 1- 6 are the HPLC chromatograms and UV scan of constituents of six VLC fractions of the ethyl acetate fraction of the seed while Fig. 7 is the HPLC chromatogram and UV scan of the crude pod extract**.**

Some of these constituents identified from *Picralima nitida* seed and pod extracts havebeen reported to have antimicrobial and hepatoprotective activities. In recent study the ethanolic extract of the fruit-rind of *Picralima nitida* was analyzed by Gas chromatography-mass spectrometry (GC-MS). Seven different phytochemical compounds were characterized, including : 1,2,3,5-cyclohexanetetrol(23.04%), alpha-methyl mannofuranoside(70.61%), hexadecanoic acid, methyl ester(1.46%), tetradecanoic acid ethyl ester(0.70%), 12-octadecenoic acid methyl ester(1.28%), Z,E-3,13-octadecadien-1-ol (1.65%) and N,N-dimethyldodecanamide (1.26%) Okenwa and Yusuf (2017). Pyranonigrin A has been reported to have antifungal and antioxidant activities (Schlingmann, 2007). Cerebrosides were characterized as antigenic molecules directly or indirectly involved in cell growth or differentiation in *P. boydii* (Pinto *et al*., 2002), *C. albican* (Pinto *et al*., 2002), *A. nidulans* (Levery *et al*., 2002) and *A. fumigatus* (Levery *et al*., 2002). The cerebroside characterized from the fruit of *Lycium chinense* Miller (Solanaceae), possessed hepatoprotective activity on cultured rat hepatocytes exposed to carbon tetrachloride (CCl_4) (Kim *et al*., 1999). Pestalamides are bioactive metabolites from the plant pathogenic fungus *Pestalotiopsistheae* with antimicrobial activity (Gang *et al*., 2008). Scorzodihydrostilbene is a natural phenol and one of the stilbene derivatives; it has antimicrobial activity in *Scorzonera radiate* (Wang *et al*., 2009). Septicine is an alkaloid found in plants, they have both antimicrobial and hepatoprotective activity on the methanol extract of *Tylophoraindica* (Reddy *et al*., 2009). Shanzin methyl ester is an ester and has been reported to have hepatoprotective activity (Gaurav *et al*., 2009). Enniatins are bioactive compounds produced by several strains of *Fusarium sp*. It exhibits antibacterial activity against

Escherichia coli, *Enterococcus faecium*, *Salmonella enterica*, *Shigella dysenteriae*, *Listeria monocytogenes*, *Yersinia enterocolitica*, *Clostridium perfringens*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, (Meca *et al*., 2011). Enniatin B is an antibiotic and fungal metabolites generated by multiple *Fusarium* strains (Behm *et al*., 2009). Enniatin B has been reported to have hepatoprotective activity (Ozbeke *et al*., 2003). Cycloananyl tryptophan is a tryptophan rich hexapeptides, which has antimicrobial activity and selectivity of arginine- and tryptophan-containing hexapeptides (Dathe *et al*., 2004). It also possesses hepatoprotective activity (Modgil, 2004). Neurolenin B is a class of sesquiterpene found in *L. Mexicana*, hepatoprotective activity (Berger *et al*., 2001). Papuamine is a pentacyclic alkaloid with antifungal and anti-mycobacterial activity. Papuamine has been shown to inhibit the growth of the dermatophyte *Trichophyton mentagrophytes* and *Mycobacterium marinum* (Barker *et al*., 2007). Nigricinol is a bioactive metabolite with antimicrobial activity (Akpotu *et al*., 2015). Fettsaure are esters and exhibit antibacterial activity for both Gram positive and Gram negative bacterial (Pandey *et al*., 2006).

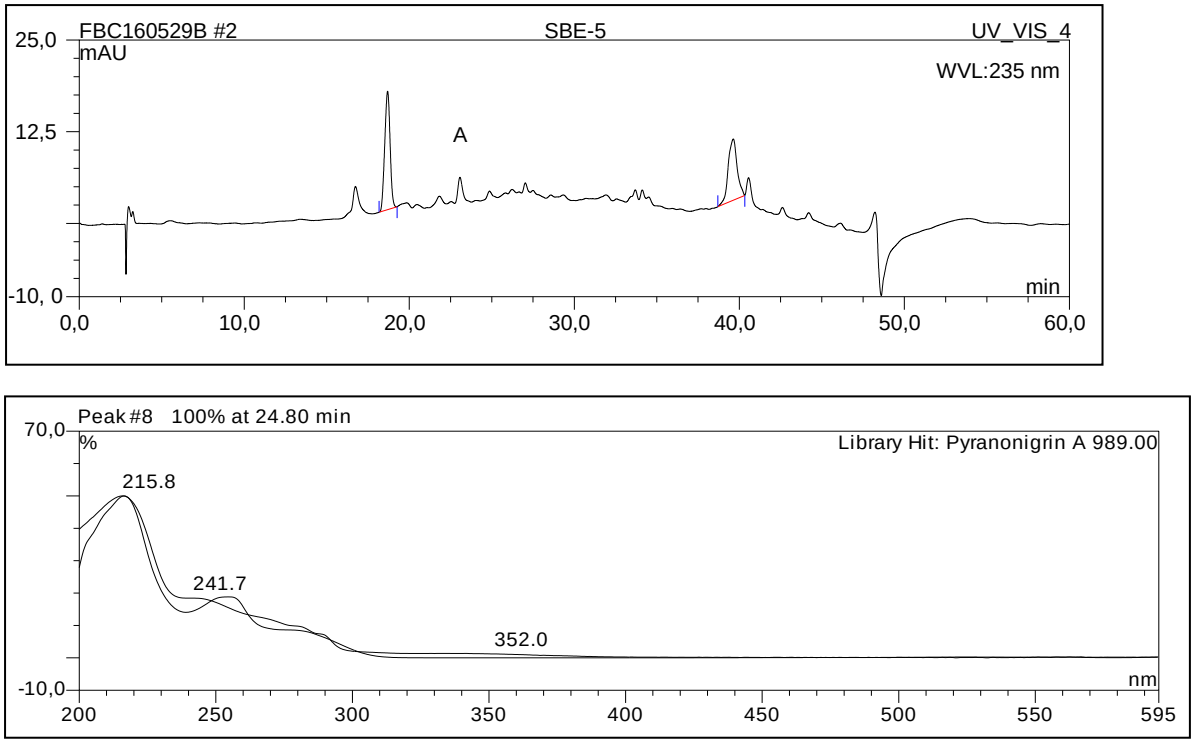


Figure 1: HPLC Chromatogram of SBE 5 (6:4), correlation ≥ 987
KEY; A- Pyranonigrin A Hit 989.00 (Rt = 24.80 min)

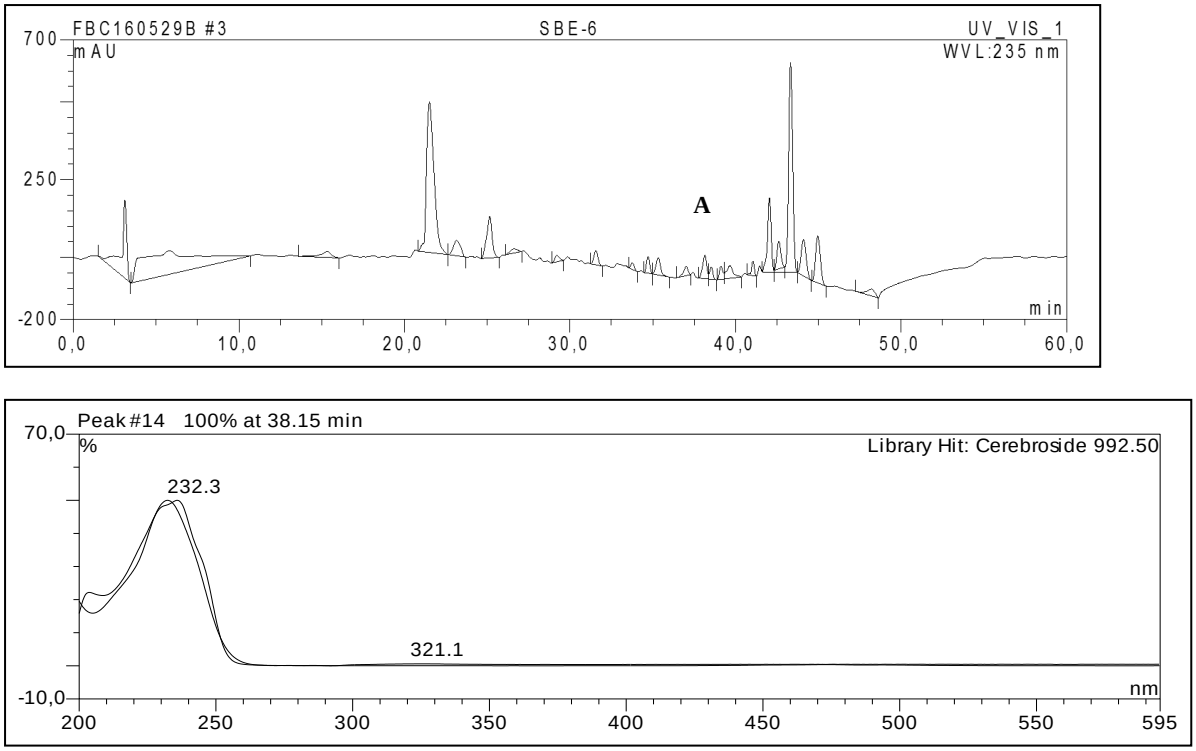
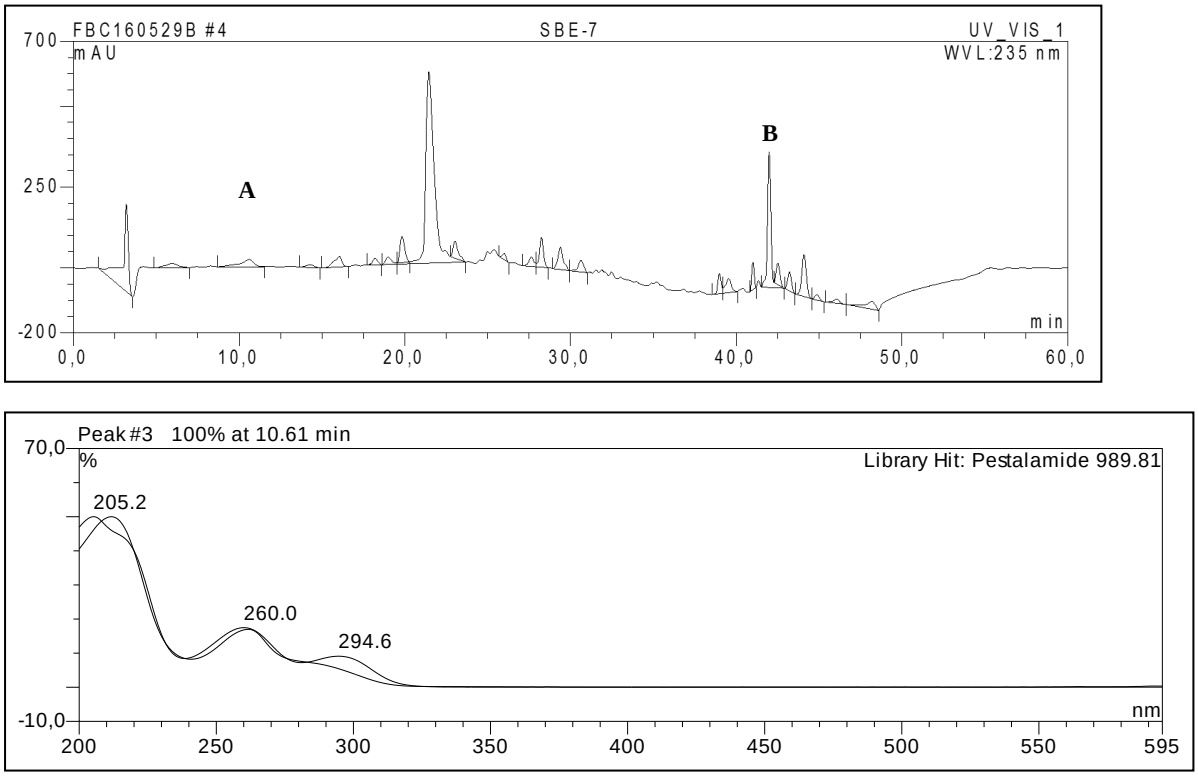


Figure 2: HPLC Chromatogram of SBE 6(5:5), correlation ≥ 987
KEY; A- Cerebroside Hit 992.50 (Rt = 38.15 min)



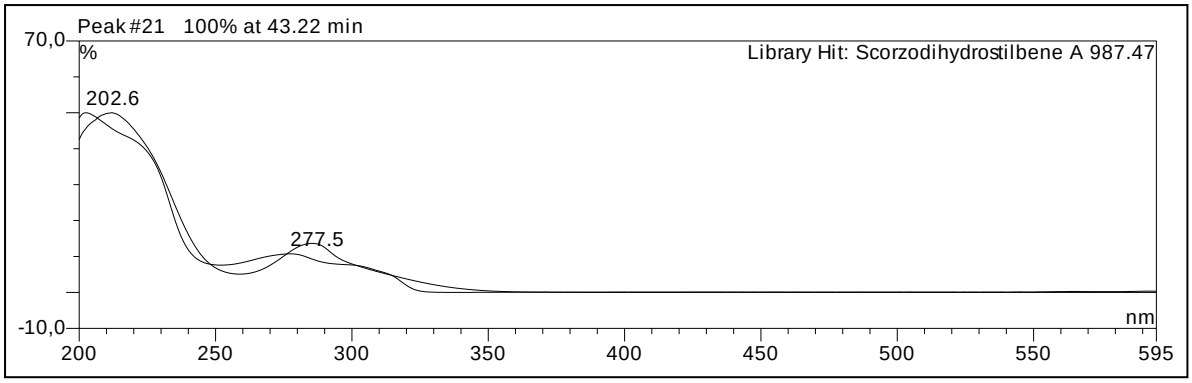
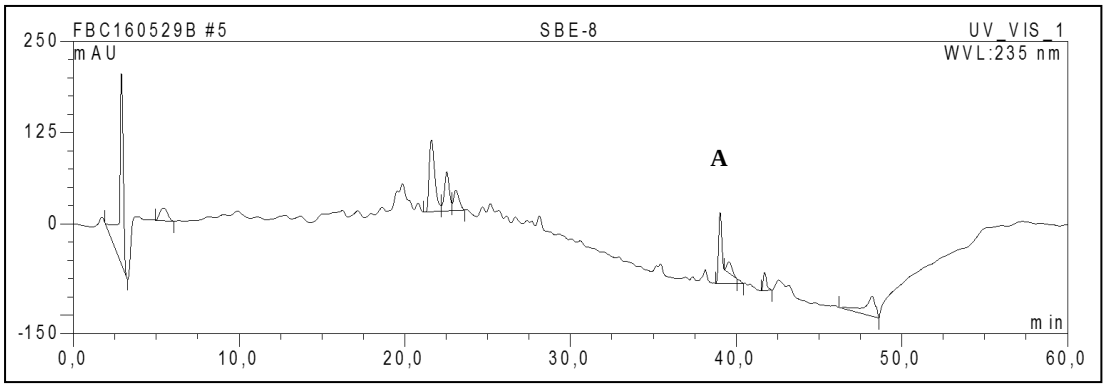


Figure 3: HPLC Chromatogram of SBE 7(4:6), correlation ≥ 987
KEY; A- Pestalamide Hit 989.81 (Rt = 10.61 min)**, B**- Scorzodihydrostilbene A Hit 987.47 (Rt = 43.22 min)



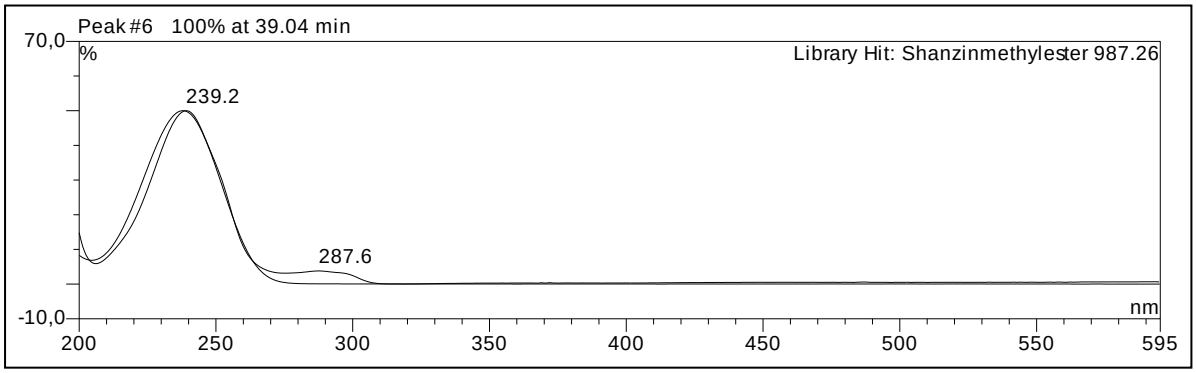


Figure 4: HPLC Chromatogram of SBE 8(3:7), correlation ≥ 987
KEY; A- Shanzi methyl ester Hit 987.26 (Rt = 39.04 min)

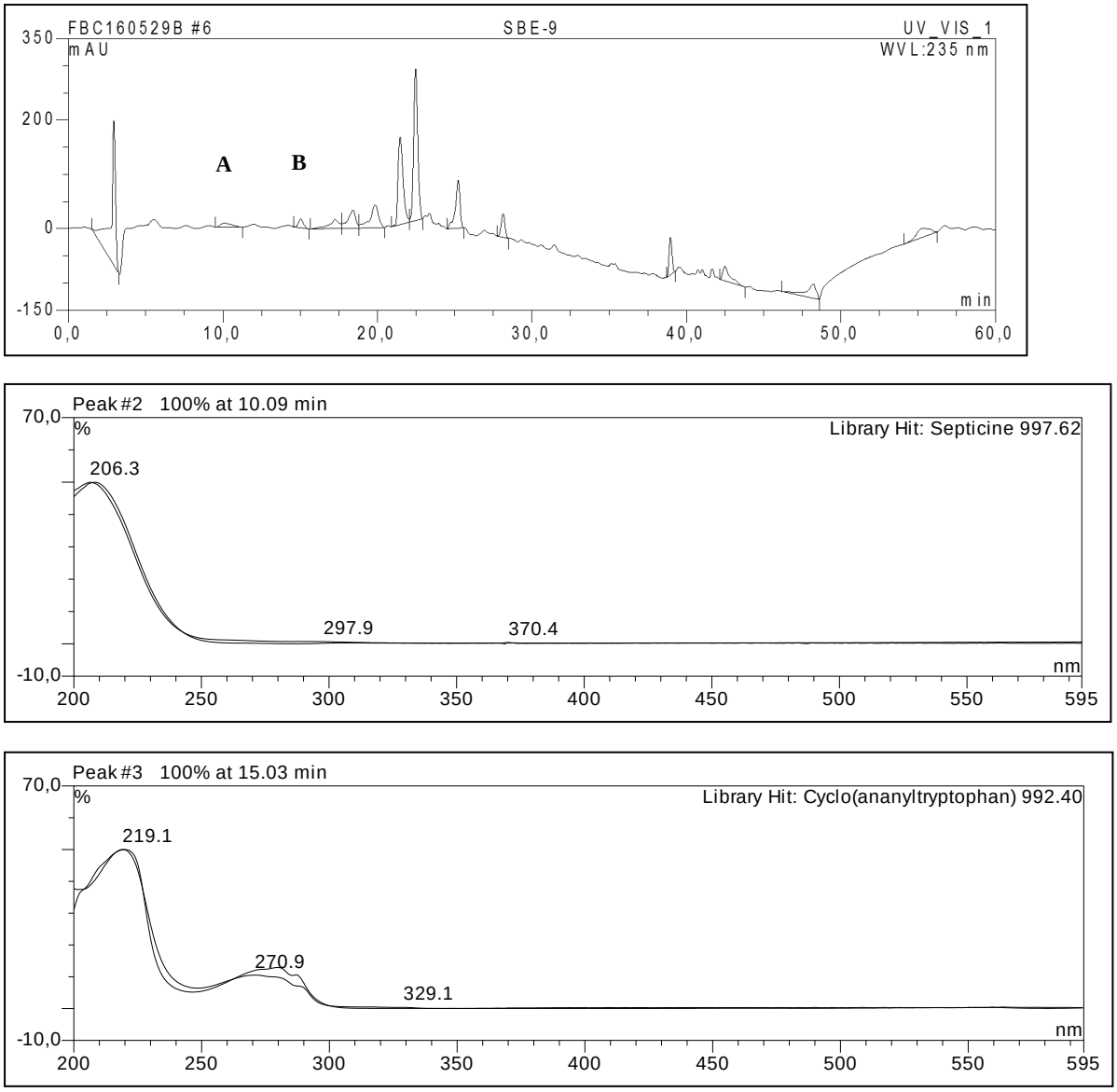
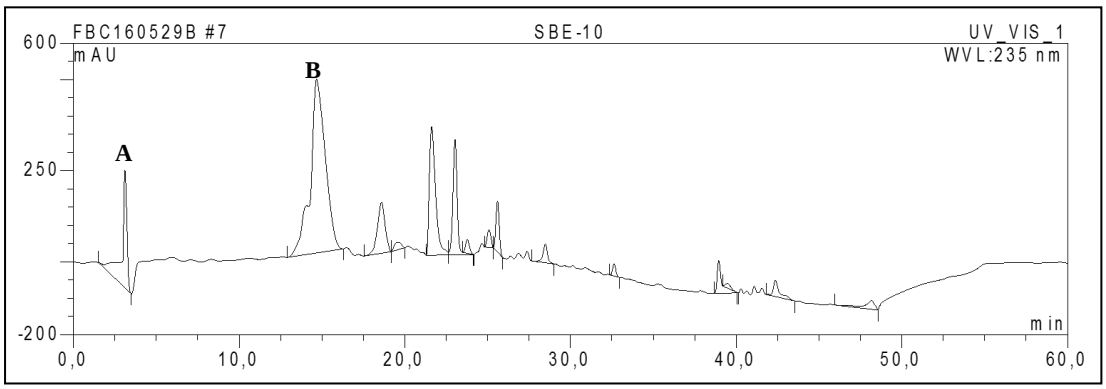


Figure 5: HPLC Chromatogram of SBE 9(2:8), correlation ≥ 987
KEY; A- Septicine Hit 998.37 (Rt = 55.32 min)**, B**- Cyclo (ananyl tryptophan) Hit 992.4 (Rt = 15.03 min)



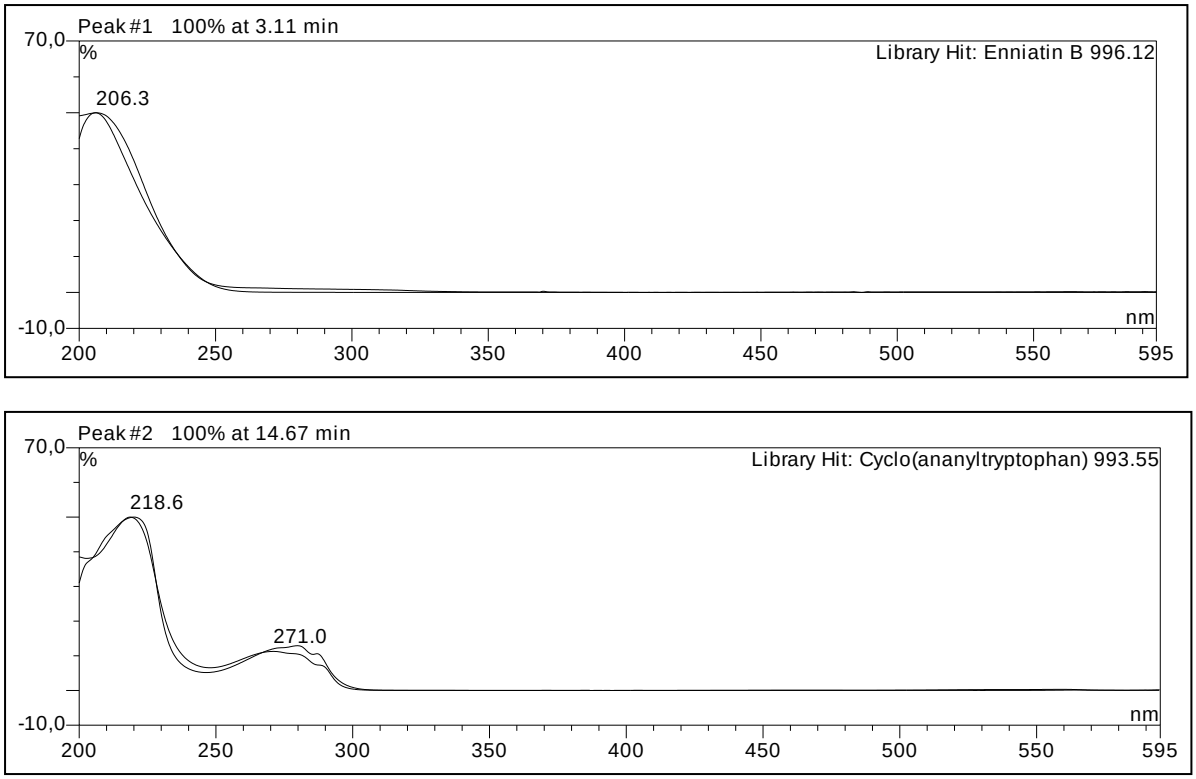
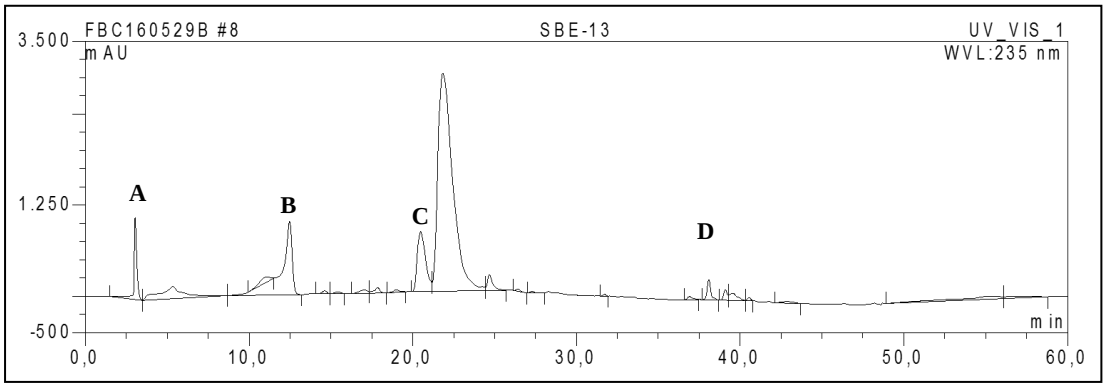
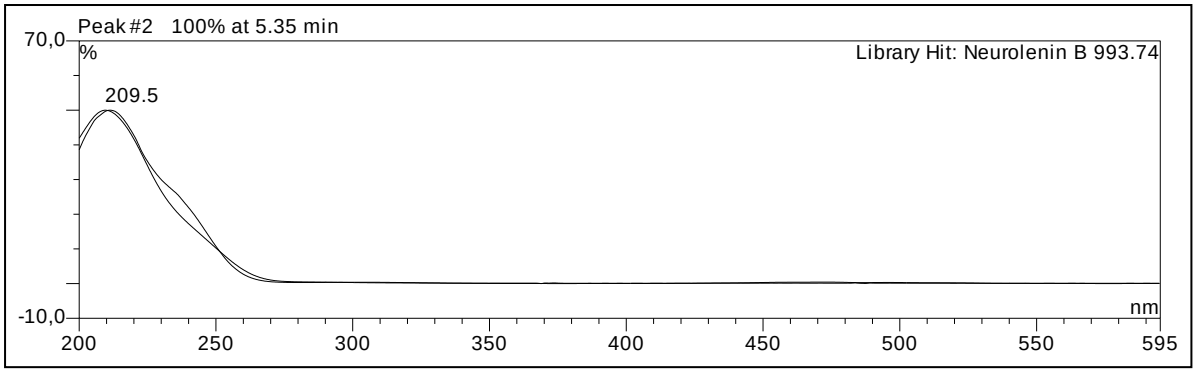


Figure 6: HPLC Chromatogram of SBE 10(1:9), correlation ≥ 987

KEY; A- Enniatin B Hit 996.12 (Rt = 3.11 min), **B-** Cyclo (ananyl tryptophan) Hit 993.74 (Rt = 14.67 min)





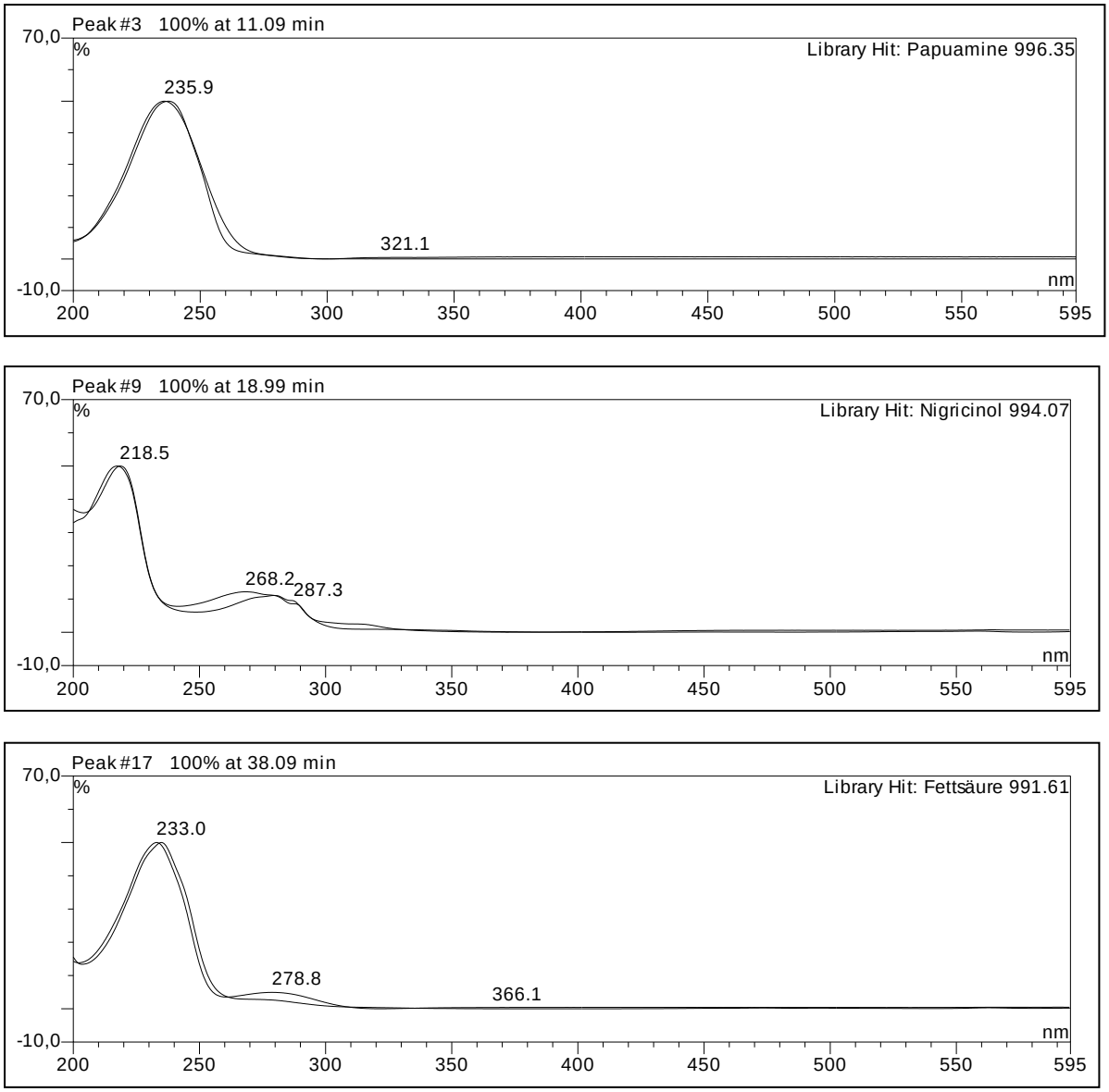


Figure 7: HPLC Chromatogram of SBE 13 (*P. nitida* pod extract), correlation ≥ 987
KEY; A- Neurolenin B Hit 993.74 (Rt = 5.35 min), **B**- Papuamine Hit 996.35 (Rt = 11.09 min), C-Nigricinol Hit 994.07 (Rt = 18.99 min), **D**- Fettsaure Hit 991.61 (Rt = 38.09 min)

CONCLUSION

This study shows *P. nitida* seed and pod have hepatoprotective activity at 400 mg/kg once daily for 14 days in CCl_4 induced liver damage or injury. This justifies its use in traditional medicine in Southern Nigeria to treat liver problem.The crude extracts and the ethyl acetate fractions also have antimicrobial activity at 400 mg/ml and 50 mg/ml respectively. They may thus have a role in the treatment of infections caused by *E. coli and P. aeruginosa*. The ethyl acetate seed extract of *P. nitida*contains polyphenols, alkaloids, peptide, amide and ester. While the pod extract of *P. nitida* contains alkaloids, terpenoids, phenolic compounds and esters.

ETHICAL STATEMENT

All authors hereby declare that the principles of laboratory animal care "(NIH publication No 85-23, revised 1985) [22], were adopted.

REFERENCES

Akindahunsi A.A., Salawu S.O. (2005). Phytochemical screening and nutrient-antinutrient composition of selected tropical green leafy vegetables Afr. J. Biotech., 4: 497 – 501.

Akpotu M.O., Eze P.M., Ajaghaku D.L., Okoye F.B.C., Esimone C.O. (2015).

Antimicrobial activity of endophytic fungi isolated from Catharanthusroseus and Euphorbia hirta. Planta Med, p 81.

Alan L., Miller N.D. (1996). Antioxidant flavonoids: structure, function and chemical usage. Alt. Med. Rev., **1**: 103 – 111.

AOAC (2005).*Methods of Analysis of Association of Official Analytical Chemists*. 16th Edn., AOAC, Washington, DC, USA., pp: 600-792.

Baheti J.R., Goyal R.K., Shah G.B. (2006). *Hepatoprotective activity of Hemidesmusindicus*R. Br. (Asclepiadaceae) in rats. Ind. J. Exp. Biol. **44**: 399-402.

Barker, L.P., *et al* (2007).*Papuamine- a pentacyclic alkaloid and antifungal agent*.Planta Med. **73**: 559-563.

Behm C., Degen G.H., Föllmann W. (2009). *The Fusarium toxin enniatin B exerts nogenotoxic activity, but pronounced cytotoxicity in vitro*. Mol. Nutr. Food Res. **53**: 423 – 430.

Berger I., Passreiter C.M., Ca´ceres A., Kubelka W. (2001). *Antiprotozoal activity of Neurolaenalobata*.Phytother. Res. **15**: 327–330.

Chong, K.T., Pagano P.J. (1997).*In vitro combination of pnv-140690, a human immunodeficiency virus type 1 protease inhibitor with ritonavir against ritonavir-sensitive and resistant clinical isolates*. Antimicrob. Agents Chemo **41**(2): 2367-2377.

Clinical and Laboratory Standards Institue, *Performance Standards for Antimicrobial*

Susceptibility Testing; Twentieth Informational Supplement M100-S20, CLSI, Wayne, Pa, USA, 2010.

Dathe M.I., Nikolenko H., Klose J., Bienert M. (2004). *Cyclization increases the antimicrobialactivity and selectivity of arginine- and tryptophan-containing hexapeptides*. Biochemistry.US Nat Library of Med. NIH.**43** (28): 9140-50.

Deshwal N., Sharma A.K., Sharma P. (2011). *Review on hepatoprotective plants*. Int J Pharm Sci Rev Res. **7**: 15–26.

Duwiejua M., Woode E., Obiri D.D. (2002). *Pseudo-akuammigine, an alkaloid from Picralimanitida seeds has anti-inflammatory and analgesic actions in rats.* J Ethnopharmacol; 8;73-79.

Edeoga HO, Gomina A (2000). Nutritional values of some noncomventional leafy vegetables of Nigeria. J. Econ. Taxon. Bot. 24:7-
13.

Ekpo, M.A., Etim, P.C. (2009). Antimicrobial activity of ethanolic and aqueous extracts of *Sidaacuta* on microorganisms from skin infections. Journal of Medicinal Plant Research, **3** (9): 621-624.

Ezeamuzie I.C., Ojinnake M.C., Uzygna E.O., Oji S.E. (1994). *Anti-inflammatory, antipyretic and antimalarial activity of a West African medicinal plant-Picralimanitida*.Afr J Med Sci; 23; 85-89.

Fakeye T.O., Itiola O.A., Odelila H.A. (2000).*Evaluation of the antimicrobial property of stembark of Picralimanitida (Apocynaceae)*.Phytother Res; **14**: 386-390.

Farombi E.O. (2003).*African indigenous plants with chemotherapeutic potentials and biotechnological approach to the production of bioactive prophylactic agents*. African Journal of Biotechnology; **2**: 662-671.

Gang D., Lihua J., Liangdong G., Xulin C., Hua Z., Yongsheng C.(2008). *Pestalazines and Pestalamides, Bioactive Metabolites from the Plant Pathogenic Fungus Pestalotiopsistheae*. J. Nat. Prod. **71** (11), pp 1861–1865.

Gaurav L., Hemant K.S., Kamlesh K.P., Zeashan H. (2009).*Hepatoprotective effects of Calotropisgigantea extract against carbon tetrachloride induced liver injury in rats*. ActaPharm. ;**59**:89-96.

Handa S.S., Sharma A., Chakraborti K.K. (1991).*Natural products and plants as liver protecting drugs*. Fitoterapia.**57**: 307-45.

Horax R., Hettiarachchy N., Islam S. (2010). *Total Phenolic contents and phenolic acid constituents in 4 varieties of bitter melons (Momordicacharantia) and antioxidant activities of their extracts*. J Food Sci, **70**: 275-280.

Hugo, W.B., Russell A.D. (2003). *Pharmaceutical microbiology*, 6thEdn.Blackwell Science Publishers.

Hudzicki, J. (2009).*Kirby-Bauer Disk Diffusion Susceptibility Test Protocol*.ASM Microbe Library. American Society for Microbiology.http://www.microbelibrary.org/component/resource/laboratory-test/3189-kirby-bauer-disk-diffusionsusceptibility-test protocol

Hussain, K.; Ismail, Z.; Sadikun, A.; Ibrahim, P. (2009).*Proximate and qualitative analysis of different parts of Piper sarmentosum, and quantification of total amides in various extracts*. Phcog. Res. 1, 113–119.

Ibrahim M.B., Owolabi M.O., Onaolapo J.A. (1997). *Antimicrobial effects of extracts of leaf, stem and root bark of Anogiessusleicarpus on Staphylococcus aureus, Escherichia coli and Proteus vulgaris*. J. Pharm. Res. Dev., **2**: 20-26.

Jean A.A., Robert P.B., Quirico J., Nelson Z., Giselle T., Guy H.H. (2001). *Separation of crude plant extracts with high speed CCC for primary screening in drug discovery. J. Liquid Chromatogr*.**24** (11 & 12):1827-1840.

Kim S.Y., Lee E.J., Kim H.P., Kim Y.C., Moon A., KimY.C. (1999). *A novel cerebroside from Lyciumfructus preserves the hepatic glutathione redox system in primary cultures of rat hepatocytes*. Biol. Pharm. Bull. **22:** 873-875.

Leo MA, Arai M (1982). *Hepatotoxicity of vitamin A and CCl_4.* Gastroenterology, **82**: 194-205.

Levery S.B., Momany M., Lindsey R., Toledo M., Shayman J., Fuller M., Brooks K., Doong R.L., Straus A.H., Takahashi H.K. (2002).*Disruption of the glucosylceramide biosynthetic pathway in Aspergillusnidulans and Aspergillusfumigatus by inhibitors of UDP-Glyceramideglucosyltransferase strongly affects spore germination, cell and hyphal growth*. FEBS Lett**525**: 59-64.

Lorke D. (1983).*A new approach to practical acute toxicity testing*. Arch Toxicol.;**54**:275–87.

Meca G.I., Sospedra I., Valero M.A., Mañes J., Font G., Ruiz M.J. (2011). *Antibacterial activity of enniatin B, produced by Fusariumtricinctum in liquid culture, and cytotoxic effects on Caco-2 cells.*ToxicolMech Methods.**21**: 503-512.

Modgil M., Modgil R., Kumar R.(2004). *Carbohydrate and mineral content of chyote (Sechiumedule) and bottle gourd (Lagenariasiceraria)*. J. Hum. Ecol; **15**(2):157-159.

Nabavi S.M., Ebrahimzadeh M.A., Nabavi S.F., Bahramin F. (2009). *In vitro antioxidant activity of P. americana berries*. Pharmacol. Online, **1**: 81-88.

Okeke M.I, Iroegbu C.U., Eze E.N., Okoli A.S., Esimone C.O. (2001).*Evaluation of extracts of the root of Landolphiaowerrience for antibacterial activity*. *J. Ethnopharmacol*., **78**: 119-127.

OkenwaUchenna Igwe1 and Yusuf OzoveheUsman (2017).*Studies of the Chemical Constituents of the Fruit-rind of Picralimanitida (Apocynaceae) and Screening of its Antimicrobial Potency*.Pharma research library. Kaduna, Nig.

Osborn A.E. (2003), "*Saponins in Cereals*", Phytochemistry**62**: 1-4. WHO Traditional

Medicines Strategy, WHO/EDM/TRM/2001, 2002-2005.

Özbek H., Ugras S., Bayram I., Uygan I., Erdogan E., Öztürk A., Zübeyir H. (2004).

Hepatoprotective effect of Foeniculumvulgare essential oil: A carbon-tetrachloride induced liver fibrosis model in rats.Scand. J. Lab. Anim. Sci. No. 1. Vol. 31

Pandey A., Trivedi P., Kumar B., Palni L.M. (2006). *Characterization of a phosphate solubilizing and antagonistic strain of Pseudomonas putida (B0) isolated from a sub-alpine location in the Indian Central Himalayas*. In: Current microbiology. Volume **53** (2): 102-107.

Paula F.S., Kabeya L.M., Kanashiro A., Figueiredo A.S.G.D, Azzolini AE, Uyemura SA,
Lucisano-Valim YM (2009). *Modulation of human neutrophil oxidative metabolism and degranulation by extract of T. indica L. fruit pulp*, Food and Chem. Tox.**47**: 163–170.

Pinto M.R., Rodrigues M.L., Travassos L.R., Haido R.M.T., Wait R., Barreto-bergter E. (2002). *Characterization of glucosylceramides in Pseudallescheriaboydii and their involvement in fungal differentiation.*Glycobiology**12**: 251-260.

Reddy B.K., Balaji M., Reddy P., Reddy P.U., Sailaja G., Vaidyanath K. (2009). *African Journal of Biochemistry Research.* India. 3 (12); 393.

Schlingmann G., Taniguchi T., He H. , Bigelis R., Yang H.Y., Koehn F.E., Carter G.T.and Berova N. (2007). *Reassessing the structure of pyranonigrin*. J. Nat. Prod. **70** (7): 1180–1187

SofoworaA. (1993). *Medicinal plants and traditional medicine in Africa*, Spectrum Books, Nigeria. 2nd Ed. pp: 10-158.

Subbulakshmi G.K., Thalavaipandian A., Ramesh V., Bagyalakshmi V., Rajendran A (2012) *Bioactive endophytic fungal isolates of Biota orientalis (*L) Endl., *Pinusexcelsa* Wall. and*Thujaoccidentalis* L. Int J Adv Life Sci**4**:2277–7581

Ubulom P., Akpabio E., Udobi C.E., Mbon R. (2011). Antifungal Activity of aqueous and ethanolic extracts of *Picralimanitida* seeds on *Aspergillusflavus*, *Candida albicans*and *Microsporumcanis*. Research in Pharmaceutical Biotechnology, **3** (5): 57-60.

Wang Y., Edrada- Ebel R.A., Tsevegsuren N., Sendker J., Braun M., Wray V., Lin W., Proksch P.
(2009). *Dihydrostilbene derivatives from the Mongolian Medicinal Plant Scorzonera radiate*. Journal of Natural products.**72**: 671-675.

Yoshikawa M., Xu F., Morikawa T., Ninomiya K., Matsuda H. (2003). *Anastatins A and B, new skeletal flavonoids with hepatoprotective activities from the desert plant Anastaticahierochuntica*. Bioorg. Med. Chem. Lett., **13** (6): 1045-1049.