GC-MS ANALYSIS OF LEAF POWDER OF *PSYCHOTRIA MICROPHYLLA* AND ITS ACUTE TOXICITY ON *CLARIAS GARIEPINUS* (AFRICAN CATFISH) JUVENILES

[1]IBIAM, U. A, [1]Orji, O. U.; [1]AJA, P. M. and [2]Chukwu, C.J.

1. Department of Biochemistry, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria
2. Department of Chemistry, Evangel University, Akaeze, Ebonyi State, Nigeria

PRESENTATION OUTLINES

- Background Information
- Local Uses of *Psychotria Microphylla* Plant
- Phytochemical analysis of the plant extract
- GC-MS analysis of the leaf, stem and root extracts
- The acute toxicity Test for lethal concentration
- Findings
- Conclusion
- Acknowledgement

INTRODUCTION

- *Psychotria microphylla* Elmer is one of the *Psychotria species* found in the Eastern part of Nigeria.
- The genus *Psychotria* is one of the largest genera of flowering plants and the largest within Rubiaceae, with estimated 1000 to 1650 species distributed worldwide (Nepkroeff *et al*., 1999).

INTRODUCTION

- Native name is *Akwukwo Iyi* or *Oye* (Igbo)
- Some of its species are important in herbal medicine and have been used to treat various diseases (Khan *et al,* 2001; Kato *et al,* 2012).
- Infusion of the whole plant is used in Afikpo South Area of Ebonyi State, Nigeria, for fishing and prevention of insects from destroying crop vegetables

INTRODUCTION....

- Locally, this plant species are used for treating infections of the female reproductive system
- Bronchitis
- Gastrointestinal disturbances
- skin infections
- Fever
- headaches, earaches
- Eye disturbances

DR U. A. IBIAM HOLD SOME OF PLANT UPROOTED FROM THE SWAMP

DR U. A. IBIAM POSING WITH THE RESEARCH TEAM MEMBERS AND A LOCAL GUIDE

FIGURE 1. PSYCHOTRA MICROPHILLA PLANT

ROOT SYSTEM OF PSYCHOTRA MICROPHYILLA

FIGURE 2. PSYCHOTRA MICROPHYILLA ROOTS AND STEM PART

FIGURE 3. PSYCHOTRIA MICROPHYLLA LEAF (OYE LEAF OR AKWUKWO IYI)

INTRODUCTION: CLARIAS GARIEPINUS (AFRICAN CATFISH)

- The African catfish, *Clarias gariepinus* (Burchell, 1822) is the most common commercially available fish in Nigeria
- The business is a source of income to many youths and ageing, retired group of the Nigerian population
- Globally. it is widely cultivated and used as experimental fish (Musa and Omoregie, 1999).
- Hence it was selected for this study

INTRODUCTION CONT...

- Till date the chemical constituents of this species of psychotra is not known to the scientific community
- To the best of our knowledge, no GC-MS analysis of the plant extracts has been conducted or reported
- This works was carried out to fill this gap

COLLECTION AND IDENTIFICATION OF THE PLANT

- The fresh samples of *Psychotria microphylla* were collected from the wild at Afikpo South L.G.A of Ebonyi State, Southeastern Nigeria.

- The plant was identified and authenticated by Mr. Ozioko of the International Bioresources and Research Centre, Nsuka, Nigeria.

PROCUREMENT AND ACCLIMATIZATION OF FISH

- A total of 72 healthy fresh water fish *C. gariepinus* (mean weight 205±13.08g and body length of 30.10±3.44 cm were procured from Chiboy's Farm, Abakaliki, Ebonyi State.
- They were safely brought to the Department of Biochemistry Laboratory and stocked in 200 litre capacity rubber tanks.
- The fish were acclimatized to laboratory conditions for 14 days before the exposure period using plastic aquaria.
- During the acclimation period the fish were feed twice daily using standard commercial fish feed.

METHODS

- Preparation of lyophilized aqueous extract
- The plant parts were washed and shade-dried
- Dry samples were then pulverized and sifted using 0.25 mm sieve.
- The leaf powder thus obtained was stored in a sealed bottled and used for all the phytochemical and GC_MS analyses

PHYTOCHEMICAL INVESTIGATIONS OF PLANTS

- Phytochemical screening was carried out according to established procedures by Sofoworo (1980) and Cuiled (1982) for the presence of:
- alkaloids,
- flavonoids,
- saponins,
- tannins,
- glycosides.

GC-MS ANALYSIS

- The extracts were obtained by Soxhlet extraction using 40g of the powdered leaves in 250 ml of 70% methanol and n-hexane.
- GC –MS analysis
- 2 µl each of the methanol, hexane or extracts of *Psychotria microphylla were* employed for GC-MS analysis (Merlin *et al.,* 2009).
- The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library (Stein, 1990).

ACUTE TOXICITY TEST

- Acute toxicity tests to determine the, 24, 48 and 96 hour LC_{50} value of the plant extract were conducted in semi-static system in laboratory according to the OECD guideline NO 23 (OECD, 1992).
- Preliminary screening was carried out to determine the appropriate concentration range for testing chemical as describe by Colbe (1995).

ACUTE TOXICITY CONTINUE...

- A complete randomized design was used in the experiment with three aquaria set up for each dose of the plant leaf powder: 0.0, 2.5. 3.15, 4.38, 6.25 and 12.50 mg
- and each aquarium contained six (8) fish in forty (40) litres of tap water as described by Solbe (1995) and Rahman et al. (2002).

STATISTICAL ANALYSIS

- The median lethal concentration (LC50) at 24, 48 72 and 96 h were computed using the probit analysis method as described by Finney (1997).

- Data were expressed as mean ±SD of three replicates and were subjected to one way ANOVA followed by Ducan multiple range test to determine significant differences in all parameters using SPSS for windows version 20. Values were considered statistically significant at $p < 0.05$.

Result Of Quantitative Phytochemical Analysis Of *Psychotria Microphylla* Leaves

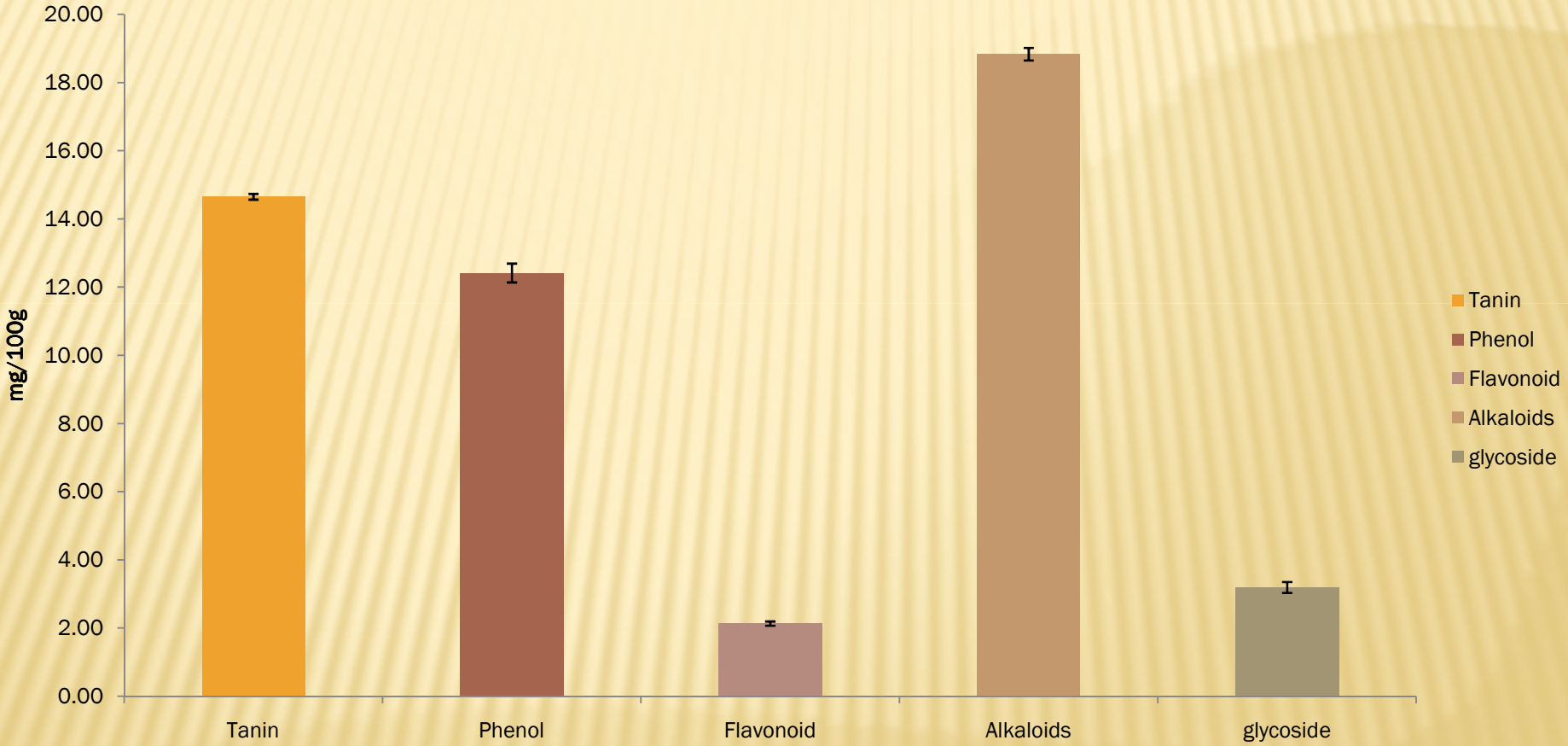


Figure 4 Quantitative phytochemical constituent of the plant leaves

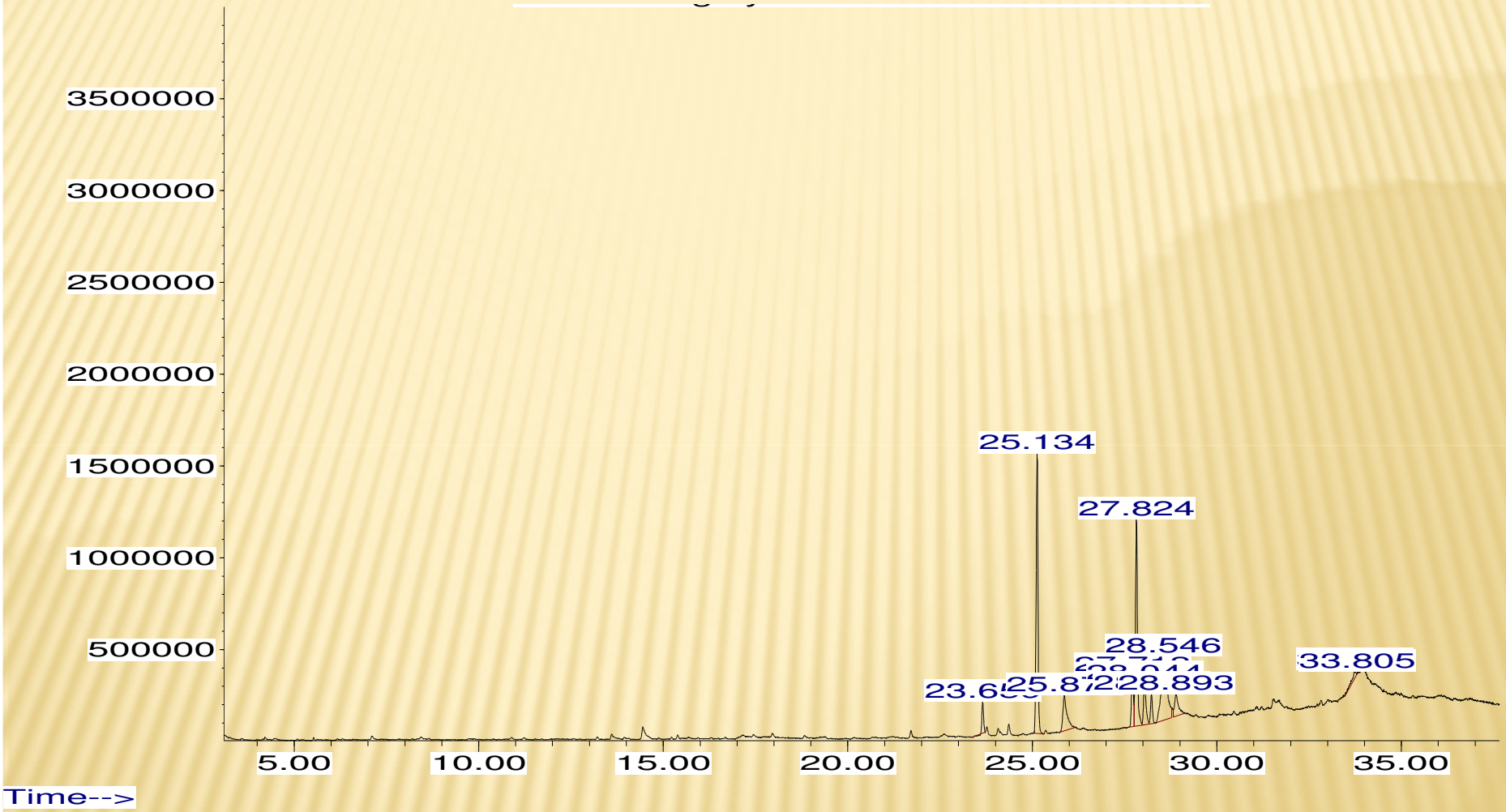


Figure 5: GC-MS chromatogram of the methanol crude extract of the Psychotria microphylla leaves

Table 1: Phytoconstituents identified in methanol crude extract of *Psychotria microphylla* leaves

NO	RT	NAME OF COMPOUND	MOLECULAR FORMULAR	MWT	BASE PEAK	PEAK AREA
1	20.783	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	73.0	12.82
2	22.417	E-9-Octadecenoic acid	$C_{19}H_{36}O_2$	296	55.05	1.96
3	22.742	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298	74.05	0.54
4	23.750	E-9-Octadecenoic acid	$C_{18}H_{34}O_2$	282	55.05	46.21
5	23.917	Stearic acid	$C_{18}H_{36}O_2$	284	73.05	14.32
6	25.042	Hexadecanoic acid, 2 -hydroxy-1,3-propane diyl ester	$C_{35}H_{68}O_5$	568	57.05	4.72
7	25.475	4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6, 6a,6b,7,8,8a,9,10,11,12, 12a,14,14a,14b-octadecahydro-2H-Picen-3-one.	$C_{30}H480$	424	218.20	8.06
8	26.425	Oleic acid, 3-hydroxy-Propyl ester.	$C_{21}H_{40}O_3$	340	55.05	1.91
9	26.875	Cis-13-Octadecenal	$C_{18}H_{34}O$	266	55.05	3.92
10	27.067	Glycerol-1,2-dipalmitate	$C_{35}H_{68}O_5$	568	57.05	0.99
11	28.792	2,3-Bis[(9E)-9-Octade-Cenoyloxy]propyl(9E)-9-octadecenoate	$C_{57}H_{104}O_6$	884	55.05	1.84
12	29.208	NIL	NIL	218.20	2.72	

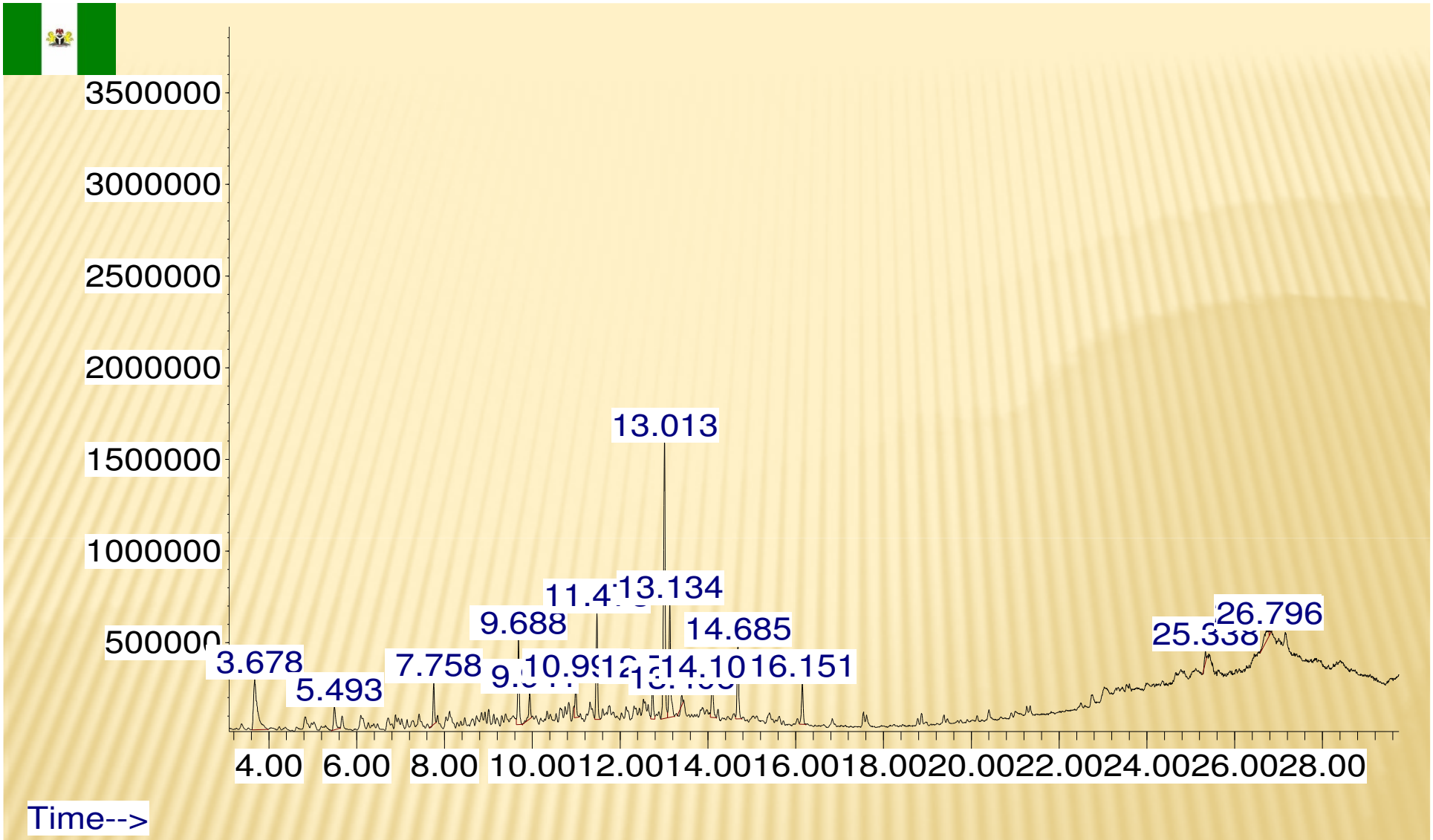


Figure 6 GC-MS chromatogram of the n-hexane crude extract of the Psychotria microphylla leaves

Table 2: Phytoconstituents identified in hexane crude extract of *Psychotria microphylla* leaves by GC-MS

NO	RT	NAME OF COMPOUND	MOLECULAR FORMULAR	MWT	BASE PEAK	PEAK AREA
1	20.683	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	73	8.71
2	22.433	E-9-Octadecenoic acid	$C_{19}H_{36}O_2$	296	55.05	3.32
3	23.133	Lupeol acetate	$C_{35}H_{52}O_2$	468	43.00	4.97
4	23.533	E-9-Octadecenoic acid	$C_{18}H_{34}O_2$	282	55.05	27.30
5	23.742	Stearic acid	$C_{18}H_{36}O_2$	284	55.05	6.85
6	23.943	Z,Z-3,15-Octadecadien-1-ol acetate	$C_{20}H_{36}O_2$	308	43.00	11.05
7	24.467	Cyclohexanol, 2-methyl-5-(1-methylethenyl)-acetate	$C_{12}H_{20}O_2$	196	43.00	8.93
8	26.425	A'-Neogammacer-22(29)-en-3-ol, acetate, (3b, 21b)	$C_{32}H_{52}O_2$	468	189.20	21.96
9	25.908	Tetrapentacontane	$C_{54}H_{110}$	758	57.05	4.97
10	26.867	Acetic acid, 10-aceto-1,6a.6b, 9,9,12a.hexamethyl-2-methylen -eicosahydro-picen-4a-ylmethyl Ester	$C_{34}H_{54}O_4$	526	43.00	1.95

Table 3: Phytoconstituents identified in methanolic extract of *Psychotria microphylla stem bark*

NO	RT	NAME OF COMPOUND	MOLECULAR FORMULAR	MWT	BASE PEAK	PEAK AREA
1	15.725	methyltridecanoate	$C_{14}H_{28}O_2$	228	74.05	
2	18.133	Z-9-Octadecenoic acid	$C_{18}H_{34}O_2$	282	41.00	
3	16.433	pentadecanecarboxylic acid	$C_{16}H_{32}O_2$	256	43.00	
4	17.433	methyl(E)-11-octadecanoate	$C_{19}H_{36}O_2$	296	55.00	
5	17.633	methyl heptacosanoate	$C_{28}H_{56}O_2$	424	74.05	
6	18.283.	cyclopentane undecanoic acid	$C_{16}H_{30}O_2$	254	41.0	
7	19.242	Docylfluoride	$C_{10}H_{21}F$	160	43.00	
8	20.358	Hexy(Z)-9-Octadecenoate	$C_{24}H_{46}O_2$	366	43.00	3.92
9	20.733	(E,E)-9-,12-Octadecadienoyl chloride	$C_{18}H_{31}ClO$	298	55.00	

SPECTRA DATA OF STEM BARK

SPECTROSCOPIC DATA OF THE CHEMICAL CONSTITUENT IN (VALUE MEOH OXTRACT OF OYE STEM BARK

Compound	Spectral data (ms/m/2 (%)
Methyl tridcecanate	74(100%) 87(60) 43(40) 41 (30) 55 (25) 57(20) 143(15) 185 (10) 101(5) 197(0.5) 228(0.2)
Pentadecanecarboxylic acid	43(100%) 73(90) 69(80) 41(20) 57 (65)55 (60) 29(50)129(30) 256(20) 213(15) 115 (10)185(0.5)
Methyl(E) –II-Octacle-cenoatic	55(100%) 41(70)69(50)74(40)29(35)87(30) 264 (20)137(10)222(5)180(.05)
Methyl heptacosan-oratic	74(100%) 43(90)87(70)57(60)41(40)143(15) 424(10)101(5) 185 (05)31(0.2) 199 (0.1)
(2) -9-octaclecenoic acid	41(100%) 55(80)43(50)29(45)69(40)83(35) 97(30)27(25)264(10)123(0.5)137(0.2)
Cyclopentanic undecanoic acid	41(100%)55(50)29(40)27(35)67(30)69(25)73(15) 129(5)185(0.5).
Decyl fluorielic	43(100%)57(60)41(50)55(45)29(30)29(25)69(20)97(15) 112(10)
Hexyl (2)-9-octadecanoatic	43(100%) 55 (50) 41(45)29(30)27(25)69(20)83(15)98 (10)264(5)229(0.5)
(E,E)-9x12-octadecaclicenayl-chloride	55(100%)67(80)41(70)81(50)95(45)43(40)29(35)98(30) 111(15)123(10)135(5)151(0.5)

Table 4: Phytoconstituents identified in methanol extract of *Psychotria microphylla root by* GC-MS

NO	RT	NAME OF COMPOUND	MOLECULAR FORMULAR	MWT	BASE PEAK	PEAK AREA
1	15.725	Methyl tridecanoate	$C_{14}H_{28}O_2$	228	74.05	
2	16.442	Pentaclosanecarboxylic acid	$C_{16}H_{32}O_2$	256	43.00	
3	17.433	Methyl Octadecanoate	$C_{19}H_{36}O_2$	298	55.00	
4	18.125	Z-9-Octadecenoic acid	$C_{18}H_{34}O_2$	282	41.05	
5	17.642	Methyl heptacosanate	$C_{28}H_{56}O_2$	428	74.05	
6	19.242	1,2-di-2-amino-ethyl hydrogen phosphate palmitin	$C_{37}H_{74}NO_{8P}$	691	85	
7	20.358	E-13-Docosenoic acid	$C_{22}H_{42}O_2$	338	55.00	
8	20.733	(E,E)-9,12-Octa-decandienyl chloride	$C_{18}H_{31}ClO$	298	55.00	

Table 5: Phytoconstituents identified in water extract of *Psychotria microphylla root* by GC-MS

NO	RT	NAME OF COMPOUND	MOLECULAR FORMULAR	MWT	BASE PEAK	PEAK AREA
1	15.725	methyl octanoate	$C_9H_{18}O_2$	158	74	
2	22.433	Z-9-Octadecenoic acid	$C_{18}H_{34}O_2$	282	41.00	
3	16.433	penta decanecarboxylic acid	$C_{16}H_{32}O$	256	43.00	
4	23.533	Methyl (Z)-6-Octadecenoate	$C_{19}H_{36}O_2$	296	55.00	
5	23.742	5-Hydroxymethylundecane	$C_{12}H_{26}O$	186	43.00	
6	20.358	(E)-13-Docosanoic acid	$C_{22}H_{42}O_2$	338	55.00	
7	20.742	(E,E)-9,12-Octadecadienyl chlorate	$C_{18}H_{31}ClO$	298	55.00	
8	20.900	Decyl Fluorate	$C_{10}H_{21F}$	160	43.00	

SPECTRA DATA OF ROOT EXTRACT

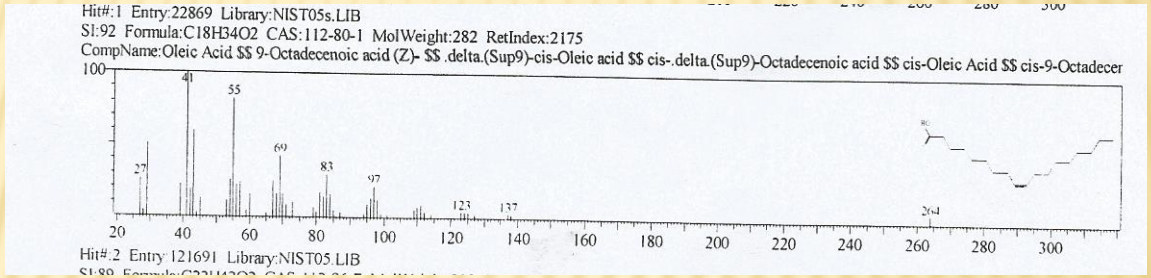
SPECTROSCOPIC DATA OF THE CHEMICAL CONSTITUENT IN CRUDE AND EXTRACT OF OYE ROOT

Compound	Spectral data (ms/m/2 (%))
Methyl tridcecanate	74(100%)87(60)43(40)41(35)55(30)57(25)185(20)143(15) 129(10)101(5)19(2)228(0.5)
Pentadecanecarboxylic acid	43(100%) 41(80)60(60)73(55)55(50)57(45)256(20)129(15)85(10)213(5)115(0.5) 199(0.2)171(0.1)
Methyl(E) –II-Octacle-cenoatic	55(100%) 41(80) 69(50) 74(40) 87(35) 118(30)264(20)180(5)137(0.5)
Methyl heptacosan-oratic	74(100%)43(90)87)(70)57(50)41(40)143(20)424(5)381 (0.5)325(0.2)
(Z) -9-octaclecenoic acid	41(100%)55(80)43(70)
1,2-di,2-aminoethyl hydrogen phosphate palmitin	85(100%)43(90)57(70)98(60)41(50)29(45)239(40)69 (35)71(30)313(25)129(20)112(15)150(10)297(5)
(E) -13-Docosenoic acid	55(100%)41(95)98(50)69(40)81(35)29(30)137(10)152 (5)
(E,E) -9, 12-octadecadienoyl chloirde	55(100%)41(80)67(70)81(50)43(45)95(40)31(30),129 (20)109(15)116(10)

SPECTRA DATA OF ROOT EXTRACT

SPECTROSCOPIC DATA OF THE CHEMICAL CONSTITUENT IN CRUDE AND EXTRACT OF OYE ROOT

Compound	Spectral data (ms/m/2 (%))
Methyl tridcecanate	74(100%)87(50)43(30)41(25)29(20)55(15)57(10)127 (5)115(2)158(0.5)
Pentadecanecarboxylic acid	43(100%)73(95)60(80)41(75)55(65)57(60)29(40) 129(30)256(20)85(15)115(10)85(5)157(0.5213(0.2).
Methyl(2) –6-Octacle-cenoatic	55(100%)41(95)43(70)74(50)67(45)84(40)98(30) 264(25)29(20)123(15)222(10)180(5)137(0.5)
(Z) -9-octaclecenoic acid	41(100%)55(80)29(50)69(40)83(35)97(30)264(10) 137(5)
(Z)-9-octadeneoic acid	41(100%) 55(80)29(50)69(40)83(35) 97(30)264(10) 137(5).
5 - Hydroxymethylunclela nce	43(100%)57(99)41(70)29(60)71(50)85(40)111(15) 126(10)140(5)168(0.5)
(E) -13- Docosenoic acid	55(100%)41(98)98)(60)69(50)67(40)29(30)27(25)81 (20)112(15)137(10152(5)
(E,E) -9x12-octaclecaeliunonyl chloride	55(100%)67(70)41(60)81(50)43(45)95(40)29(35)111 (20)135(15)151(10)
Decyl fluoridic	43(100%)57(70)55(65)41(60)a71(40)97(20)98(15) 112(10).


Hit#:1 Entry:22869 Library:NIST05s.LIB
SI:92 Formula:C18H34O2 CAS:112-80-1 MolWeight:282 RetIndex:2175
CompName:Oleic Acid $$ 9-Octadecenoic acid (Z)- $$ .delta.(Sup9)-cis-Oleic acid $$ cis-.delta.(Sup9)-Octadecenoic acid $$ cis-Oleic Acid $$ cis-9-Octadecer
100
41
55
69
83
97
27
123
137
264
20 40 60 80 100 120 140 160 180 200 220 240 260 280 300
Hit#:2 Entry:121691 Library:NIST05.LIB

ACUTE TOXICITY TEST

- Six graded concentrations of 0, 2.50, 3.125, 4.375, 6.25, and 12.50 mg/l of the leaf powder were applied to *C. gariepinus* juveniles (mean weight: 180 g and length 25 cm) in plastic containers. The 24, 48, 72 and 96 h LC_{50} values (with 95 % confidence limits) estimated by probit analysis were 6.06 (5.369-7.269), 4.995(4.238-6.118), 3.827(3.083-4.639) and 3.259(2.481-3.915) mgl^{-1}, respectively.

Table 7: Data on fish survival of C. gariepinus at different test concentrations of leaf powder of *P. microphylla* leaf.

Exposed concentration (mgl^{-1})	Number exposed	Number of fish alive at different time intervals (hours) 24	48	72	96	% survival
0.00	18	18	18	18	18	100
2.5	18	18	15	12	12	67
3.50	18	18	15	12	09	50
4.75	18	15	12	09	06	33
6.25	18	09	07	04	03	17
12.50	18	00	00	00	00	00

Table 6 Physico-chemical properties of the test water

Parameters	Value
Temperature (℃)	26.71±1.92
Dissolved Oxygen (mg/l)	7.02±0.44
pH	7.13±1.31
Total alkalinity (mg/l)	18.10±1.22
Total hardness (mg/l)	17.88±1.1

Behavioral response of fishes to aqueous extract of *P. microphylla* leaves

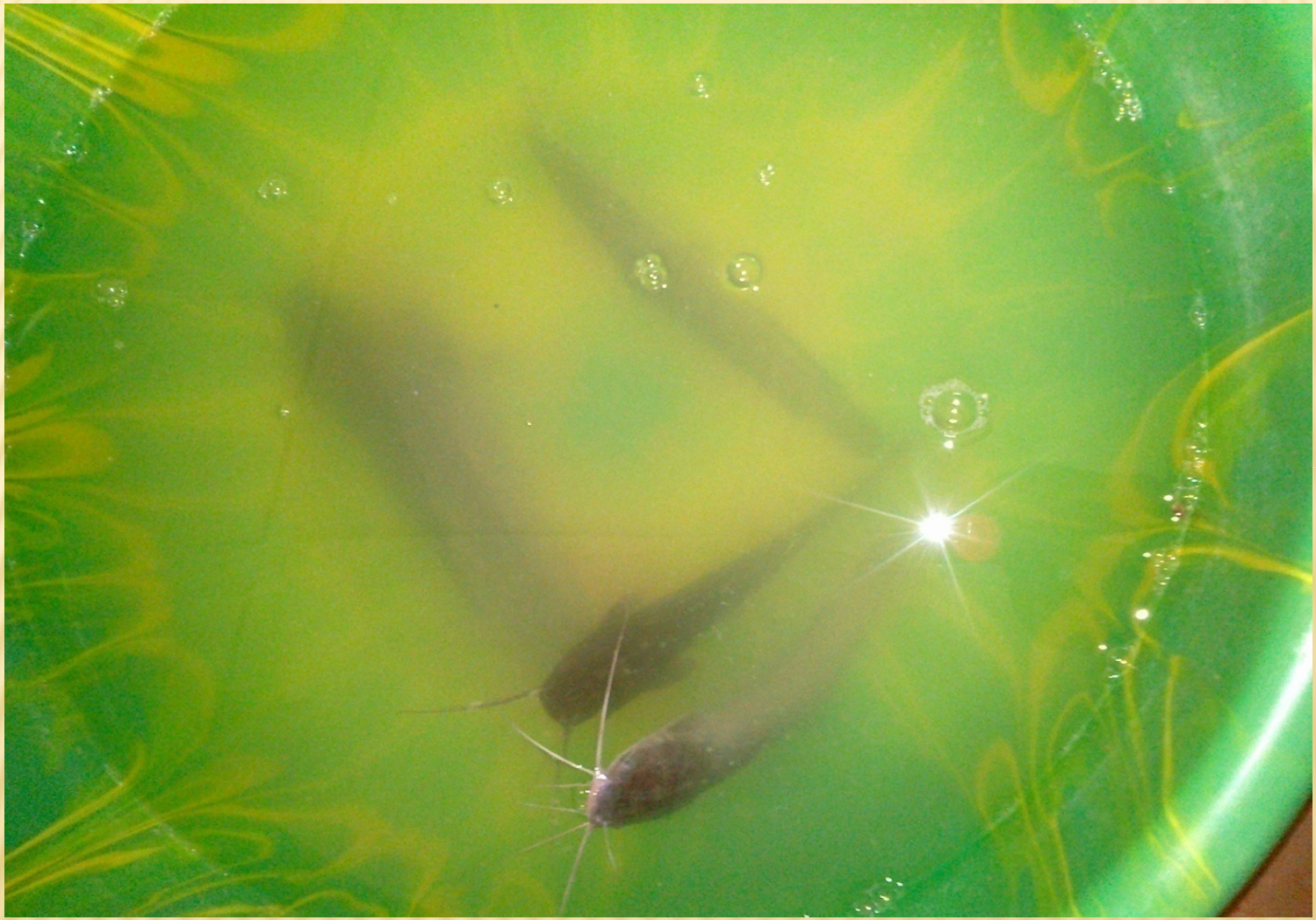


Figure 7: showing fish exposed to the plant extract

Figure 8: showing dead fish by the plant extract

Table 8: the 24, 48, 72, and 96h LC50 values of leaf powder of *P. microphylla leaf at* different time intervals to the fish, *C. gariepinus*

Exposure period	Effective dose (mg/l)	Limits (mg/l)	
(Hour)		LCL	UCL
24	**LC_{50} = 6.06**	**5.369**	**7.269**
48	**LC_{50} = 4.995**	**4.238**	**6.118**
72	**LC_{50} = 3.827**	**3.083**	**4.639**
96	**LC_{50} = 3.25**	**2.48**	**3.92**

SOME FINDINGS

- *P. microphylla* leaves are rich in alkaloids, tanins, phenol, saponins, glycosides and flavonoids
- Sixteen chemical identified from both methanol and hexane extracts of *P. microphylla* leaves by GC-MS were found to have various biological activities ranging from therapeutic effects (anti-microbial, anti-cancer, anti-androgenic, hypocholesterolemic) to toxic effects to aquatic organism.

FINDINGS CONT'D....

- Comparatively, methanol gave a better yield of the phytoconstituents
- The 24, 48, 72 and 96h LC_{50} value of the aqueous extract of the plant were and 6.06, 4.995, 3.82 and 3.25 mg/l, respectively, indicating that the extract is very toxic to the fish.

FINDINGS

- (9Z)-Octadec-9-enoic acid is one of the chemicals suspected to be one of the causes of P. microphylla toxicity
- It has been reported to induce lung damage in certain types of animals and used for the testing new drugs
- Specifically in sheep, intravenous administration of oleic acid causes acute lung injury with corresponding plumonary edema Julien et al., (1986).

PROJECTED USE OF THE PLANT EXTRACT

- Prior to stocking of pods with fish, the ponds are cleaned to get rid of competing fish species from previous stock
- Synthetic toxicants like cyanide and rotenone are often used (Guerreo and Guerreo, 1986)
- This, however is not biodegradable, and has a lot of environmental toxic effects
- ..

PROJECTION

- There is need for local alternatives to chemicals used for cleaning ponds prior to stocking of new fish
- Psychotra microphylla plant extract is a potential candidate to replace chemicals in this respect
- However, more research is needed to establish this.

ACKNOWLEDGEMENT

- TERTIARY EDUCTION TRUST FUND NIGERIA (TETFUND NIGERIA): for providing the fund
- EBONYI STATE UNIVERSITY, ABAKALIKI: for providing enabling environment for the research
- To my students and colleagues who actively participated in the work
- To my wife and Children for their supports

THANK YOU FOR LISTENING!!!
REMAIN RAPTURABLE!

ON GOING RESEARCH

ON

HISTOLOGICAL

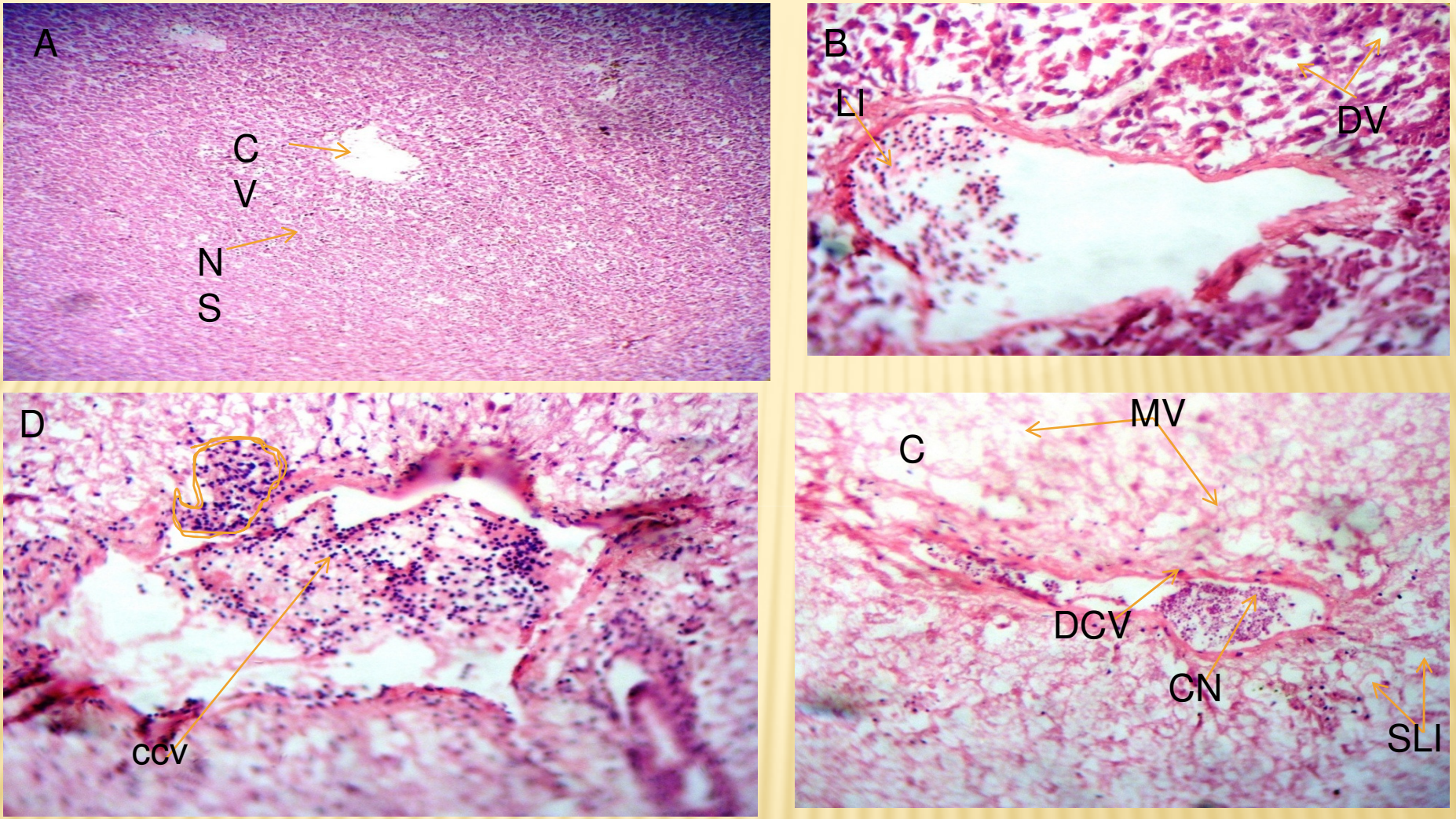
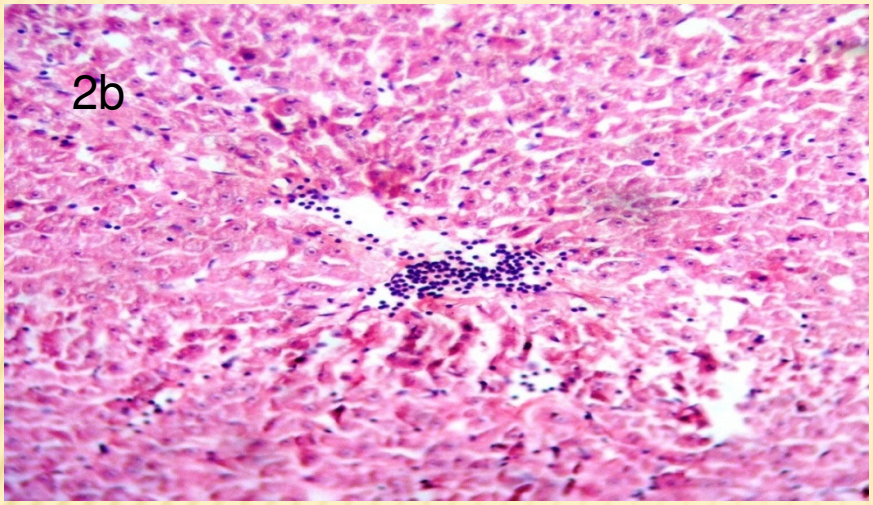
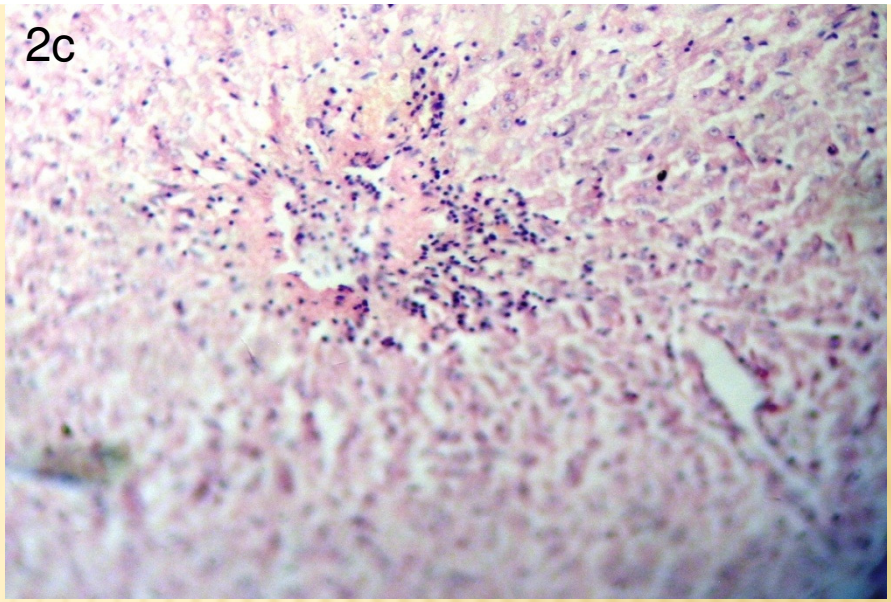
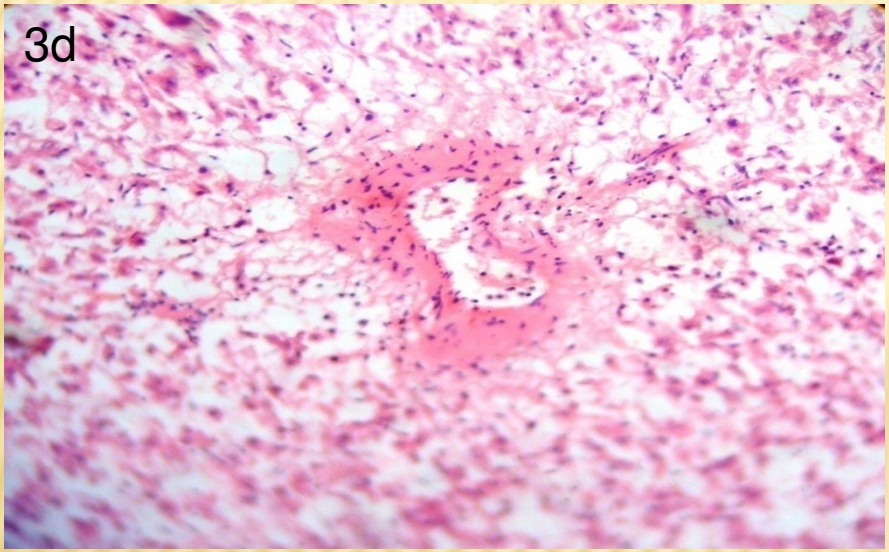


Figure 55 A is a photomicrograph of control experiment (0.000mgl-1).hepatic tissue showed normal lattice network of parenchymatous cells. Central vein (CV), prominently shown without a central nucleus, with normal sinusoid (NS). Showed unremarkable changes around a central vein, **B** is a photomicrograph fish liver exposed to 0.016 mg/l. showed diffused vacuolation of hepatocytes, distorted central vein and mild lymphotic infiltration. **C** is photomicrograph of fish liver treated with 0.03 mg/l showed scanty lymphocytic infiltration (SLI) of the portal area, marked vacuolation of hepatocytes (MV) and slight degeneration of central vein (DCV) became evident with mild congestion of nuclei (CN) and **D** is the photomicrograph of liver of fish exposed to 0.065 mg/l showing marked lymphocytic infiltration (circle) of the portal area and marked vacuolation of hepatocytes (MV), congestion of the central vein (CCV), and an elaborate sinusoidal distortion (SD)(H &E stain x 40).







2c is a photomicrograph of fish liver treated with 0.016 mg/l after 15 day showing moderate lymphocytic infiltration (LI) of the portal area, slight degeneration of central vein (DCV), and distortion of the sinusoids. **3c** is a photomicrograph of fish liver treated with 0.03mg/l for 15 days showing marked lymphocytic infiltration of the portal area (H &E stain x 100). **3d** is a photomicrograph of fish liver treated with 0.065 mg/l showed loss of normal lattice network of parenchymatous cells, marked vacuolation hepatocytes around an arteriole, slight degeneration of central vein (DCV) (H &E stain x 100).