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GC-MS Analysis, Acute Toxicity and Oxidative Stress Potentials (Effects) of *Albizia chevalieri* Extract on Juvenile African Catfish (*Clarias gariepinus*)

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Abstract: Stem-bark of *Albizia chevalieri* is traditionally used to harvest fish from streams in Ebonyi State, Nigeria. Here the phytochemical constituents, the GC_MS analysis of the phytochemicals and acute toxicity of aqueous extract of the stem-bark and the oxidative stress potential of *Albizia chevalieri*in *C. gariepinus*were carried out using standard methods. A total of one hundred and twenty (120) *C. gariepinus* juveniles were purchased from Chiboy Farms Abakaliki, Ebonyi State and acclimatized for two weeks in laboratory aquaria. For the acute toxicity test, 80 fish juveniles were divided into eight groups in a semi-static tank, each containing ten fish. The groups were exposed to 800, 400, 200, 120,60, 40, 10 and 0mg l^{-1} of the aqueous stem-bark extract of *A. chevalieri* for 96 hours. The 24, 48, 72 and 96 hour LC_{50} values calculated by Probit analysis were107.289 (91.160-132.461), 96.244 (81.470-117.524), 83.807 (70.697-99.802) and 77.915 (66.584-90.492) mg l^{-1}, respectively. Phytochemical screening showed the presence of phenols, alkaloids, flavonoids, saponins, tannins, cardiac glycosides, phlobatannin and steroids. The GC-MS analysis of the ethanol extract revealed the following compounds 1-dodecene (0.36 %), 1-fluorodecane (9.00 %), 9, 12-octadecadienoyl chloride (30.35 %), hexadecanoic acid (9.43 %), 1, 2-dimethylbenzene (10.63 %), n-nonane (19.97 %), hexadecane (1.55 %) and 2-propylheptanol (1.50%), while n-hexane extract unveiled the presence of Sec-butylcyclohexane (9.68%), 2-methyloctane (6.80%), 1, 2-dimethylbenzene (10.63%), n-nonane (19.97%), pentafluoropriopionic acid (tridecyl ester) (9.53%), n-decane(20.70 %), 4-methyldecane(5.81%), n-Dodecane(0.83%), hexadecane(1.55%), 2-propylheptanol (1.50%), 2-methylnonadecane (1.41%), 2-methyloctadecane (2.71%), 9, 12 octadecadienoylchloride (6.55%) and hexadecane(2.31%), For the biochemical studies, a total of 36 fish were divided into three groups each containing twelve (12) fish and exposed to three sub-lethal concentrations (18.22, 9.11 and 7.29 mg l^{-1}) of the aqueous stem-bark extract of *A. chevalieri*, corresponding to the ¼th LC_{50}, $^{1}/_{8th}$ LC_{50} and $^{1}/_{10th}$ $LC_{50,}$ for 16 days. A set of 12 fish were simultaneously maintained in an aquarium (0.00 mg l^{-1}) to serve as the control. Blood samples were collected from exposed fish every four days and analysed for oxidative stress biomarkers. The results showed a significant decrease ($P<0.05$) in superoxide dismutase (SOD), catalase (CAT) and glutathione S-transferase (GST) activities while glutathione reductase (GSR) decreased significantly ($P<0.05$). Malondialdehyde (MDA) concentration also increased significantly ($P<0.05$) showining that lipid peroxidation occurred. The results show that *Albizia chevalieri* holds great potential for novel drug discovery.

Key words: *Albizia chevalieri* · GC/MS analysis · Phytochemical screening · Toxicity testing · Oxidative stress · *C. gariepinus*

INTRODUCTION

Fish farming is a vital source of livelihood in Ebonyi State, Nigeria, as in many other parts of the world. Over the years many plants have been used by the local fishermen to harvest fish from streams and lakes [1-3]. In Ebonyi State, *Albizia chevalieri* is one of the plants used locally to harvest fish from streams and ponds.

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It is prepared by pounding the cut stem-bark alone or mixed with the root of the plant. The stream or pond is demarcated and the ground plant material is poured into the demarcated water body and allowed to stay till the following day. On the following morning, dead fishes are found floating on the surface of the water and are hand-picked by the local fish farmers.

In addition to its use in fish farming, *Albizia chevalieri* is also used for tanning in many parts of Nigeria and Senegal [4-6]. It is also used to treat many ailments such as stomach upsets, cough, dysentery and diabetes mellitus [7-9].

Clarias gariepinus or African catfish is a species of the family *Clariida*, the air-breathing catfishes. They are found throughout Africa and Middle East and live in freshwater lakes, rivers and swamps and migrate from within streams and rivers [10, 11]. They secrete mucus to prevent drying and are able to burrow in the muddy substrate of a drying body of water [12-16] and thus can adapt to extreme environmental conditions such as poorly oxygenated waters [17-20]. In Nigeria, it is one of the most widely cultivated or farmed fish (Personal account). The fish is widely used as biological indicators in ecotoxicological studies [21-25].

Though *A. chevalieri*has been used over the years by the local fishermen to harvest fish and for other purposes, there has not been any scientific report on its chemical constituents (via Gc-MS analysis)and toxicity to fish. Therefore, this study was designed to fill this gap by providing such information to the scientific community.

MATERIAL AND METHODS

Materials, Chemicals and Reagents: the major equipment used were Spectrophotometer (Spectro 21D PEC MEDICALS USA) and GC-MSQP2010 Shimadzu system. All the chemicals and reagents used in this research were of the purest analytical grade commercially.

Sample Collection and Preparation: A total of 150 juveniles of *Clarias gariepinus* (catfish) were purchased from Chiboy Farms, Abakaliki, Ebonyi State and were acclimatized in the Department of Biochemistry Laboratory aquaria of Ebonyi State University, Abakaliki, Nigeria for two weeks before the experiment. The fresh stem-bark of *Albizia chevalieri* was collected from Nkaliki Enyibuchiri in Ikwo Local Government Area of Ebonyi State, Nigeria. The plant was identified and authenticated by Prof. S. S. Onyekwelu of the Applied Biology Department, Ebonyi State University, Abakaliki. The stem-bark was dried under laboratory condition, ground to powder and stored in a sealed container before use.

Preparation of Aqueous Extract: About 200 g of the ground stem-bark of *A. chevalieri* was soaked in 800 ml of cold distilled water and allowed to stand for 24 hours in an air-tight container. The mixture was filtered using a clean, white muslin clothe and squeezed thoroughly to let all the filtrate out and then through Whatman No. 1 filter paper, while the residue was discarded. The filtrate was evaporated in an oven at 60 °C for 5 days to get the dry aqueous extract of the *A. chevalieri* stem-bark.

Acute Toxicity Test: A total of one hundred and twenty eight (128) acclimatized juveniles of *Clarias gariepinus* weighing between 240.0 ± 20.0 g and 180.0 ± 60.0 g, with lengths of 30.8 cm to 25.5 cm, were selected for this study. Acute toxicity test to determine the 24, 48, 78 and 96 hour LC_{50} values of aqueous stem-bark extract of *A. chevalieri* on *C. gariepinus* was conducted in a semi-static system in the laboratory according to the OECD guideline NO 23 (OECD 1992). The fish selected for the acute toxicity study were grouped into eight, each group containing ten (10) fish juveniles and exposed to 800, 400, 200, 120, 80, 60, 40 and 0 mg l^{-1} of the aqueous stem-bark extract of *A. chevalieri;* to obtain the 24 h, 48 h, 72 h and 96 h LC_{50}. The water with *A. chevalieri* concentrations were changed after every 24 hours by adding fresh *A. chevalieri*, in order to counter balance their decreasing concentrations. The experiment was conducted in aquaria containing 40 L of aerated tap water.

Phytochemical Analysis: Phytochemical screenings were carried out on the aqueous extract of the powdered samples using standard procedures to identify the constituents as described by Sofowara (1993).

Preparation of Samples for Gas Chromatography/Mass Spectrophotometer Analysis (GC-MS analysis): The extracts were obtained by Soxhlet extraction using 40g of the powdered leaves in 250 ml of 70% methanol and hexane. The extract was collected and evaporated to dryness by using vacuum distillation unit. The final residue thus obtained was then subjected to GC-MS analysis. About *2* µl each of the methanolic and hexane extracts of *A. chevalieri were* employed for GC-MS analysis.

Instruments and Chromatographic Conditions: GC-MS analysis was carried out on GC-MSQP2010 Shimadzu system comprising a gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column VF-5MS fused silica capillary column (30.0m x 0.25mm x 0.25µm, composed of 5% phenyl/95% dimethylpolysiloxane), operating in electron impact mode at 70 eV. Helium (99.999%) was used as carrier gas at a constant flow of 1.5ml/min and an injection volume of 1µl was employed (split ratio of 10:1); injector temperature was 240°C and ion-source temperature was 200°C. The oven temperature was programmed from 70°C (isothermal for 3min), with an increase of 10°C/min, to 240°C, then 5°C/min to 300°C, ending with a 9 min isothermal at 300°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 40 to 440 Da. Total GC running time was 35min.

Identification of Components: The identity of the components in the extracts was assigned by comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library. Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained (Stein, 1990).

Sub-acute Toxicity Test: The 96 h LC_{50} of *A. chevalieri* on *C. gariepinus* obtained was 77.915 mg l^{-1}. The LC_{50} value was used for the sub-acute toxicity test for the biochemical studies. A total of 36 acclimatized fish were divided into three groups, each containing twelve (12) fish in each aquarium. The three groups were respectively exposed to 19.47, 9.74 and 7.79 mg l^{-1} of *A. chevalieri*, corresponding to their ¼th, $^{1}/_{8th}$ and $^{1}/_{10th}$ LC_{50} for 16 days. A set of 12 fish were simultaneously maintained in an aquarium (0.00 mg l^{-1}) to serve as the control. The water with the stem-bark extract concentrations were changed every 24 hours by adding fresh stem-bark extract in order to counter balance their decreasing concentrations. At the end of every 4 days, three fish were taken from each aquarium and blood sample collected from the head and caudal fin region. The blood sample was centrifuged at 3000 g for 30 minutes and the serum collected was used for biochemical assays.

Determination of Biochemical Parameters: Standard methods were used for all the biochemical analysis. The glutathione transferase (GST) and glutathione reductase activities were determined according to the method of Habig *et al.*, (1974). Lipid peroxidation was determined according to the method of Wallin *et al.* (1993). Superoxide dismutase (SOD) and catalase activities were determined using the methods of Xin *et al.* (1991) and Aebi (1983), respectively.

Statistical Analysis: The percentage survival and mortality were calculated, while the LC_{50} was determined following the Probit analysis (Finney, 1977). Statistical analysis was performed using one-way Analysis of Variance (ANOVA) followed by Ducan's multiple range test procedure of SAS software version 9.1. All the results obtained were expressed as mean ± Standard Deviation (S.D.) of three replicates of each sample and the differences between means were regarded significant at $P < 0.05$.

RESULTS

Acute Toxicity Effect on *Clarias gariepinus* juvenile Exposed to Aqueous Stem-bark Extract of *Albizia chevalieri*: The results of the acute toxicity of *Albizia chevalieri* stem bark extract to *Clarias gariepinus* juvenile is presented in Table 1. The mean lethal concentrations ($LC_{50\ s}$) expressed in mg l^{-1} for 24, 48, 72 and 96 h exposure periods (95% confidence intervals) calculated by Probit analysis were 107.289 (91.160-132.461), 96.244 (81.470-117.524), 83.807 (70.697-99.802) and 77.915 (66.584-90.492)mg l^{-1}, respectively. The result showed that there was no death (100 % survival) recorded on the fish exposed to the plant extract of concentration 0 to 60 mgl^{-1}*A. chevalieri*. A total of 8 fish died in the aquaria exposed to 80 mgl^{-1} (20 % survival) and 9 fish died (10 % survival) in the aquaria containing 120 mgl^{-1} of the plant extract; whereas all the fish died (0 % survival) in the aquaria containing 200 to 800 mgl^{-1} within 96 hours.

Qualitative Phytochemical Constituents of *Albizia chevalieri* Stem-bark: The results of the qualitative analysis of aqueous extract of *A. chevalieri* stem back are presented in Table 2. The result revealed that*A. chevaleri*plant is rich in phenols, alkaloids and glycosides, whereas tannins and flavonoids were moderately present.

Table 1: Acute toxicity test of *Clarias gariepinus* juvenile exposed to aqueous stem-bark extract of *Albizia chevalieri* collected from Ikwo, Ebonyi State, Nigeria

Albizia chevalieri Conc. (mgl^{-1})	Number of fish exposed	Number of deaths				%mortality			
		24hrs	48hrs	72hrs	96hrs	24hrs	48hrs	72hrs	96hrs
00	10	10	10	10	10	00	00	00	00
40	10	10	10	10	10	00	00	00	00
60	10	10	10	10	10	00	00	00	00
80	10	02	04	07	08	20	40	70	80
120	10	06	07	08	09	60	70	80	90
200	10	10	10	10	10	100	100	100	100
400	10	10	10	10	10	100	100	100	100
800	10	10	10	10	10	100	100	100	100

Table 2. Phytochemical screening of Stem-bark extract of *A. chevalieri*

Phytochemicals	Results
Phenols	+
Alkaloids	++
Flavonoids	+++
Saponins	+
Tannins	+
Cardiac glycosides	++
Reducing sugar	+
Phlobatanin	+
Steroid	+
Terpenoid	-

Key: + = present,-= absent.

Table 3: Identified chemicals present in the ethanol extract of the stem-bark of *Albizia chevalieri* using GC-MS

Name of Compound	Retention Time	Molecular Formula	Molecular Mass	%Peak Area
2-Ethoxypropane	3.10	$C_5H_{12}O$	88	3.96
1-Dodecene (Dodec-1-ene)	14.12$C_{12}H_{24}$	168	0.36	
Methyl cis-6-octadecenoate	22.55$C_{19}H_{36}O_2$	296	2.38 9	
octadecenoic acid (Z)-(oleic acid)	22.78	$C_{18}H_{34}O_2$	282	19.35
1 Fluorodecane	25.16	$C_{10}H_{21}F$	160	9.00
9, 12-Octadecadienoyl chloride,	27.00	$C_{18}H_{31}ClO$	298	30.35
Hexadecanoic acid	27.58	$C_{19}H_{38}O_4$	330	9.43
13-Tetradece-11-yn-1-ol	55.05	$C_{14}H_{24}O$	208	25.18

Table 4: Identified chemical present in the n-hexane extract of the stem-bark of *Albizia chevalieri* using GC-MS

Name of Compound	Retention Time	Molecular Formula	Molecular Formular	% Park Area
Sec-butylcyclohexane	3.14	$C_{10}H_{20}$	140	9.68
2-methyloctane	3.30	C_9H_{20}	128	6.80
1, 2-dimethylbenzene	3.56	C_8H_{10}	106	0.63
n-Nonane	3.69	C_9H_{20}	128	19.97
Pentafluoropriopionicacid (tridecyl ester)	4.22	$C_{16}H_{27}F_5O_2$	346	9.53
n-Decane	5.10	$C_{10}H_{22}$	142	20.70
4-methyldecane	5.39	$C_{11}H_{24}$	156	5.81
n-Dodecane	4.18	$C_{12}H_{26}$	170	0.83
Hexadecane	20.65	$C_{16}H_{34}$	226	1.55
2-Propylheptanol	22.31	$C_{10}H_{22}O$	158	1.50
2-methylnonadecane	24.92	$C_{20}H_{42}$	282	1.41
2-Methyloctadecane	25.98	$C_{19}H_{40}$	268	2.71
9,12-Octadecadienoylchloride	26.99	$C_{18}H_{31}ClO$	298	6.55
Hexadecane	27.83	$C_{16}H_{34}$ 226	2.31	

Table 5: Effect of *Albizia chevalieri* stem-bark extract on Oxidative Stress parameters in *Clarias gariepinnus* Juveniles

Days [LC50 = 77.915 (mg l-1)]	Superoxide Dismutase (SOD) (IU/g)	Catalase (CAT) (IU/g)	Glutathione S-transferase (GST (M/min)	Glutathione reductase (GR) (Umol/ml)	Malondialdehyde (MDA) (mg/ml)
4th Day					
Control	10.33 ± 0.01^{g}	18.07 ± 0.00^{g}	4.75 ± 0.09^{g}	0.48 ± 0.00^{g}	7.25 ± 0.01^{a}
1/10th LC50	10.01 ± 0.00^{c}	17.49 ± 0.00^{e}	4.11 ± 0.03^{e}	0.43 ± 0.00^{e}	7.56 ± 0.00^{c}
1/8th LC50	9.92 ± 0.00^{b}	17.04 ± 0.00^{b}	3.70 ± 0.03^{c}	0.40 ± 0.00^{c}	8.23 ± 0.05^{e}
1/4th LC50	9.39 ± 0.00^{a}	16.63 ± 0.01^{a}	3.18 ± 0.02^{a}	0.29 ± 0.00^{a}	9.82 ± 0.00^{g}
8th Day					
Control	10.43 ± 0.00^{g}	17.28 ± 0.00^{g}	4.17 ± 0.00^{g}	0.46 ± 0.00^{f}	7.97 ± 0.00^{a}
1/10th LC50	9.11 ± 0.00^{d}	14.98 ± 0.01^{c}	3.05 ± 0.01^{e}	0.36 ± 0.01^{d}	10.21 ± 0.00^{e}
1/8th LC50	8.99 ± 0.00^{b}	14.39 ± 0.01^{b}	2.86 ± 0.02^{c}	0.35 ± 0.00^{c}	10.75 ± 0.00^{f}
1/4th LC50	8.93 ± 0.00^{a}	14.12 ± 0.00^{a}	2.64 ± 0.01^{a}	0.27 ± 0.01^{a}	10.97 ± 0.00^{g}
12th Day					
Control	10.44 ± 0.00^{f}	16.98 ± 0.00^{g}	4.14 ± 0.02^{e}	0.43 ± 0.00^{f}	7.99 ± 0.00^{a}
1/10th LC50	8.30 ± 0.01^{c}	13.23 ±. 00^{c}	2.36 ± 0.02^{b}	0.28 ± 0.00^{d}	11.11 ± 0.00^{e}
1/8th LC50	7.99 ± 0.00^{b}	12.85 ± 0.01^{b}	2.44 ± 0.02^{c}	0.27 ± 0.00^{c}	11.65 ± 0.01^{f}
1/4th LC50	7.91 ± 0.00^{a}	12.26 ± 0.00^{a}	2.22 ± 0.01^{a}	0.24 ± 0.00^{a}	11.83 ± 0.00^{g}
16th Day					
Control	10.28 ± 0.01^{g}	15.94 ± 0.00^{g}	4.10 ± 0.01^{f}	0.42 ± 0.00^{e}	8.86 ± 0.00^{a}
1/10th LC50	6.99 ± 0.00^{e}	11.76 ± 0.29^{c}	2.06 ± 0.02^{d}	0.25 ± 0.00^{d}	12.46 ± 0.00^{e}
1/8th LC50	6.06 ± 0.00^{b}	11.46 ± 0.00^{b}	1.95 ± 0.03^{c}	0.23 ± 0.00^{b}	12.56 ± 0.00^{f}
1/4th LC50	5.89 ± 0.00^{a}	10.42 ± 0.00^{a}	1.55 ± 0.03^{a}	0.22 ± 0.00^{a}	12.86 ± 0.00^{g}

Values are mean ± Standard deviation. Values with different superscripts per column are significantly different ($p < 0.05$).

Table 6: Identified phytochemical bio active compounds present in the n-hexane extract of the stem-bark of *Albizia chevalieri* using GC-MS

Name of Compound	Retention Time	Molecular Formula	Molecular Formular	%Park Area
Sec-butylcyclohexane	3.14	$C_{10}H_{20}$	140	9.68
2-methyloctane	3.30	C_9H_{20}	128	6.80
n-Nonane	3.69	C_9H_{20}	12	19.97
Pentafluoropriopionicacid (tridecyl ester)	4.22	$C_{16}H_{27}F_5O_2$	346	9.53
n-Decane	5.10	$C_{10}H_{22}$	142	20.70
4-methyldecane	5.39	$C_{11}H_{24}$	156	5.81
n-Dodecane	4.18	$C_{12}H_{26}$	170	0.83
Hexadecane	20.65	$C_{16}H_{34}$	226	1.55
2-Propylheptanol	22.31	$C_{10}H_{22}O$	158	1.50
2-methylnonadecane	24.92	$C_{20}H_{42}$	282	1.41
2-Methyloctadecane	25.98	$C_{19}H_{40}$	268	2.71
9,12-Octadecadienoylchloride	26.99	$C_{18}H_{31}ClO$	298	6.55
Hexadecane	27.83	$C_{16}H_{34}$	226	2.31

Chemical Constituents of Ethanol Crude Extract of *Albizia chevalieri* Stem-bark by GC-MS Analysis: The result of the GC-MS analysis of ethanol stem-bark extract of *A. chevalieri* is presented in Table 3. The result revealed the presence of 8 bio-active compounds which include 2-Ethoxypropane (3.96 %), Dodec-1-ene (0.36 %), Methyl cis-6-octadecenoate (2.38 %), 9-octadecenoic acid (19.35 %), 1-Fluorodecane (9.00 %), 9, 12-Octadecadienoyl chloride (30.35 %), Hexadecanoic acid (9.45 %) and 13-Tetradece-11-yn-1-ol (25.18 %).

Chemical Constituents of Hexane crude extract of *Albizia chevalieri* Stem-bark by GC-MS Analysis: The result of the GC-MS analysis of hexane stem-bark extract of *A. chevalieri* is presented in Table 4. The results revealed the presence of 13 bioactive compounds with 14 peaks which include Sec-butylcyclohexane (9.68 %), 2-methyloctane (6.80 %), 1, 2-dimethylbenzene (10.63 %), n-nonane (19.97 %), Pentafluoropriopionicacid (9.53 %), n-Decane (20.70 %), 4-methyldecane (5.81 %), n-Dodecane (0.83 %), hexadecane (1.55 %) and 2-propylheptanol

(1.50 %). 2-methylnonadecane (1.41 %), 2-Methyloctadecane (2.71 %), 9,12-Octadecadienoylchloride (6.55 %) and Hexadecane (2.31 %).

Oxidative Stress Enzymes: The activities of antioxidant enzymes-superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), glutathione reductase (GSR) decreased with increasing concentrations of *A. chevalieri*while the concentrations of Malondialdehyde (MDA) increased (P<0.05) with increasing concentrations of the aqueous stem-bark extract (Table 5).

The GC-MS Analysis of N-HEXANE Extract of *Albizia chevalieri* STEM-BARK: The result of the GC-MS analysis of ethanol stem-bark extract of *A. chevalieri* is presented in Table 6. The results revealed the presence of 13 bioactive compounds with 14 peaks which include Sec-butylcyclohexane (9.68 %), 2-methyloctane (6.80 %), 1, 2-dimethylbenzene (10.63 %), n-nonane (19.97 %), Pentafluoropriopionic acid (9.53 %), n-Decane (20.70 %), 4-methyldecane (5.81 %), n-Dodecane (0.83 %), hexadecane (1.55 %) and 2-propylheptanol (1.50 %). 2-methylnonadecane (1.41 %), 2-Methyloctadecane (2.71 %), 9,12-Octadecadienoylchloride (6.55 %) and Hexadecane (2.31 %).

DISCUSSION

The result of the acute toxicity study showed that *C. garipinus* exposed to high concentrations (120 to 800 mg l^{-1}) of aqueous stem-bark extract of *A. chevalieri* displayed erratic swimming, quick and sudden movement, settling down at the bottom of the aquaria and finally died. The fish in the aquaria with lower concentrations of aqueous stem-bark extract of *A. chevalieri* displayed less erratic and quick movements but were weak after 96 hours based on the exposed concentrations of the stem-bark extract. The control (0.00 mg l^{-1}) displayed normal movements after the 96 hours. The 24 h, 48 h, 72 h and 96 h LC_{50} of the aqueous stem-bark extract of *A. chevalieri* on juveniles of *Clarias gariepinus*were107.289, 96.244, 83.807 and 77.915 mg l^{-1}. The results show that *A. chevalier* was toxic to *C. gariepinus* junenile.The 24 h LC_{50} of the aqueous stem-bark extract of *A. chevalieri* obtained from this study is close to the one obtained by [25-27] in which the 24 h $LC_{50}$of acetone stem-bark extract of *Thevetia peruviana* on the fish,*Catla catla*,in a laboratory condition was 99.43 mg l^{-1}. In a rat model study, [25-26] reported LD_{50} of the extract of *Albizzia chevalieri* to be > 3000 mg kg^{-1} body weight. In this study, the secondary metabolites found in *A. chevalier* stem-bark were tannins, phenols, flavonoids, alkaloids, glycosides, saponins and phlobatanin (Figure 1). Among the phytochemicals revealed, saponins have been implicated as fish poison [15, 28, 29] www.primitiveways.com/fishpoison.html). Saponins are one of a group of glucosides found in many plant species with known foaming properties when mixed with water [30-32]. Thus the relatively high foaming nature of *A. chevalier* stem-bark can be attributed to the presence of saponins suggesting that saponins could be involved in the death of the fish exposed to the plant extract. It has been reported that fish take in saponins directly into their bloodstream through their gills where they act on the respiratory organs of the fish and cause the breakdown of red blood cells, via complexation with cholesterolsto form pores in red cell membrane bilayers which leads to hemolysis [33-36].

The GC-MS analysis of the stem-bark of *Albizia chevalieri* indicated the presence of 1-dodecene (0.36 %), 1-fluorodecane (9.00 %), 9, 12-octadecadienoyl chloride (30.35 %), hexadecanoic acid (9.43 %), 1, 2-dimethylbenzene (10.63 %), n-nonane (19.97 %), hexadecane (1.55 %) and 2-propylheptanol (1.50 %) as shown (Tables 1 and 2). Paradoxically, most of the chemicals revealed by the GC-MS analysis have many useful health benefits. However, it has been reported that 9-Octadeconoic acid (Oleic acid) is used to induce lung damage in certain types of animals, for the purpose of testing new drugs and other means to treat lung diseases. Specifically in sheep, intravenous administration of oleic acid causes acute lung injury with corresponding pulmonary edema [37]. Also, 1-Fluorodecane and hexadecanoic acid thereby causing ataxia, lungs, thorax, or respiration cyanosis and dispnea resulting in the death of the fish by suffocation [30].

Our results also showed a significant (P<0.05) decrease of the antioxidant enzymes: SOD, CAT, GSR and GST activities in the serum of *C. gariepinus* exposed to sub-lethal concentrations ($^{1}/_{10}$ to ¼th LC_{50}) of aqueous stem-bark extract of *A. chevalieri* from the 4th to the 16th day compared to that of the controls. The effects were both concentration and time dependent. These enzymes acts as cellular defense against reactive oxygen species (ROS) and free radicals that cause oxidative stress and hence, cellular injury. The decrease in activity might be attributed to inactivation of the antioxidant enzymes by the chemical contents of *C. gariepinus.* It has been reported that inefficient scavenging of ROS might be implicated in oxidative inactivation of these enzymes [4].

Superoxide dismutases (SOD) are enzymes that catalyze the dismutation of superoxide (O_2^-) into oxygen and hydrogen peroxide. Reduced activity of SOD could lead to immune system damage [13] and [15]. Catalase catalyses the decomposition of hydrogen peroxide to water and oxygen [12]. It is a very important enzyme in protecting the cell from oxidative damage by ROS. The decrease in the activity of catalase observed in this work indicates inefficient scavenging of H_2O_2 which could be as a result of inhibition of the enzymes' activities by the plant extract.

Glutathione reductase (GSR) catalyzes the reduction of glutathione disulfide (GSSG) to the sulfhydryl form glutathione (GSH), which is a critical molecule in resisting oxidative stress and maintaining the reducing environment of the cell [12, 18, 24, 28]. GSTs catalyse the conjugation of GSH-via a sulfhydryl group-to electrophilic centers on a wide variety of substrates in order to make the compounds more soluble [8, 16, 19, 20]. The decreased activities of GSR and GSTs observed in this work are indicative of 'oxidative stress state' of the cell due to exposure to the plant extract.

The above is supported by the observed significant (P<0.05) increase in malondialdehyde (MDA) in this study which is indicative of lipid peroxidation and hence accumulation of ROS in the serum of *C. gariepinus* exposed to *A. chevalieri.*Lipid peroxidation can occur if the rate of production of reactive oxygen species is higher than that of the antioxidants. That is, when the antioxidants level in the cell is low such that they cannot combat and overcome the free radicals, it results to lipid peroxidation. This leads to cell membrane damage.

In conclusion, this study has revealed the chemical constituents of *A. chevvalieri* via GC-MS analysis. This is the first time this is being reported in scientific literature. The toxicity of this plant to *C. gariepinus* may be attributed to the combined effects of the chemical constituents reported here. *A. chevaleeri* is a plant of great phytopharmaceutical importance.The results obtained here show that *Albizia chevalieri*extract exerts its toxicity possibly by inducing oxidative stress in exposed fish. However, further studies will need to be undertaken to ascertain fully its bioactivity, toxicity profile and oxidative stress potentials.

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