ANTI-VENOM POTENTIALS OF FRIEDELIN ISOLATED FROM HEXANE EXTRACT FRACTION OF *ALBIZIA CHEVALIERI* HAMS (MIMOSACEAE)

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ABSTRACT

Albizia chevalieri Hams (Mimosaceae), mostly found in the Northern Sahel Savannah regions of Nigeria as well as in Nigér Republic and Senegal is a tree of the acacia type with a long list of folklore therapeutic claims, which include its plant parts use as purgative, taenicidal, cough remedy, dysentery, cancer, diabetes mellitus, tuberculosis and snake bite remedy. In this report, an attempt has been made to evaluate the bioactive molecules in the plant that are anti-venom agents. Consequently, the stem bark was exhaustively extracted with hexane and subsequently with methanol. The methanolic extract was fractionated into ethyl acetate (EA) and n-butanol (NB) soluble parts after which the hexane extract were subjected to silica gel gravity column chromatography for the isolation of pure bioactive molecules. The major compound isolated, HXC_1 (friedelin), determined using spectrometric analysis of HR-ESIMS, ^{1}HNMR, ^{13}CNMR and IR spectra was investigated for anti-venom activity, including the extract fractions in experimental albino mice using standard methods. The extent of cobra venom (*Naja nigricollis* venom) induced lethal activity (minimum lethal dose of 5mgkg^{-1} or MLD) in the positive control group (venom-only) was compared with the mortality rate in the treatment group so as to evaluate the likely inhibitive/prophylaxis potentials of HXC_1 and those of the extract fractions in the animals within 24 hours. The results showed that the isolated compound, HXC_1 offered the highest protection by prolonging time of death of HXC_1 envenomated animal treated group at a dose dependant amount of 4mgkg^{-1} and 8mgkg^{-1} with a minimum survival rate of 20%, as compared to the positive control and plant extracts treated groups where about 80% of the animals died within an average time of 17minutes. This study went to show the likely anti-venom potentials in the isolated compound as well as those of NB and hexane extracts of *Albizia chevalieri,* which is being reported for the first time.

Keywords: *Albizia chevalieri*, Friedelin, *Naja nigricollis*, Anti-venom agent, Hexane-Extract Fraction, LD_{50}.

INTRODUCTION

Information from literature has indicated that there are over 420 species of snake in Africa, 100 of which occur in Nigeria of which 40 are venomous, 10 being very deadly (Pugh and Theakston, 1980). Both poisonous and non-poisonous snakes occur throughout Africa (Warrell, 1995). However, statistics on snakebites are hard to come by especially in Nigeria (Pugh and Theakston, 1980).

In the Sahel it could be estimated that 23,000 people are killed each year by snakes. It will be easy to verify that those who die are the rural poor, who walk at night without lights, have no proper shoes, cultivate the soil using short-handle tools and have little or no access to medical care (Abubakar, 2004). Hence, Warrell (1995) had earlier opined that in more remote areas of Africa, snakebite can be a significant medical risk. *Naja nigricollis* (Figure 1), also called the black spitting cobra or black necked cobra is among the common spitting cobras found in Nigeria with quite a number of species. The venom is usually collected by the milking method of Markfarlane, 1967 from adult *Naja nigricollis* (Figure 2). This deadly snake is occasionally found in the semi-desert areas of Northern Nigeria particularly in Sokoto (Russell, 1980). In a similar behaviour of treatment remedy by folks of unlettered society, Newman *et al.* (1997) had noted that most victims usually prefer traditional treatment when bitten by venomous snakes and cases of such are not reported to hospitals unless conditions have reached critical stages. Plants with reputations for use against poisonous snake venoms are found worldwide, especially in areas inhabited by poisonous snakes (Abubakar *et. al.*, 2006). Consequently, the use of plants in poisonous snakebites is practiced worldwide (Nuno *et al.,* 1994; Walter *et al.,* 1994;

Selvanayagam *et al.*, 1995).

Quite a good record of plant anti-venom agents used among the various ethnic groups and regions of Nigeria has been established through ethnobanical and ethnomedical records. Notably among the survey data are plants with therapeutic claims on cancer and venoms in the predominant Hausa-Fulani tribe of Northern Nigeria (Abubakar *et. al.*, 2006; Abubakar *et al.*, 2007). Records has also showed plants used in the treatment of poisonous snakebites as spanning genera and species of plants, their uses usually depending on the type of poisonous snake found within the locality (Abubakar *et. al.*, 2006). Some of the methods of therapy use in traditional medicine have been pointed to have scientific basis (Selvanayagam *et al.*, 1995), hence to this end, quite a number of anti-venom compounds have been isolated from some of these plants (Haruna and Choudry, 1995; Tsai *et al.*, 1980).

Figure1. Adult *Naja nigricollis* (Black Spitting Cobra);

Figure 2. Method of venom collection

Therefore, in continuation of attempts to scientifically validate folklore therapeutic claims of ethno-medical survey records, our candidate plant, *Albizia chevalieri* Hams (Mimosaceae) became a plant of choice having earlier been reported of its versatile traditional use (among which is its plant portion used as an anti-venom agent. *Albizia chevalieri* is a tree of the acacia type native to tropical and subtropical regions including Nigeria and Niger Republic, with loose balls of whitish fragrant flowers and flat brown pods. It is a tree that grows up to 12 meters high but could be a shrub under harsher conditions of the dry savannah from Senegal, Nigér and Northern Nigeria. Its detailed taxonomy has been clearly documented by Aubréville, (1950). Although the plant *A. chevalieri* has been scientifically evaluated for its hypoglycaemic and hypolipidaemic, hematotoxicity (Saidu *et al.,* 2007; 2010) as well as antioxidant studies of the leaves (Aliyu *et al.,* 2009), empirical research leading to the investigations of pure bioactive molecules in the plant has not been carried out, despite all the afore-mentioned ethnobotanical exploits.

MATERIALS AND METHODS

Instrumentation

The experiments were recorded in $CDCl_3$ and TMS as internal standard on a Top spin 300 MHz and 400 MHz Bruker spectrometer. The UV was carried out on a Specord® 200 plus analytic jena spectrometer, while IR was performed on Perkin Elmer spectrum Rx IFT-IR system. Thin layer chromatography (TLC) was performed on precoated silica gel plates (0.25mm) Merck, column chromatography was carried out on silica gel-60 (0.063-0.200 mm of 70-230 mesh) Merck.

Collection and Identification of the Plant Material

Albizia chevalieri stembark were collected in the month of July 2013 in Zuru Kebbi State, Nigeria and authenticated by Mr B.D. Musa of Herbarium section, Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. A voucher specimen (900247) was deposited.

Preparation of Extract

Air-dried and powdered stem-bark (250g) was

exhaustively extracted by maceration with n-hexane (2.5L) for 24hours at ambient temperature. The dried marc from the later was then extracted with absolute methanol (2.5L) at room temperature, and the total alcoholic extract was evaporated to dryness under reduced pressure to give a dark-brown mass residue (50g). A portion of the later (30g) was suspended in distilled water (100ml) and successively partitioned with 1L each of EtOAc and n-BuOH (five times each with 150ml) to give ethyl acetate soluble part EA (3.11g), and n-butanol soluble part NB (5.01g). Consequently, four extract fractions labelled: HX, EA, NB and ME (corresponding to hexane, ethyl acetate, n-butanol and methanol fractions respectively) resulted.

Isolation of Compound from Hexane Extract

Silica-gel (150 g) was wet packed in glass column (3.0 x 60 cm) using 100% Hexane. A portion of the Hexane extract (3g) in a fine powdered form (pre-absorbed with silica in DCM and concentrated to obtain a fine powder) was loaded onto the packed column and allowed to stabilize for 2 hours before elution commenced. The column was gradiently eluted with 100% Hexane, Hexane/EtOAc mixtures (19:1; 9:1; 4:1; 2:1; 1:1; 1:2; 1:4; 1:9), and ethyl acetate (100%). Finally, 10% methanol was used to wash the column. In all, a total of 151 fractions were collected at 5 ml each. The fractions were monitored on TLC and similar ones from the column were pooled to give eight (8) fractions (grouped) designated HA to HH. The TLC chromatogram of fraction HF (58-80) eluted with 19:1 hexane/ethyl acetate gradient indicated it was a mixture of few components. On addition of a 100% hexane to the later in a 50ml beaker at room temperature (20^0C), a white precipitate was produced. This was decanted to obtain the sediments (the precipitates) into a separate beaker, after which the precipitates were dried by evaporating out the solvent. The solubility of the precipitates was tested and found readily soluble in acetone and dichloromethane but more in chloroform. The TLC analysis of the precipitate revealed a chromatogram of one spot, but not so absolute, indicating the presence of slight impurity. The precipitates were then further purified by recrystallizing several times in hexane at an elevated temperature to yield white flakes crystalline substance from HF, which was labelled HXC_1 (46mg)

Animals and Venom

Albino mice of both sexes weighing about 18–20g and about 25–30 g were used for the lethal effect (MLD) and inhibition/prophylaxis activity assays, respectively. Lyophilized *Naja nigrocollis* Broadley venom (NNBV) was provided by Prof M.S. Abubakar of the Department of Pharmacognosy and Drug Development, ABU Zaria-Nigeria.

Anti-snake Venom Activity

Lethality Assay of the crude Venoms of *Naja nigricollis.*

The median lethal dose (MLD) of NNBV was determined according to the method developed by Broad *et al.* (1979) and Theakston and Reid (1983). Crude venom was reconstituted with normal saline to obtain concentrations raging 5-11mgml^{-1}. Five groups of mice (n=6) were injected intraperitoneally with 0.2 ml of different doses of the reconstituted venom. The control group received only normal saline (0.2 ml, each i.p.). For each of these groups the average time of death and number of deaths was recorded within 24 hours. The LD_{50} value (MLD) of venom was determined by probit analysis. Mice that survived were euthanatized at the end of the experiment and examined.

Median lethal dose determination (LD_{50}) of extract of *Albizia chevalieri*

The methods of Lorkes (1983) was used: a total of 13 mice in two phases: phase one (3 groups, n=3) and phase two (4 groups, n=1).

Evaluation of Inhibitory/Prophylaxis Effect of Crude Extracts/Fractions of *A. chevalieri* on Lethal Dose of the Crude Venom.

The effect of the extract fractions (HX, EA, and ME) on lethal doses of venom was determined by grouping the albino mice into three groups of five animals (n=5); group 1 was given i.p. dose of MLD venom only, group 2 (duplicated into three with n=5 for each fraction) was given an i.p. dose of 200 mg kg^{-1} of extract in normal saline followed 1hour later by MLD of venom. Group three (the negative control) was given normal saline and followed 1 hour later by an i.p dose of 100 mg kg^{-1} of extract. The average time of death and

mortality after 24 hours was determined (Abubakar, 2004). The procedure was then repeated using a lower concentration of 100mg kg^{-1} for extract fractions that showed activity at 200mgkg^{-1} concentration

Studies of in-vivo anti-venom effect of pure isolated compound (friedelin) from hexane extract fractions of *A. chevalieri* on *Naja nigricollis* envenomation.

Isolated compound from ethyl acetate at 4 and 8 mgkg^{-1}, was investigated of its anti-venom properties according to the method described by Abubakar, 2004. Three groups of n=5 mice were used in this protocol. The first group (G1, control) received 0.2 ml of MLD of venoms ($LD_{99;}$ 5mgkg^{-1}) only. The second group (G2) received 4mgkg^{-1} of pure isolated compound, (HXC_1); while the third group (G3) received 8 mgkg^{-1} of HXC_1. Mortality and average time of death was determined over a period of 24 hours. Mice that survived the experiment were euthanatized at the end of the study and examined (Abubakar, 2004).

RESULTS

Structure Determination of Compound HXC_1

Compound $HXC_{1,}$ was obtained as white crystalline flakes (46mg). The ^{1}H NMR (Fig. 3) revealed signals for seven siglets of methyl at δ 0.93 (H-28), 0.85 (H-27), 0.85 (H-26), 1.00 (H-30), 1.00(H-29), 0.85(H-25) and 0.70 (H-24), a doublet of methyl at δ 1.18 (d, J = 7.7 Hz, 1H), a methine proton (CH) at δ 2.25 (q, J = 6.7 Hz, H-4) and methylene protons (CH_2) at δ 0.85-1.23(m, 23H).

The ^{13}C NMR showed a total of thirty carbons, including the significantly diagnostic ketonic (carbonyl) C-3 carbon at δ = 213.45ppm

The FTIR spectrum of compound HXC_1 showed IRυ_{max} cm^{-1}:1700.9 (C=O stretching), which confirmed the ketonic (carbonyl) C-3 carbon at δ = 213.45ppm in the ^{13}CNMR; also observed are 2918 (C-H stretching) and 1461-1373 (C-H bending and rocking respectively).

The electro-spray ionisation mass spectroscopy (ESI-MS) spectrum showed a molecular ion (*m/z*) peak at 427.15 in the positive ion mode $[M+H]^+$, which corresponds to a compound with molecular formula $C_{30}H_{50}O$. Other important fragment ions (m/z) are as seen in the spectrum presented in Figure 3.

Hence, based on the spectroscopic data of compound HXC_1 gathered above, (summarised in Table 1 as well as seen from the spectra in Figure 3) which were in agreement with those reported in literature (Abreu *et al*., 2013, Sousa *et al*.**,** 2012 Putra *et al.,* 2008 and Boonyaratavej & Petsom, 1991), HXC_1 was identified as Friedelin (octadecahydro-4, 4a, 6b 8a, 11, 11, 12b, 14a-octamethylpicen-3(4H, 6bh, 14bH)-one). The structure is presented in Figure 4.

Table1. Spectroscopic data of HX_{C1} (Friedelin)

Spectroscopic technique	**Data**								**References (Compare with)**
UV λmax:	No absorption								Sousa *et al*., 2012
IRυ_{max} cm^{-1} [FTIR]	2918.0 - (C-H str)	2844.0	1700.9 (C=O str)	1461.90	1158.20	1022.04			
^{1}HNMR(CDCl$_3$) [400 MHz]	1.77 – 1.32: Signals overlap	δ 2.32 – 2.17 (m, 2H), 1.51 – 1.32 (m, 5H), 1.30 – 1.18 (m, 3H), 1.23 (s, 4H), 1.18 (d, J = 7.7 Hz, 1H), 1.16 (s, 3H), 1.05 – 0.93 (m, 10H), 0.93 (s, 4H), 0.94 – 0.78 (m, 15H), 0.70 (s, 4H).							Putra *et al.,* 2008;
^{13}CNMR (CDCl$_3$) [101 MHz]	δ 22.28 δ36.22 δ 30.03 δ 18.04	δ41.74 δ59.54 δ 42.36 δ18.88	**δ 213.46** δ 35.24 δ 32.63 δ 19.45	δ 58.14 δ 29.88 δ 28.18 δ 29.92	δ 41.88 δ 39.20 δ 32.00 δ 32.30	δ 35.83 δ 38.51 δ 39.01 δ 32.30	δ 18.16 δ 30.21 δ 7.04	δ 52.26 δ 35.55 δ 14.41	Abreu *et al*.,2013
HREIMS: m/z (rel. % abundance)	427.15 (2)	[M+H]$^+$;	411.94 (4)	341.19 (3)	273.15 (15)	206.96 (27)	122.99 (54)		

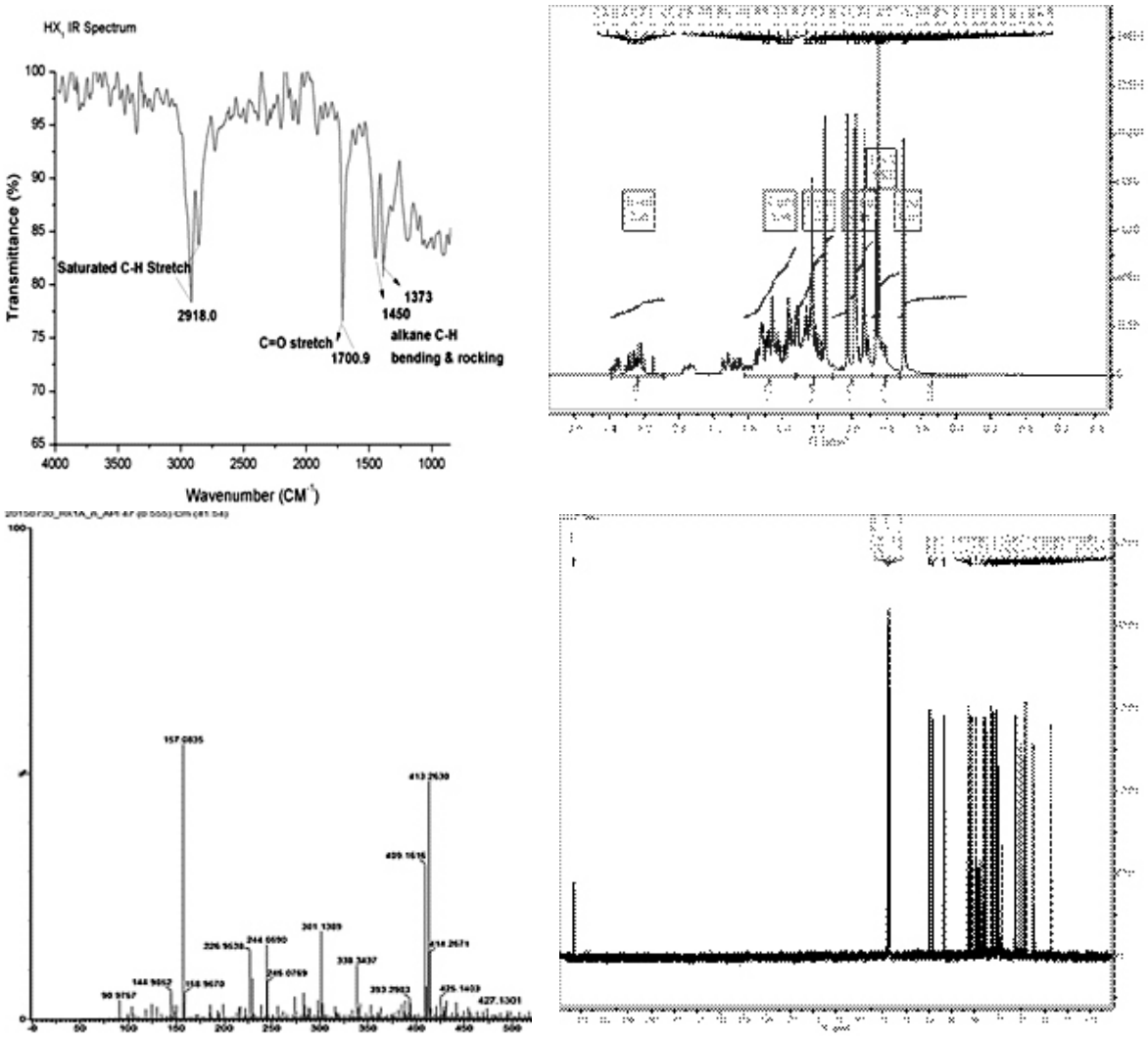

Figure 3. IR, ^{1}HNMR, ^{13}CNMR and EIMS Spectra of HX_{C1}

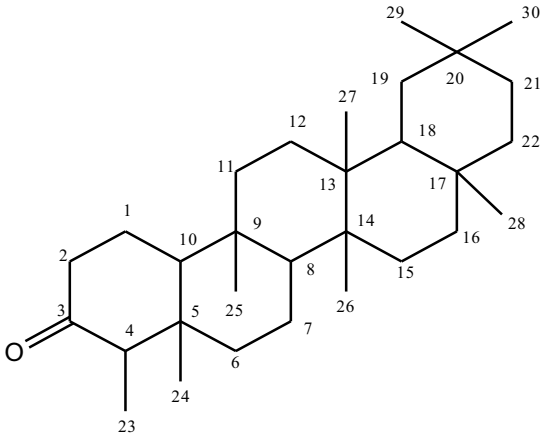

Figure 4. The chemical structure of HXC_1 (Friedelin)

Lethality Assay of the Crude Venoms of *Naja nigricollis*

The lethality assay shows the LD_{99} of crude venoms of *Naja nigricollis* to be at 5.0 mg kg^{-1}. The mice treated with MLD of *Naja nigricollis* venom showed principally neurotoxic symptoms. Table 2 present result for the determination of MLD of *Naja nigricollis* venom using probit analysis.

Table 2. Determination of Minimum Lethal Dose (MLD) of venom of *Naja nigricollis on mice*

Group	Treatment	No. of deaths / No. of mice used	Survival % (within 24 hr)	Average time of Death
1	5mgkg^{-1}	4/6	33.3	527min
2	7mgkg^{-1}	6/6	0	20min
3	9mgkg^{-1}	6/6	0	15min
4	11mgkg^{-1}	6/6	0	10min
5	N-Saline	6/6	100	>24hrs

MLD of Venom = 5.0mgkg^{-1} **(**using probit analysis)

Ld_{50} of Extract of *Albizia chevalieri* Using the Lorke's Method

Table 3 present the estimated LD_{50} for the crude methanol extract *of A. chevalieri* as 0.5657 gkg^{-1}, using the Lorke's method. This shows them to be well tolerated (at a venom protection doses of 200 mg/kg).

Table 3. Determination of LD_{50} of *A. chevalieri* by Lorke's Method:
[Phase One]

Groups/Conc	Ave. Wight. (g)	Ave. Doses	No. of deaths / No. of mice used	Survival % (within 24 hr)	Ave. time of Death
10mgkg^{-1}	21.30	0.02	0/3	100	No dead
100mgkg^{-1}	14.00	0.02	0/3	100	No dead
1000mgkg^{-1}	10.33	0.02	2/3	33.3	6hrs

[Phase Two]

Groups/Conc	Wight. (g)	Doses	No. of deaths / No. of mice used	Survival % (within 24 hr)	Ave. time of Death
200mgkg^{-1}	36.0	0.1	0/1	100	No dead
400mgkg^{-1}	38.0	0.2	0/1	100	No dead
800mgkg^{-1}	40.0	0.4	1/1	0	5hrs
1600mgkg^{-1}	20.0	0.4	1/1	0	6.12hrs

$Ld_{50} = \sqrt{D^0 * D^{100}} = \sqrt{400 * 800} = \mathbf{565.7mgkg^{-1}}$

Evaluation of the Extract Fractions of *A. chevalieri* for Antivenom Activity

Table 4 below shows the effect of 200 mgkg^{-1} extracts challenged with MLD envenomated mice. The dose was deduced preliminarily based on the results of the LD_{50} of *A. chevalieri* determined using the Lorkes method as a guide, which showed that the dose level is nontoxic for all the plant extracts on the MLD of venom. 'Percentage Survival' (protection) was calculated after 24 hrs. Extract fractions of n-Butanol and Hexane offered the highest protection (both showing 16.7%) as compared to those of Methanol and Ethyl acetate (both showing 0%).

Table 4. Evaluation of extract fractions of *A. chevalieri* at 200 $mgkg^{-1}$

Groups/Conc	Ave. Wght. (g)	Ave. Doses/vol.	No. of deaths / No. of mice used	Survival % (Within 24 hr)	Ave. time of Death
MeOH 200$mgKg^{-1}$	17.30	3.5mg/0.2	6/6	0	60min
n-Butanol 200$mgkg^{-1}$	17.50	3.6mg/0.2	5/6	16.7	253.5min
EtOAc 200$mgkg^{-1}$	18.17	3.7mg/0.2	6/6	0	13.3min
Hexane 200$mgkg^{-1}$	18.00	3.8mg/0.2	5/6	16.7	250.1min
N-Saline	27.70	NS-mg/0.2	6/6	0	35.5min

Table 5 shows results for the evaluation of Hexane and n-Butanol extract fraction at a lowered dose of 100$mgkg^{-}1$. This dose could not protect the animal, as both extracts gave a survival (protection) of 0%; the animals died at an average time lower than the MLD (positive control) group. However, almost all animals from the group treated with normal saline (the negative control) survived.

Table 5. Evaluation of Hexane and n-Butanol Extract fraction of *A. chevalieri* at100$mgkg^{-1}$on MLD of Venom

Groups/Conc	Ave. Wght.(g)	Ave. Doses/vol.	No. of deaths / No. of mice used	Survival % (Within 24 hr)	Ave. time of Death
Venom only (MLD)	28.20	0.2	4/5	20	17.5min
Hexane 100$mgKg^{-1}$ + MLD	28.20	0.3	5/5	0	2.6min
n-But 100$mgKg^{-1}$ + MLD	27.20	0.3	5/5	0	10.2min
N/S + 100$mgkg^{-1}$ Hex	26.20	0.3	0/5	100	>24hrs
N/S + 100$mgkg^{-1}$ n-But	24.80	0.3	1/5	80	1170min (19.5hrs)

Tables 6 shows the *in vivo* activity of the isolated compound, HXC_1. This offered some protection by prolonging the time of death of the mice, as seen when compared with the average time of death of the MLD group. However, this protection is dose or concentration dependant.

Table 6. Evaluation of compound HXC_1 of *A. chevalieri* on MLD of venom

Groups/Conc	Ave. Wght.(g)	Ave. Doses/vol.	No. of deaths / No. of mice used	Survival % (Within 24 hr)	Ave. time of Death
Venom only (MLD)	28.20	0.2	$\frac{4}{5}$	20	17.5min
HXC_1 4mgKg^{-1} + MLD	16.80	0.1	$\frac{5}{5}$	0	21.2min
HXC_1 8mgKg^{-1} + MLD	17.30	0.2	$\frac{5}{5}$	0	38.4min

DISCUSSION

The plant *A. chevalieri* has been reported to be bioactive from the various evaluations of its extracts. However, the active constituent responsible for some of these activities has not been investigated. Isolation of pure active lead molecule from the extracts of this plant is reported for the first time. Friedelin is a triterpenoid common in the plant Kingdom and have been isolated by many other researchers. Its distribution in the plant Kingdom has been mainly in the plant families of Celastraceae, Guttiferae and Flacourtiaceae, as reported by Gunatilaka, (1984). In addition, friedelin has been reported in many species of the genus *Calophyllum*: *Calophyllum innophyllun* (Ali *et al*., 1999; Yimdjo, *et al*. 2004), *C. gracilipes* (Cao *et al*., 1997), *C. lankaensis*, *C. thwaitesii* (Dhamaratne, *et al*., 1984), *C. calaba* (Gunatilaka *et al*., 1984), *C. amoenum* (Banerji, *et al*., 1977) and *C. cuneifolium* (Gunasekera, *et al*., 1977), *C. soulattri* (Susidarti *et al*., 2014); others are *Dombeya torrida*, family Sterculiaceae (Ndwigah, 2013), *Bridelia tomentosa*, family Euphorbiaceae (Boonyaratavej and Petsom, 1991), but only for the first time in *Albizia chevalieri*, family Mimosaceae.

The results of the assay in Table 5 showed that the dose of 100mg/kg could not protect the animal against lethal MLD dose of venom, as they died earlier than those injected with MLD only. The Hexane and n-butanol extracts earlier showed protection at 200mg/kg as against those of ethyl acetate and Methanol extract fractions at the same dose. This informed their selection for investigation at a much lower dose of 100mg/kg.

However, going in line with bioassay-guided studies, the compound HXC_1 (friedelin) was isolated from the hexane extract fraction and assayed to confirm the result of the activity of the crude hexane extract earlier investigated. The result of the later showed that the pure compound, friedelin was more potent than the extract fractions of hexane and n-butanol (put together) that earlier showed activity (Table 5). The ethyl acetate earlier recorded the least activity (Table 4).

The result of this study as seen in Table 4, where both n-butanol and the hexane crude fractions showed activity at 200mg/kg but almost non at 100mg/kg can be explained by reasoning that the anti-venom agent(s) in the plant may be synergistic. However, it is evidently seen from Table 6 that the bioactive antivenom molecule in the plant is dose/concentration-dependant. Consequently, an increase of dose from 4mgkg^{-1} to 8mgkg^{-1} prolonged time of death of mice challenged with lethal dose of venom, thereby performing a prophylaxis function to the animals. This implies that higher doses (doses > 8mg per weight), although not investigated, may offer a maximum protection, which may be higher enough to lead to a 100% survival of animal within or even beyond the observed period of 24 hours. In the interim, the pure compound, HXC_1 recorded some 64% protection time, which is 30% higher than the animals treated with MLD only (Table 6). This showed its potential as prophylaxis with promising potency as an antivenom agent.

The seeming alleviation of toxic symptoms and survival of laboratory animals (within a short time frame) after being challenged with lethal doses of venom is in good agreement with the findings of Okonogi *et al*., (1979), Haruna *et al*., (1995), Mors, *et al* (2000), Gomes *et al.,* (2007), Santhosh *et al*., (2013) and Kadiyala *et al*., (2014), where different classes of plant constituents have been shown to

possess *in vivo* activity against some snakes venom; these including sterols, triterpenes, phenolic compounds (flavonoids and tannins), aristolochic acids etc. The presence of some of these active classes of compounds with antivenom effect and their mechanism of actions have also been suggested (Tsai *et al*., 1980).

CONCLUSION

Chromatographic separation of the hexane extract portion led to the isolation of the compound friedelin and its investigation for anti-venom property in the plant *A. chevalieri* for the first time. Although the solubility of friedelin in water may pose a challenge, this pentacyclic triterpenoid often occurring in some plant species may just rightly be a potent bioactive molecule, serving as a lead candidate drug of anti-venom agent as well as other potentials it may have. These results therefore, justified the folklore therapeutic claims of the use of *A. chevalieri* as a snake bite remedy among its other associated medicinal uses.

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