International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 8, Issue 5, 2016

Original Article

PENTACYCLIC TRITERPENOIDS FROM THE STEM-BARK OF *ALBIZIA CHEVALIERI* HAMS (MIMOSACEAE)

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Received: 01 Feb 2016 Revised and Accepted: 30 Mar 2016

ABSTRACT

Objective: This research is focused towards isolation and identification of pure compounds from the extracts fractions of *Albizia chevalieri* through the means of gravity column chromatography and other chromatographic processes.

Methods: the stem bark was extracted exhaustively 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 extracts were subjected to silica gel gravity column chromatography for the isolation of pure bioactive molecules.The major compounds isolated, were then determined and identified by the use of spectrometric analysis of HR-ESIMS, ^{1}HNMR, ^{13}CNMR, IR and UV spectra.

Results: Investigation of the stem bark hexane extract fraction of *A. chevalieri* led to the isolation of three known pentacyclic triterpenoids: friedelin (HXC_1), Friedelan-3-ol (HXC_2) and Lupeol (HXC_3), for the first time in the plant.

Conclusion: The results obtained will be useful in the evaluation (bioassay) of the isolated compounds against the list of folklore therapeutic claims of *A. chevalieri* (which include its use as purgative, taenicidal, remedies of cough, dysentery, cancer, diabetes mellitus, tuberculosis and snake bite), and thereby providing scientific basis for its used for treatment of the aforementioned ailments.

Keywords: Friedelin, Friedilan-3-ol, Lupeol, Isolation, Spectrometry

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INTRODUCTION

The genus *Albizia* comprises approximately 150 species, mostly trees and shrubs native to tropical and subtropical regions of Asia and Africa. *Albizia* is a large genus of tree, of the pea family (Fabaceae). *Albizia chevalieri* Hams used to belong to the sub-family of the Fabaceae known as Mimosoideae, however, the Cronquist System' treat the Fabaceae in a narrow sense, raising the Mimosoideae to the rank of the family as Mimosaceae. Hence, *A. chevalieri* (Mimosaceae) is a tree of the acacia type native to tropical and subtropical regions including Nigeria and the 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, [1].

A. chevalieri is a plant with a long list of folklore therapeutic claims which include its plant part use as a purgative, taenicidal; cough remedy, dysentery, cancer, diabetes mellitus, tuberculosis and snake bite remedy. Bioactive evaluation of its leave constituent has shown that it has antioxidant [2], anti-hypoglycemic and hypolipidaemic [3] properties. Hepatotoxicity study [4] also revealed that the leaves might be rightly be considered safe in the tested doses in alloxan induced diabetic rats. Consequently, literature of various biological evaluations of *A. chevalieri* extracts have affirmed its bioactivity but however, until this work, the active pure constituent responsible for some of these activities have not been investigated. Isolation of pure active phytomolecule from the extracts of this plant is reported for the first time.

Ethnobotanical survey information revealed that the bark of *A. chevalieri* was reported to contain alkaloids and also tannins sufficient for use in tanning in Nigeria and Senegal [2]; it is used in Borno, North-Eastern Nigeria as purgative, taenicidal and also remedy for coughs [2]. A decoction of the leaves is used in Northern Nigeria as remedy for dysentery. There are also reports on the local use of the leaves extract for cancer treatment in Zaria city, Kaduna State North-western Nigeria, while in the Northern Sahel Savannah of Sokoto-Nigeria and Niger Republic, the leaf extract is used either as cold water decoction or the dried ground sieved leaf powder is mixed with pap and consumed for the management of diabetes mellitus by traditional medical practitioners [4]. Meanwhile, an ethnomedical survey conducted in the course of this research revealed the use of the root and stem barks of *A. chevalieri* for therapy against tuberculosis, toothache, and inflammations among the Zuru people of Kebbi State, Nigeria.

Comparative phytochemical researches in various laboratories have indicated that plants found in the tropics/tropical rain forests of the world have three to four times more biologically active compounds than their temperate counterparts. Consequently, it has been observed that the primary sources of chemical compounds that exhibit novel pharmacological activities essential for new drug development are likely to come from marine invertebrates, insects and vascular plants from the tropics/tropical rain forest [5]. It is, therefore, the overwhelming consensus of Phytochemists that tropical plants are understudied and contain many undiscovered drugs.

Hence, credibility for the choice of the candidate plant is supported from it ethnobotany; owing also to the fact that researchers have found that chances of success in finding useful drugs can be increased threefold if the search for a medicinal plant is concentrated on plants used for medicinal purposes by indigenous peoples of regions who have preserved their traditional culture [5]. This paper has presented research on isolation of pure phytomolecules from *A. chevalieri* without evaluating their bioactivity. The latter aspect is however, presented in our subsequent paper.

MATERIALS AND METHODS

Instrument

The experiments were recorded in $CDCl_3$ and TMS as internal standard on a Topspin 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. The compounds isolated were analysed using an electrospray ionisation quadrupole time-of-flight mass spectrometry (ESI-QTOF MS) with a scanning range of 100 to 1200 Da kDa), and was operated in both the positive and negative mode, SYNAPT G1 facility of Chemistry Department, University of Pretoria, Hatfield, South Africa. Thin layer chromatography (TLC) was performed on precoated silica gel plates (0.25 mm) Merck while column chromatography was carried out on silica gel-60 (0.063-0.200 mm of 70-230 mesh) Merck in a glass column (60 x 3 cm).

Plant material

Albizia chevalieri stembark was 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 stembark (250g) were extracted exhaustively with hexane by maceration in 2.5L hexane for 24hours at room tempt, after which it was filtered and separated from the marc. The filtrate was concentrated in vacou to obtain the hexane extract (HE). The marc was air-dried and subsequently extracted by maceration in absolute methanol (5L) at room tempt for 48hours to give the methanolic extract (ME). 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 (100 ml) and successively partitioned with 1L each of EtOAc and n- BuOH (five times each with 150 ml) to give ethyl acetate soluble part EA (3.11g), and n-butanol soluble part NB (5.01g). Consequently, four extract fractions labelled: HE, EA, NB, and ME (corresponds to hexane, ethyl acetate, n-butanol and methanol fractions) resulted.**Isolation**

A portion of the Hexane extract (3g) was parked in a column (60 cm x 3 cm) with silica gel (150g), and eluted with n-hexane and n-hexane**:** ethylacetate gradient mixtures (19:1; 9:1; 4:1; 2:1; 1:1; 1:2; 1:4; 1:9), ethyl acetate (100%) and finally 10% methanol was used to wash the column. In all, a total of 151 fractions were collected at 5 ml each. Column fractions 58-80, 81-93 and 94-100 eluted with 19:1, 9:1 and 4:1 hexane-ethyl acetate gradient respectively, were found similar in each case and pooled, which showed a yellowish sticky mass on concentration. On addition of 100% hexane to each of the concentrated solids of the three groups, a white crystal which was insoluble in 100% hexane was precipitated in each case. On checking using TLC (solvent system, hexane/ethyl acetate, ratio4:1), the crystals from the group 58-80 and 81-93 showed single spots with similar Rf and were labelled HXC_1 (46 mg), while those obtained from the group 94-100 showed single spot of different Rf from the later and labelled HXC_2 (27 mg).

Column fraction 94-151 (≈ 1g) was subjected to re-column chromatography on 50g silica packed in a smaller column (1.5 cm i. d x 65 cm) of 50 ml capacity. An eluent gradient of 49:1, 19:1 and 4:1hexane-EtOAc was utilised for 100 collections at 5 ml. On analysis of the fractions, F62-80, it showed a TLC chromatogram of single spots with the same Rf. These were pooled and concentrated before repeating the TLC run to ascertained component purity. The TLC of the later (appearing creamy white) gave a chromatogram with a single spot and was labelled HXC_3 (9 mg).

RESULTS

Compound HXC_1 was obtained as white flakes crystalline solids (46 mg). Table 1 below gives a summary of spectral information.

Table 1: Spectroscopic data of HXC_1 (Friedelin)

Spectroscopic Technique	Data									References (Compared)
UV λmax:	No absorption									[6]
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]	Signals overlap	1.77–1.32:	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).							[7]
^{13}CNMR ($CDCl_3$) [101 MHz]	δ22.28		δ41.74	δ 213.46	δ 58.14	δ 41.88	δ 35.83	δ 18.16	δ 52.26	[8]
	δ36.22		δ59.54	δ 35.24	δ 29.88	δ 39.20	δ 38.51	δ 30.21	δ 35.55	
	δ 30.03		δ 42.36	δ 32.63	δ 28.18	δ 32.00	δ 39.01	δ 7.04	δ 14.41	
	δ 18.04		δ18.88	δ 19.45	δ 29.92	δ 32.30	δ 32.30			
HREIMS: m/z (rel. % abundance)	427.15 [M+H] +; (2)			411.94 (4)	341.19 (3)	273.15 (15)	206.96 (27)	122.99 (54)		

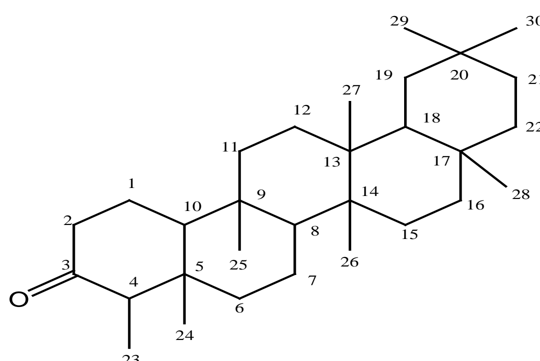

Fig. 1: The chemical structure of HXC_1 (Friedelin)

Compound HXC_2, was obtained as a white crystalline solid (27 mg). Table 2 below give a summary of spectral information.

Table 2: Spectroscopic data of HXC_2 (Friedelane-3-ol)

Spectroscopic technique	Data								References (Compared)
UV λmax:	No absorption								[6]
IRυ_{max} cm^{-1} [FTIR]	3342.3 (O-H str),	2937.5 (Aliphatic str),	2844.0	1461.9	1382.96	1158.2	1022.04		[9]
^{1}HNMR ($CDCl_3$) [400 MHz]	Signals overlap	δ 2.39 (d, *J* = 5.1 Hz, 0H), 1.77–1.68 (m, 1H), 1.50 (d, *J* = 16.4 Hz, 3H), 1.44 (d, *J* = 2.4 Hz, 1H), 1.41 (d, *J* = 10.6 Hz, 1H), 1.42–1.30 (m, 7H), 1.33–1.22 (m, 5H), 1.16 (s, 6H), 1.05–0.95 (m, 18H), 1.00–0.89 (m, 1H), 0.93 (s, 7H), 0.69 (s, 0H).							[10]
^{13}CNMR ($CDCl_3$) [101 MHz]	δ17.92	δ36.06	δ 72.16	δ 50.08	δ 38.22	δ42.06	δ17.19	δ 53.26	[11]
	δ37.68	δ61.47	δ 35.66	δ 30.50	δ 39.20	δ39.01	δ11.80	δ16.44	
	δ30.03	δ42.65	δ 35.32	δ 28.18	δ 32.78	δ39.01	δ33.35	δ33.35	
	δ17.37	δ19.41	δ19.45	δ 32.11	δ33.35	δ33.35			
HREIMS: m/z (rel. % abundance)	429.7; 411.3 [M+H]$^+$[M+H−18]$^+$		413.2 (30)	273 (11)	205 (6)	123 (7)			

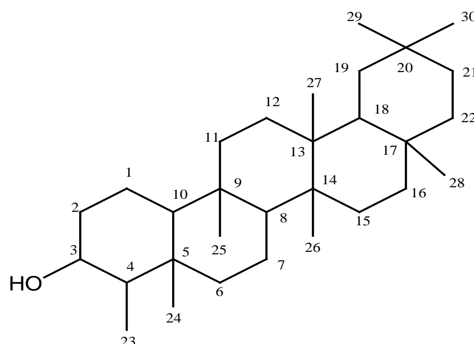

Fig. 2: The chemical structure of HXC_2 (Friedelinol)

Compound HXC_3 was obtained as a white amorphous solid (9 mg). Table 3 below give a summary of spectral information.

Table 3: Spectroscopic data of HXC_3 (Lupeol)

Spectroscopic technique	Data								References (Compared)
UV λmax:	370								[12]
IRυ_{max} cm^{-1} [FTIR]	3253.4 (O-H str),	2907.5- (C-H Alkyl str)	2851.4	1680 (C=C str)	1012- =CH bend	880			
^{1}HNMR ($CDCl_3$) [400 MHz]	δ 4.75 (dp, *J* = 2.0, 0.9 Hz, 3H), 4.69 (d, *J* = 2.2 Hz, 1H), 3.44 (td, *J* = 7.0, 5.0 Hz, 3H), 2.07–1.94 (m, 5H), 1.82–1.72 (m, 1H), 1.72 (s, 3H), 0.92 (s, 6H), 0.84 (d, *J* = 18.9 Hz, 12H), 0.77 (d, *J* = 14.3 Hz, 12H), 0.65 (t, *J* = 6.9 Hz, 3H).								
^{13}CNMR ($CDCl_3$) [101 MHz]	δ38.01,	δ29.81,	δ79.02,	δ38.83,	δ55.25,	δ17.98,	δ34.24,	δ40.79,	[13]
	δ50.39,	δ37.14,	δ20.90,	δ25.09,	δ38.66,	δ42.80,	δ29.65,	δ35.55,	[14]
	δ42.98,	δ47.97,	δ48.26,	δ150.98,	δ31.92,	δ39.98.	δ29.70	δ29.70,	
	δ16.11,	δ15.95,	δ14.52,	δ18.29,	δ109.30,	δ19.28			
HREIMS: m/z (rel. % abundance)	427.3; [M+H]$^+$	410 (25)	409 (30)	383 (5)	207 (3)	189 (5)			

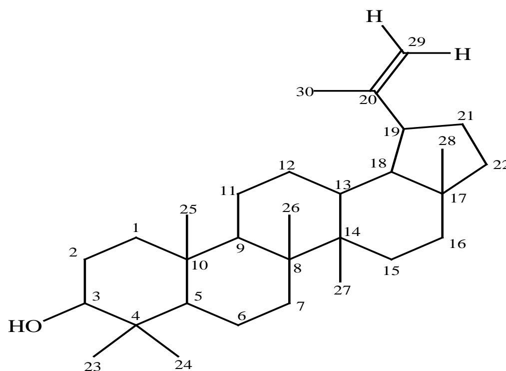

Fig. 3: The chemical structure of HXC_3 (Lupeol)

DISCUSSION

The plant *A. chevalieri* from various evaluations of its extract has been reported to be bioactive. However, the active constituent responsible for some of this activity was not evaluated. Isolation of pure active phytomolecules from the extracts of this plant is reported for the first time. The hexane extract of the stembark of *A. chevalieri* was separated on silica gel column and purified by washing to precipitate white solid compounds HXC_1, HXC_2 and HXC_3. The ^1H and ^{13}C NMR spectra data for these compounds revealed typical triterpenoids spectra features.

Compound HXC_1, a white crystalline powder, gave the following: the MS spectrum showed a molecular ion peak at *m/z* 247.4 in the positive mode (*m/z* 427.3969 $[M+H]^+$); The ^1H-NMR 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) and a doublet of methyl at δ 1.18 (d, *J* = 7.7 Hz, 1H), suggesting that there are eight methyl groups present in the compound. The signals at δ1.25 and 1.2, δ1.70 and 1.45, δ 1.25 and 1.4, δ 1.25 and 1.23 were due to un-equivalent methylene protons (CH_2) attached to carbon C-2, C-1, C-6 and C-22 respectively. The presence of a cross-correlation between a quartet methine proton (CH) at δ 2.25 (q, *J* = 6.7 Hz, H-4) and a doublet methyl signal at δ 0.81 (at C-23) in the COSY spectrum as well as the presence of signals due to one secondary and seven quaternary methyl in the ^1HNMR spectrum suggested the friedelane skeleton of pentacyclic triterpenoid (PTT) [15]. These data are supported by ^{13}C-NMR spectrum. The ^{13}C-NMR showed the presence of thirty carbon resonance and also gave a signal at δ_c (ppm) 213.45 (C-3) which is characteristic of a ketone carbonyl or a carbon bearing an oxygen in a triterpenoidal structure. The DEPT spectrum further reaffirmed the later and also showed eight methyls, eleven sp^3 methylene, four sp^3 methines and six quaternary sp^3 carbons. The ^{13}CNMR (table 1) of the compound HXC_1 is in agreement with published literature [8]. The FTIR spectrum showed an intense band at $IR\upsilon_{max}$ 1700.9 cm^{-1} consistent with a six-membered ring ketone for C=O stretching. There was no absorption observed in the UV spectrum, suggesting the absence of unsaturation in the compound. This information derived from the MS, NMR and IR are in agreement with those reported in the literature [8, 6, 13&11] for Friedelin, a PTT.

Compound HXC_2 was isolated as a white crystalline powder, highly soluble in chloroform, with a melting point of 287-290°C. The mass spectral data showed a molecular ion peak $[M+H]^+$in the positive mode with m/z 429, indicating the quasimolecular ion, with other characteristic fragment ions peaks seen at m/z 413, 409, 342, 273, 205 and 123 (table 2). The ^1H-NMR displayed signal for eight tertiary methyl proton singlet at δ_H (ppm) 0.89 (23H), 0.93 (24H), 0.99 (25H), 0.89 (26H), 0.86 (27H), 0.97 (28H), 0.93 (29H) and 0.79 (30H). This suggests that there are eight methyl groups present in the compound. The presence of downfield signals at δ_H (ppm) 4.34 (1H, br.) suggest the presence of hydroxyl group. The appearance of the signal showing a double doublet at 4.34 δ_H (J=7.1 and 4.9Hz 1H) is typical of a hydrogen bonded to an oxygenated carbon, further suggesting the existence of a hydroxyl group in the structure [10]. The ^{13}C-NMR spectrum showed the presence of thirty carbon resonance and gave important signal peaks at δ_c (ppm) 72.89 (C-3), is typically indicative of a carbon bearing hydroxyl group in a triterpenoidal structure. The DEPT 135 spectrum further reaffirmed the later by showing eight methyls, eleven sp^3 methylenes, four sp^3 methines and six quartenary sp^3 carbons. The ^{13}CNMR spectrum data (table 2) of the compound HXC_2 are in agreement with published literature [11 & 10]. The FTIR spectrum showed a strong, broad peak at $IR\upsilon_{max}$ 3342 cm^{-1} affirming to an OH group at C-3, which is characteristic of an aliphatic OH stretching. Other signals at 2920 cm^{-1} (alkyl C-H stretching) and 1600 cm^{-1} (C-C stretching in ring) further help to indicate the absence of unsaturation in the structure. There was no absorption observed in the UV spectrum, hence sopporting the other spectra information. This information derived from the MS, NMR and IR are in agreement with those reported in literature [10, 11, 6 & 9] for 3β-friedelinol

Compound HXC_3 was isolated as a creamy white powder, highly soluble in chloroform. The mass spectral data showed molecular ion (quasimolecular ion) peak $[MH]^+$with m/z 427, with other peaks of fragment ions at m/z 410, 409, 383, 207 and 189. The ^1H-NMR showed seven tertiary methyl (CH_3) singlet signals at δ_H (ppm) 0.75, 0.78, 0.83, 0.86, 0.92, 0.97 and 1.23 (3H each, s, CH_3). This suggests that there are seven methyl groups present in the compound. Two protons appeared at δ 4.66 and 4.69 as single representing exocyclic double bond protons assigned to H-29a and H-29b respectively. The signal at δ 3.48 is typical for triterpenoids with a 3-hydroxy substitution [16], with H-3 proton appearing as a triplet of a doublet (tdd) (J = 7.0, 5.0 Hz). The assignments are in good agreement for elucidated structure of lupenol in the literature [16, 17, 18 19 &14]. The ^{13}CNMR gave 30 signals with δ value as in Table3, suggesting that the compound consists of 30 carbon atoms. The appearance of a signal at δ_c (ppm) 79.02 gave information about the carbon bonded to a hydroxyl functional group at (C-3), which has a downfield signal in the ^1HNMR. Diagnostically appearing also is the signals at δ_c 150.98 and 109.30 ppm assigned for C-20 and C-29, is characteristic of a vinylic carbon signals for triterpenoid of the lupane (skeleton) series [16]. The FT-IR spectra of the compound showed a broad peak at $IR\upsilon_{max}$ 3253 cm^{-1} (OH Stretching), and a peak at 1680 cm^{-1} accounted for the vinylic carbon-carbon double bond (C=C stretching). The UV spectrum further confirmed the presence of unsaturation in the compound by revealing a chromoporic absorption at $UV\lambda_{max}$ 370 nm associated with the olefinic mioety carbon signal observed at δ150 and δ 109 in the ^{13}CNMR. The data are consistent with published work [13 & 14] for the elucidation of the compound lupeol.

CONCLUSION

Chromatographic separation of the Hexane soluble plant extract led to the isolation of three pentacyclic triterpenoids (friedelin, friedelinol, and lupeol) for the first time in *A. chevalieri*. Triterpenoids have been reported to exert several biological activities, among which may be associated with some of the folklore therapeutic claims of *A. chevalieri,* when properly validated through an experimental bioassay research. More so, the compounds isolated are somehow water soluble, which offers them a formulation advantage. This findings has therefore provided lead compounds for the proper evaluation of the candidate plant against its traditional (folklore) use in Nigeria.

ACKNOWLEDGMENT

We are grateful to the Department of Chemistry, University of Pretoria, South Africa, for the use of their LC-MS (SYNAPT) facility, and other spectroscopic equipment.

CONFLICT OF INTERESTS

Declared none

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