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A Friedelane Type Triterpene From *Prosopis africana* (Guill. & Perr.) Taub. Stem Bark

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Abstract
Prosopis Africana can be found growing wild in Northern and the Middle Belt of Nigeria and it is the only known species of its genus found in Africa. In folk medicine, the stem bark is used as remedies for skin diseases, caries, fevers gonorrhoea, tooth and stomach-ache, dysentery and bronchitis. Chemical investigation of the stem bark resulted in isolation and characterisation of a friedelane type triterpene, compound J29. J29 was isolated by column chromatography from the chloroform fraction of ethylacetate extract. The J29 ^{1}H-NMR ^{13}C-NMR and DEPT135 spectra matched the characteristic data of the proposed triterpene skeleton. The compound was finally identified as friedelin on the basis of spectroscopic evidence, including 2D NMR as well as its IR spectrum. Friedelin is being reported for the first time as a chemical constituent of *P. africana*.
Keywords: *Prosopis africana*, friedelane type, phytochemical constituents, spectroscopy, chromatograph

1.0 Introduction

Prosopis Africana can be found growing wild in Northern and the Middle Belt of Nigeria. The indehiscent pods are palatable to man and animal. There are available reports on its use as local remedies for several disease conditions. Information on the general phytochemical constituents of *Prosopis africana* in the internationally accessible literatures are rare, except for scattered information on the presence of the two alkaloids, prosopine and prosopinine (Rattle, 1966) (Bourrinet P, 1968). Several 2,6-disubstituted piperidin-3-ol alkaloids have been isolated from the *Prosopis africana,* whose leaves have been used in Africa to treat toothaches (Mathew, 1999). This work is intended to report the isolation of a triterpenoid from the stem bark of the plant which has some known biological activities.

2.0 Methods

2.1 Preparation of P. africana extract

2.1.1 Collection and Preparation of Plant Material

Stem barks, fresh mature leaves and pods were collected from fully-grown plant in fields at the outskirts of Zuru town in Kebbi State, North Western Nigeria. The identity and authenticity of the plant was established by comparing its morphological characters with available literature (Vautier, 2007) and by a taxonomist of the Herbarium Unit, Department of Pharmacognosy and Ethnopharmacy, Usmanu Danfodiyo University, Sokoto, Nigeria. A voucher specimen numbered PCG/UDUS/Mim/004 is kept in the same herbarium unit.

2.1.2 Drying and preparation of material:

The plant stem bark was shade dried for 7 days and pulverized using mortar and pestle to obtain a coarse powder. The powder was stored in appropriate container until required for use.

2.2 Extraction

Exactly 300 g of the pulverized stem of *Prosopis africana* was extracted with ethyl acetate (Sigma-Aldrich-St. Louis, MO, USA) using soxhlet extractor to obtain the ethyl acetate crude extract of the plant.

2.3 Thin Layer Chromatographic (TLC) Finger Print Profile

Ethyl acetate solution of the *Prosopis africana* crude extract was spotted on a pre-coated Thin Layer Chromatographic (TLC) plate (Silica gel 60); and developed using a solvent system composed of Petroleum Ether, Chloroform, Methanol 4:6:1 (V/V/V).The developed TLC plate was air dried and visualized by spraying

with 5% sulphuric acid and heating in an oven at 105 ^{0}C for about 10 minutes. The chromatograph was photographed. The crude extract was fractionated into petroleum ether and chloroform fractions. The TLC profiles of both fractions were determined adopting the procedure above.

2.4 Fractionation of the P. africana crude extract

Using Kern EW Electronic Scale, 4.0 g of the ethyl acetate crude extract was in water and partitioned successively with Petroleum ether (Sigma-Aldrich-St. Louis, MO, USA), Chloroform (Sigma-Aldrich-St. Louis, MO, USA) and Methanol (Sigma-Aldrich-St. Louis, MO, USA) to obtain their respective fractions. The fractions were concentrated and weighed.

2.5 Column chromatographic separation of fraction

A 100 mL capacity glass chromatographic column was wet packed with 60 gm of 60-120 mesh silica gel in n-Hexane (Merck-Germany) solvent and allowed to stabilize for 4 h. The Chloroform fraction (1.24 gm) obtained was dissolved in a small volume of n-hexane and loaded in the column allowing it for 2 h to stabilize before commencing elution. The column was eluted by gradient elution method starting with 100% n-hexane then with 0-100% ethyl acetate. Successive fractions of 10 mL each were collected in numerically labelled collection bottles. The fractions were monitored for purity using TLC with 4:6:1 (V/V/V) petroleum ether, chloroform, methanol solvent system.

2.6 Melting point determination of the isolate

Melting point of compound J29 was determined using Digimelt Melting Point apparatus model MPA 161.

2.7 Spectroscopic analysis of the isolate

The following spectroscopic instrumentations were adopted to ascertain the identity compound J29.

2.7.1 Infrared (IR) Spectroscopy of the isolate

Fourier Transform Infrared Spectroscopy (FTIR) experiments were performed using Shimadzu (Model 4800S) instrument controlled by IR solution software set at spectra resolution of 4 cm^{-1}. A KBr disc was first prepared to serve as a blank then, the sample J29 (2 mg) was dissolved in Chloroform and grounded with 5 mg KBr and pressed to make the sample disc (window). The prepared sample window was scan between 400 – 4000 cm^{-1} twenty times and the mean was printed.

2.7.2 Nuclear Magnetic Resonance (NMR) of the isolate

Both ^{1}H-NMR and ^{13}C-NMR (1D and 2D NMR) analyses were performed on 10 mg of Sample J29 dissolved in 0.5 ml of deuterated chloroform using Topspin 300 MHz, Bruker Germany and TMS as internal standard reference which was added to the sample before recording.

3.0 Results

3.1 Extraction

The ethyl acetate extraction yield profile of *P. africana* stem bark is represented in table 1.

Table 1. The ethyl acetate extraction yield profile of *P. africana* stem bark

Solvent	Quantity Loaded	Quantity of Extract	Yield
Ethylacetate	150 g	4 g	2.67 %

3.2 Column Chromatographic Separation of the Extract

Fractions number 30–35 showed single and identical spots on the TLC chromatogram and were pooled together to obtain a 12 mg sample for further analysis. This sample was designated J29. J29 is a white crystalline needle shaped compound. The mass and physical profile of J29 is represented in table. 2

Table 2. Column Chromatographic Isolate from the Stem Bark of *P. africana*

Fraction Type Loaded	Quantity Loaded	Quantity of Isolate	Yield
Chloroform	1.24 g	0.012 g	0.97%

3.3 Melting Point Analysis

The melting point analysis of the isolate indicated a melting point range of 161 ^{0}C – 163 ^{0}C as shown in table 3.

Table 3: Melting Point Analysis of the isolate (J29)

Melting point range
161 – 163 ^{0}C

3.4 Spectroscopic Analysis of J29

The various spectroscopic evaluation of sample J29 showed the following enumerated results.

3.4.1 Infra-Red (IR) Spectroscopy of Compound J29

The IR spectrum of compound J29 showed νmax (KBr): 3254.05 cm^{-1} (CH_3 stretching), 2899.11 cm^{-1} (C – H Asymmetric and Symmetric Stretching), 1751.05 cm^{-1} (C=O Stretching for Ketone), 1498.74 cm-1 (C-H Bending for CH_3). All the band types are sharp (Table 4).

Table 4: The IR spectrum of J29

Band type	Wavenumber Cm^{-1}	Possible Bond
Sharp	3254.05	CH_3 Stretching
Sharp	2899.11	C –H Asymmetric and Symmetric Stretching
Sharp	1751.05	C=O Stretching for Ketone
Sharp	1498.74	C-H Bending for CH_3

3.4.2 ^{1}HNMR spectrum of J29

The spectrum showed a cluster of signals between δ_H 0.8 to 1.2, characteristic of methyl (CH_3) protons. Also other δ_H were observed between 1.2 to 1.5 which are characteristic of methylene (CH_2) protons. The proton chemical shift of J29 is represented in Table 5.

3.4.3 ^{13}CNMR spectrum of J29

Characteristic signals representing CH_3, CH_2 and CH and other diagnostic signals were observed and reported in Table 5.

Table 5. ^{1}H-NMR and ^{13}C-NMR Chemical Shift of J29

H/C Position	δc (ppm)Ref.	δc (ppm)J29	δH (ppm)Ref.	δH (ppm) J29	Multiplicity
1	**22.3**	22.27	**1.90,1.6**	2.09,1.61	2H,m
2	**41.5**	41.52	**2.22,2.32**	2.22,2.33	2H,m
3	**213.2**	213.37	_	_	_
4	**58.2**	58.20	**2.23**	2.24	1H,m
5	**42.1**	42.15	_	_	_
6	**41.3**	41.26	**1.66,1.21**	1.65,1.21	2H,m
7	**18.2**	18.20	**1.36,1.46**	1.36,1.46	2H,m
8	**53.1**	53.07	**1.36**	1.37	1H,m
9	**37.4**	37.42	_	_	
10	**59.4**	59.44	**1.50**	1.50	1H,m
11	**35.6**	35.60	**1.23,1.25**	1.26,1.28	2H,m
12	**30.5**	30.49	**1.23,1.25**	1.23,1.26	2H,m
13	**39.7**	39.68	_	_	_
14	**38.3**	38.27	_	_	_
15	**32.4**	32.39	**1.36,1.46**	1.38,1.48	2H,m
16	**36**	35.98	**1.36,1.46**	1.35,1.45	2H,m
17	**30**	29.98	_	_	
18	**42.8**	42.75	**1.40**	1.41	1H,m
19	**35.3**	35.32	**1.60,1.20**	1.59,1.19	2H,m
20	**28.1**	28.16	_	_	
21	**32.7**	32.74	**1.41,0.90**	1.42,0.90	2H,m
22	**39.2**	39.23	**1.41,0.90**	1.39,0.91	2H,m
23	**6.8**	6.82	**0.81**	0.82	3H,s
24	**14.6**	14.64	**0.65**	0.70	3H,s
25	**17.9**	17.94	**0.80**	0.79	3H,s
26	**20.2**	20.25	**0.98**	0.98	3H,s
27	**18.6**	18.66	**0.94**	0.94	3H,s
28	**32.1**	32.07	**1.11**	1.15	3H,s
29	**35**	35.60	**0.93**	0.93	3H,s
30	**31.8**	31.77	**0.88**	0.88	3H,s

The reference used is (Rajesh, 2012).

3.5 Structure of compound J29

An integration of the NMR and IR data, which compare well with published data, (Rajesh, 2012) predicts the structure of compound J29 as Friedelin which is presented in Fig. 1.

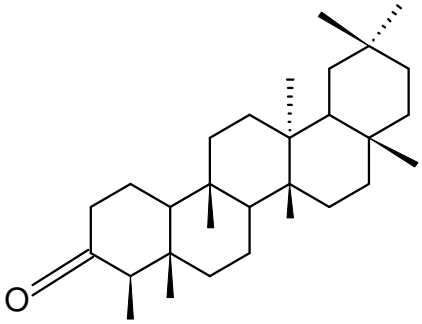

Fig.1. Friedelin

The triterpenoid compound friedelin was isolated and identified from *Prosopis africana*. Friedelin is found to be present in many plants.

4.0 Discussion

Compound J29 was isolated as white needles. The melting point of J29 is 261 – 263 ^{0}C and it is soluble in chloroform. Compound J29 was identified by comprehensive analysis of its NMR spectra in comparison with published data, (Rajesh, 2012). From Table 5, ^{1}HNMR showed seven singlet Methyl (CH_3) signals at δ_H (ppm) 1.15 (3H, 28), 0.98 (3H, 26), 094 (3H, 27), 0.93 (3H, 29), 0.88 (3H, 30), 0.79 (3H, 25), 0.70 (3H, 24), and a doublet methyl at 0.82 (3H, d, 23). It reveals methine proton at 2.24 (1H, m, 4), 1.37 (1H, m, 8), 1.50 (1H, m, 10), 1.41(1H, m, 18). The ^{13}CNMR and DEPT 135 spectra of J29 confirmed the presence of 30 carbons, one of which is δ_C (ppm) 213.37; diagnostic for carbonyl carbons. The IR spectrum (table 4) of the compound J29 showed characteristic absorption peak at V_{max} 1715.05cm^{-1}, which is a ketone stretching signal. This suggests that the carbonyl group is a ketone. The NMR data of J29 were identical with those reported in literature for Friedelin (Rajesh, 2012) (Table 5). Therefore, compound J29 was identified as Friedelin (Fig. 1).

5.0 Conclusion

On the basis of chromatographic and spectroscopic techniques the pentacyclic triterpenoid Friedelin, have been isolated characterized and reported for the first time as a phytoconstituent of *P. africana* stem bark.

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