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ISOLATION OF BETULINIC ALDEHYDE FROM N-HEXANE LEAVES EXTRACT OF *Piliostigma thonningii* (SCHUM.)

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ABSTRACT

An experiment was carried out to isolate Betulinic aldehyde and characterize its structure from the n-hexane extract using standard procedure. The n-hexane fraction was subjected to column chromatography followed by preparative thin layer chromatography which resulted in isolation of a colorless crystalline compound named as "PA". The spectral data (1H NMR, 13C NMR and FTIR) and literature comparison infer that the isolate is a pentacyclic triterpenoid namely Betulinic aldehyde. To the best of our knowledge, this is the first time Betulinic aldehyde was isolated from the leaves extract of Piliostigma thonningii (Schum.)

Key words: *Betulinic aldehyde, pentacyclic triterpenoid, isolation, crystalline compound and n-hexane*

Introduction:

Plants have been the primary source of health care for both humans and other animals for many years (Yagayata and Vijay, 2012). Approximately 80 % of the world's population depends on plant for treatment of diseases (WHO, 2000). Plants are the major source of pharmaceuticals. They provide bioactive compounds that can be used directly as drugs or as synthetic analogs. Cancer is the second most important disease leading to death in both the developing and developed countries nowadays. Numerous experimental and epidemiological studies have shown that several plant derived natural products may

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Serve as effective anticancer drugs, among which are plant triterpenes, for example Betulin and Betulinic acid (Rita *et al*., 2009).

Synthetic transformations of natural compounds for the purpose of developing biologically active agents have become the basis of the actively advancing scientific direction of perfect organic synthesis and medical chemistry. The greatest attention of researchers is attracted by native compounds with reliably established biological activity. An attractive factor is availability of natural metabolites due to frequent occurrence of the sources, and technological reasonableness of the methods of isolation of natural substances (Tolstikov*et al*., 2005).

In Nigeria, *Piliostigma thonningii* (*Caesalpinioideae*) known as monkey bread or camel's foot tree which is also known as *Kalgo* in Hausa, *Kalur* in Kanuri and its mistletoe also called *Afomo* in Yoruba, *Awuruse* in Igbo, *Kauchi* in Hausa and *Burongu* in Kanuri are widely used for treatment of various diseases. *Piliostigma thonningii* is a leguminous plant of sub-family *Caesalpinioideae,* comprising of trees, shrubs or very rarely scramblers. Different parts of *Piliostigma thonningii* such as root and twig have been described as useful medicinal plant parts. They are used for the treatment of dysentery, fever, respiratory ailments, snake bites, hook worm and skin diseases (Jimoh and Oladiji, 2005). The stem of the *Piliostigma thonningii* is used for treatment of dysentery, pile, male erectile malfunction among others. Its root is used for the treatment of gastro intestinal track problems (GIT) and dysentery. Its leaves are used in treatment of inflammation, bilharzias, and in treatment of eye diseases, while the mistletoe of the *Piliostigma thonningii* (*Tapinanthus globiferus*) is used for the treatment of breast cancer, elephantiasis and migraine.

Different parts of *Piliostigma thonningii* scum have been used medicinally. The roots and twigs have been used locally in the treatment of dysentery, fever, respiratory ailments, snakebites, and hookworm and skin infections in eastern Nigeria. The leaf extracts has been used for various ethnomedicinal purposes including the treatment of malaria all over eastern Nigeria (Kwaji *et al*., 2010).

Materials and Methods

All the reagents used were of analytical standard grade. Thin Layer Chromatography (TLC) was run on pre-coated aluminum sheets (TLC) $60F_{245}$ Sigma Aldrich, Germany

Germany. The spots were visualized using UV light from a UV lamp of wavelength 254 and 365 nm. Column chromatography was carried out using silica gel 60-120 meshreagent grade made by Lobachemie, India.

Proton Nuclear Magnetic Resonance (1H-NMR) and Carbon-13 Nuclear Magnetic

Resonance (^{13}C NMR) spectra were run on Agilent-NMR-vnmrs400 by Agilent Tech., USAusing $CDCl_3$ as solvent and TMS as internal standard. The values are recorded in δ (ppm). FTIR was run on Cary 660 FTIR Spectrometer, by Agilent Tech., USA

Sample Collection and Identification

The leaves of *Piliostigma thonningii* was collected from the plant farm of Faculty of Pharmaceutical Science, Usmanu Danfodiyo University, Sokoto, in the month of November, 2015. The plant was identified and authenticated by U.S. Gallah, a consultant taxonomist at the Department of Pharmacognosy, UDUS. The voucher specimen number for *Piliostigma thonningii is*PCG/UDUS/LEGU/0001and the specimen was deposited for future reference.

Extraction of Plant Material

The *Piliostigma thonningii* leaves were weighed using an electronic scale. The mass was recorded. The *Piliostigma thonningii* was then transferred into a separating funnel with the aid of a spatula. 300 cm^3 of n-hexane was added and allowed to stay for 24 hours. After 24 hours, the hexane was drained out of the separating funnel. The marc was then rinsed with 110 cm^3 of n-hexane until the marc is colourless. The marc was then remove out of the separating funnel and then air dried for 30 min. The n-hexane extract was allowed to stay for two days in 500 cm^3 beaker in the laboratory for the hexane to evaporate at room temperature. The extract was air dried and weighed with the electronic scale. Its mass was recorded.

The marc was transferred into the separating funnel and same procedure was followed by adding 275 cm^3 of dichloromethane, after 24 hours, the dichloromethane was removed and rinsed with 100 cm^3 of DCM. The marc was then air dried and weighed. The mass was recorded.

The marc was transferred into the separating funnel and then 200 cm^3 of ethyl acetate was added and allowed to stay for 24 hours, the ethyl acetate extract was then drained out of the separating funnel and the marc rinsed with 110 cm^3 of fresh ethyl acetate. The extract was then allowed to dry at room temperature. The marc was then removed from the separating funnel and air dried for 30 min. The extract was weighed with the electronic scale, and its mass was recorded.

The marc was transferred into the separating funnel and then 200 cm^3 of methanol added and allowed to stay for 24 hrs. The methanol extract was removed and the marc rinsed with 100 cm^3 of fresh methanol and allowed to stay for 24 hours for the methanol to dry at room temperature and the mass recorded of the extract recorded after drying at room temperature.

Thin Layer Chromatography Profile of the Mistletoe Leaves Extracts

Thin layer chromatography was run using TLC silica gel pre-coated plates by ascending manner. Capillary tubes were used to spot the samples on the base line on a 10 cm by 4 cm TLC plates; the spots were developed in an air tight chromatank at room temperature. A preliminary TLC separation of all the extracts of the mistletoe were carried out using different solvent systems, the solvent front was allowed to travel at least 75 % height on the TLC plate. Spots were visualized under day light, ultraviolet light (254 nm and 365 nm) and then by spraying with 10 % tetraoxosulphate(VI) acid followed by heating in an oven for 5 minutes at 105 ºC.

The solvent system used for n-hexane extract: n-hexane: ethyl acetate (8:2), for dichloromethane (DCM) extract: DCM: methanol (9: 1), for ethyl acetate extract: ethyl acetate: n-hexane (8:2), and for methanol extract: ethyl acetate: methanol: water (8:1:1).

Isolation of Compound (column chromatography)

One hundred and twenty gram (120 g) of silica gel (60-120 mesh) was made into slurry with 100 % n-hexane and was packed into a 2.5 × 63 cm

glass column and allowed to stand for about one hour to attain stability. About 3 g of n-hexane extract was pre-adsorbed on 3 g of silica gel and loaded onto the column. The loaded sample was eluted gradiently starting with n-hexane (100 %), n-hexane: ethyl acetate (99: 1), n-hexane: ethyl acetate (98: 2), n-hexane: ethyl acetate (97: 3) and n-hexane: ethyl acetate (96: 4). A total of 60 fractions were collected and regrouped based on their TLC profiles and R_f values, fractions 1 to 20 were combined together. The fractions consist of one major spot and some impurities. The fractions were then subjected to preparative TLC for further purification. A colourless crystalline non oily compound was isolated.

Solubility Test

The solubility of compound PA was observed in n-hexane, ethyl acetate and chloroform the compound was soluble in all the solvents tested.

Spectral Analysis of Isolated Compound

The isolated compound was subjected to 1H-NMR and 13C-NMR by dissolving 20 mg of the compound in 0.5 cm^3 deuterated chloroform ($CDCl_3$) in an NMR tube. TMS was used as an internal standard and the chemical shift recorded in ppm. The isolated compound was subjected to FTIR analysis by running 5 mgof the dried compound. Transmittance method was used with resolution 8. The result was presented as % Transmittance against wave number in cm^{-1}. The range was 4000-650 cm^{-1}.

Thin Layer Chromatography profiles

Tables 1 showed the various R_f values of the spots observed when all the 4 extracts of *Piliostigma thonningii* were sprayed with 10 % H_2SO_4. The methanolic extract of *Piliostigma thonningii* shows 2 spot. The other extracts of n-hexane, dichloromethane and ethyl acetate contain much number of compounds extracted than the methanolic extracts. This suggest that *Piliostigma thonningii* leaves contain less polar compounds in their n-hexane, dichloromethane and ethyl acetate extracts as methanol is expected to extract the most polar compounds.

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Table 1: R_f Values of the Separated Components of the *P. thonningii* Leaves Extract

n-Hexane	Dichloromethane	Ethyl acetate	Methanol
0.95	0.96	0.91	0.90
0.89	0.86	0.81	0.76
0.85	0.73	0.69	
0.78	0.62	0.52	
0.58		0.37	

Preparative Thin Layer Chromatography

Preparative thin layer chromatography carried out on the pooled fractions 1 to 20 collected from the silica gel column chromatography of the n-hexane extract led to the isolation of a compound coded 'PA'.

Spectral Analysis of Compound PA

The compound PA was a colourless crystalline solid which is soluble in n-hexane, ethyl acetate and chloroform. The proton NMR (Figure 1) and presented in Table 2 reveals the presence of CHO group with chemical shift of $^{\delta}$ = 9.68 ppm and is peculiar to only Aldehydes (Donald, *et al.*, 2001). The ^{13}CNMR spectrum (Figure 2) and presented in Table 2 show signals of $^{\delta}$ = 150.91 ppm (C-20) and $^{\delta}$ = 109.39 ppm (C-29) which are peculiar to Lupane derivatives. Furthermore, the IR spectrum (Figure 3) and in Table 3 indicates presence of absorption at 1733 cm^{-1} for carbonyl (C=O) and two bands around 2914 and 2847 cm^{-1} which is a characteristic of C-H aliphatic absorption, other prominent signal is the OH frequency of 3351 cm^{-1} attached on C-3 and shows a broad band in the hydrogen region of the spectrum. The ^{1}H NMR and ^{13}C NMR spectra suggest presence of 30 carbon atoms. The chemical shift of compound PA was the compared with that of Betulinic Aldehyde isolated by (Hongyan, 2001). The presence of CHO on C-28 of compound PA gave this strong chemical shift of $^{\delta}$ = 218.39 ppm as compared with $^{\delta}$ = 206.5 ppm, signal for CHO group was 206.69 ppm (Pathak, 1988). The CHO is a strong electronegative group that is why it was further downfield to this high value. The C-17 of the compound PA now experiences a neighboring field effect of the CHO on C-28 and it also

went down field to about $^{\delta}$ = 68.12 ppm which also show similar trend for the Betulinic Aldehyde isolated by (Hongyan, 2001). Other prominent chemical shifts especially on C-20, C29 and C3 remain in close agreement with the Betulinic aldehyde. The isolated compound PA is probably a pentacylic triterpene containing an aldehydic functional group namely Betulinic Aldehyde. This compound has been isolated by Hongyan, (2001) and to the best of our knowledge, this the first report of the presence of Betulinic aldehyde isolated from the leaves of *Piliostigma thonningii*. Based on the spectral data (^{1}H NMR, ^{13}C NMR and FTIR), the proposed structure of PA is presented in Figure 4.

Table 2: ^{1}H-NMR and ^{13}C NMR Spectra of Compound PA ($DCCl_3$)

Position	$^{\delta}$H	$^{\delta}$C (ppm)	$^{\delta}$H*	$^{\delta}$C* (ppm)
1	1.37	38.66	38.6	
2	1.66	27.37	27.8	
3	4.00	77.33	3.22	78.8
4	-	38.66		38.7
5	1.40	55.24		55.2
6	1.34	17.99		18.1
7	1.40	34.16		34.2
8	-	40.72		40.7
9	1.66	50.46		50.3
10	-	38.66		38.5
11	4.60	20.77		20.6
12	4.59	25.86		25.3
13	-	37.06		37.0
14	1.29	42.85		42.4
15	1.64	26.86		27.2
16	1.51	29.37		29.1
17	-	68.12		60.6
18	1.51	47.47		47.4
19	2.48	47.93	2.48	47.9
20	-	150.91		149.5
21	1.11	29.70		29.7
22	1.11	33.26		33.1
23	0.85	28.90	0.85	28.7
24	0.81	15.22	0.80	15.2

25	1.00	15.75	1.00	15.8
26	1.02	15.97	1.02	15.9
27	0.99	14.14	0.98	14.1
28	9.68	218.39	9.68	206.5
29	4.81 & 4.55	109.39	4.76 & 4.68	110.0
30	1.66	19.27	1.68	18.9

*(Hongyan, 2001).

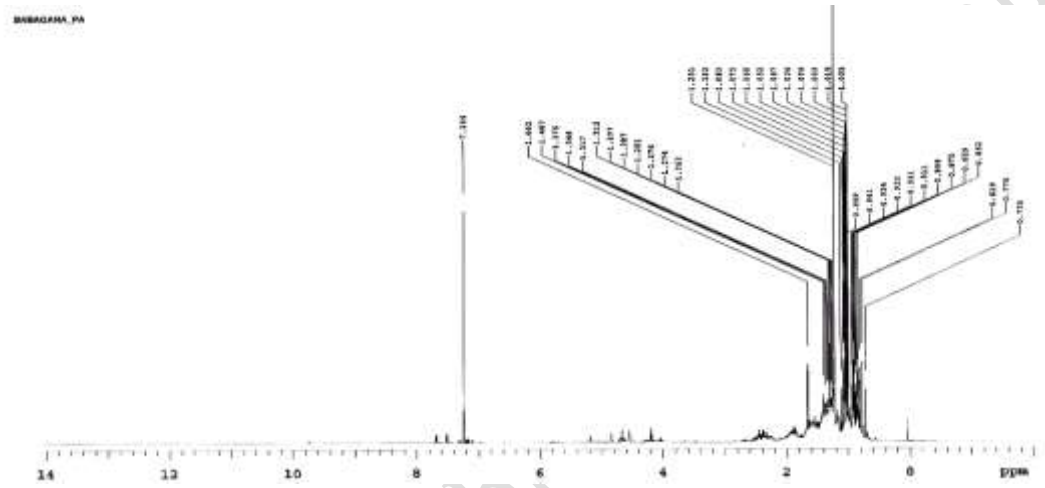

Figure 1: ^{1}H NMR Spectrum of Compound PA

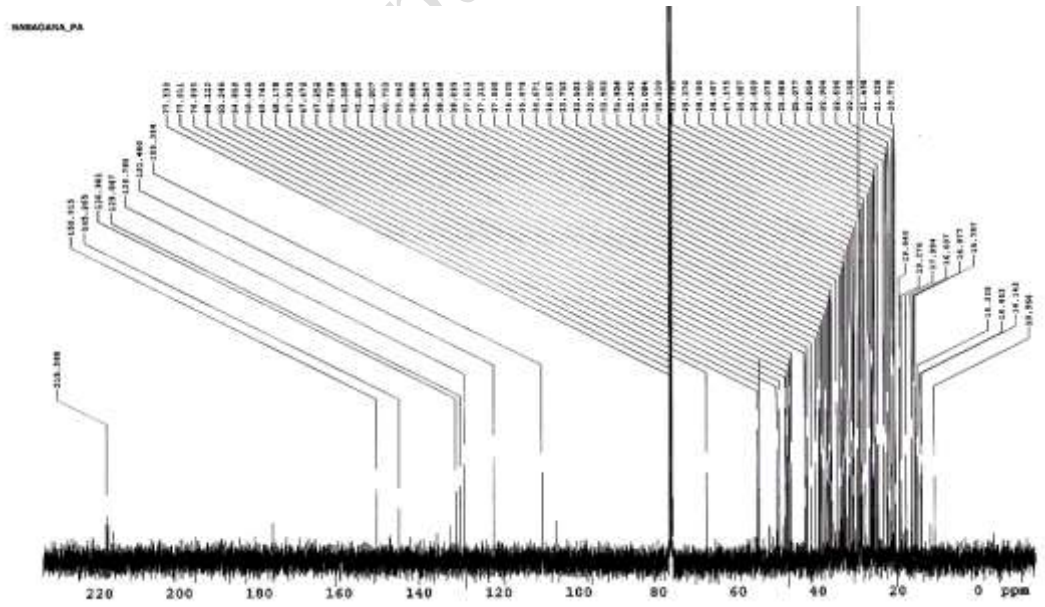

Figure 2: ^{13}C NMR Spectrum of Compound P

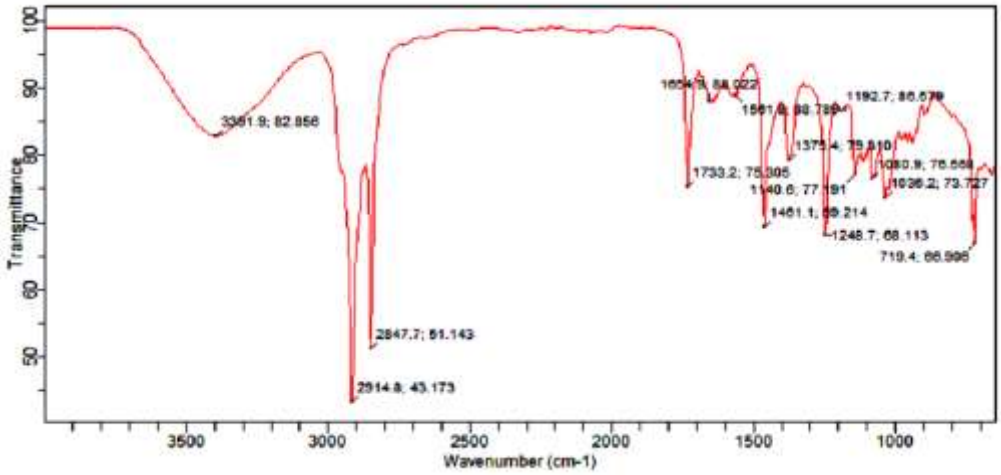

Figure 3: IR Spectrum of Compound PA

The results of the prominent signals from the IR spectra of PA is presented in Table 3

Table 3: Prominent Frequencies from the Spectra of PA

Frequency (cm^{-1})	Inference
1733	C=O
3355	OH
2914 & 2847	C-H str

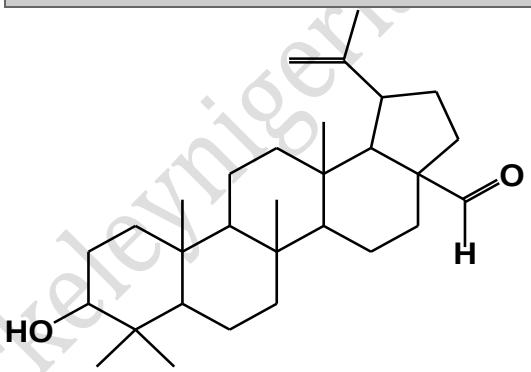

Figure 4: Proposed Structure of PA: Betulinic Aldehyde

Betulinic Aldehyde had previously been isolated from the bark of Birch (*Betula paperyfera*) (Hongyan, 2001). It has also been isolated from *Tectona grandis linn.* (Verbanaceae) and has an antitumour activity (Pathak, 1988).

Conclusion

Chromatographic separation of the n-hexane leaves extract of *Piliostigma thonningii* afforded a pentacyclic triterpene compound Betulinic Aldehyde.The presence ofBetulinic Aldehydeand potentially other phytochemicals may explain the use of the leaves of the plant traditionally to treat inflammation.

Acknowledgement

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Conflict of Interest

The author declare no conflict of interest

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