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# TWO TRITERPENOIDS FROM THE STEM BARK EXTRACT OF *Brachystegia eurycoma* HARMS (FABACEAE)

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## ABSTRACT

*Brachystegia eurycoma* is a leguminous plant that is popular amongst the people of the Southern part of Nigeria for its ethnomedicinal values to treat different aliments such as malaria, microbial infection, and inflammatory conditions among others. As part of our ongoing research in medicinal phytochemistry, we report herein, the isolation and characterization of oleanolic acid and betulinic acid from the methanol stem bark extract of *Brachystegia eurycoma*. The isolation was carried out by a combination of column chromatography over silica gel and gel filtration using sephadex LH-20. The isolated compounds were identified as Oleanolic acid and Betulinic acid *via* chemical tests, IR, 1D-$^{1}$H and $^{13}$C NMR spectral data as well as comparison with reported data.

**Keywords:** betulinic acid, *Brachystegia eurycoma*, NMR-analysis, oleanolic acid, stem bark
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## INTRODUCTION

Secondary metabolites are naturally occurring chemicals produced by living organism for the purpose of protection and defense but they have been shown to possess various pharmacological/ biological activities in different laboratory models. This is thought to be the basis why natural products, especially plants have proven effective in treating various diseases and illnesses since ancient times [1]. Several bioactive compounds have been isolated and have either been used directly as drugs or as source of new drugs [2]. Lead compounds from natural products are considered to be the cornerstone of drug development, since they provided important clues in drug discovery [3].

*Brachystegia eurycoma,* a member of *Caesalpinioideae* family, is a large tree that may reach a height of 35 m tall and 2 m diameter [4]. It has spreading branches that are either twisted or uneven in shape. It has a fibrous, tough bark that typically exudes a brownish buttery gum and peels off in places. It is commonly known as *Taura* (Hausa), *Achi* (Igbo), *Ekalado* (Yoruba) [5]. The stem bark is used in traditional medicine for the treatment of malaria, microbial infections and inflammatory conditions. Despite its varied medicinal potentials, there is paucity of data on the phytochemical constituents of the plant The isolation of 4 – (4 – phenyl-1, 4-dihydronaphthalen-1-yl) has been reported from the stem bark of the plant, bis-6,6-methylenebis (4-tert-butyl-2-methylphenol) and 3-hydroxy-2, 2-bis(6-methoxy-3-methyl-2,3-dihyrobenzoic furan-2-yl) was isolated from the stem bark exudates [6]. In this paper we report the isolation and characterization of Oleanolic acid and Betulinic acid.

## MATERIALS AND METHODS

The solvents used were of high quality and include: methanol, n -hexane, chloroform, ethylacetate and n-butanol purchased from Sigma Co. USA; Silica gel 60-120 µm (Qualikems, India) was used for column chromatography, Sephadex LH-20 (GE Healthcare), TLC was carried out on Aluminum-backed Kieselgel 60 $F_{254}$ TLC plate (Merck no. 5554, Darmstadt, Germany) and a Gallenkhamp electro thermal melting point apparatus was used to determine the melting point of the isolated compound. The IR was recorded on Agilent technologies Cary 630 fourier transform infrared spectrophotometer while the NMR spectra were obtained on a Bruker ADVANCE 400 MHz NMR spectrometer available at Multi-User Research Science Laboratory, Ahmadu Bello University, Zaria-Nigeria, using TMS as internal standard. The Chemical shift values (δh) were reported in parts per million (ppm) relative to internal solvent standard and coupling constants (*J* values) were given in Hertz. The NMR solvent used for the measurements was deuterated chloroform for both isolated compounds.

### Collection and extraction of the plant material

The stem bark of *Brachystegia eurycoma* alongside the leaves and fruits was collected in January 2022 from Tieen in Obi Local Government Area of Nasarawa State, Nigeria after identification of the plant in the field using description in the monograph [4]. The identity of the plant was confirmed and authenticated by Mal. Namadi Sanusi of the Herbarium Unit, Department of Botany, Faculty of Life Sciences, Ahmadu Bello University, Zaria, by

comparing with a specimen of voucher number V/N ABU023263. The stem bark was washed, shade dried and pulverized manually. The size-reduced stem bark (500 g) was extracted using cold maceration with methanol for 72 hours and the marc was re-extracted. The extract was filtered and concentrated *in-vacou* to produce a reddish pastry subsequently referred to as the crude methanol extract.

**Fractionation of the crude extract**

A 100 g of the crude extract was suspended in water and filtered. The insoluble portion was partitioned with n-hexane, chloroform and ethyl acetate in order of increasing polarity to obtain hexane fraction (HF), chloroform fraction (CF) and ethylacetate fraction (EAF).

**Column chromatography of chloroform fractions**

The chloroform fraction which had the best TLC profile (5 g) was subjected to column chromatography over a silica gel pre-packed column of dimension 75 cm x 3.5 cm using the technique of gradient elution [5]. The column was eluted using Hexane 100% followed by Hexane-chloroform mixture (95:5) increasing the polarity gradually up to methanol. A total of 26 collections, 100 mL aliquot each were made and the progress of elution was monitored on TLC. Aliquots with similar TLC profiles were pooled together to obtain bulk fractions coded A–Z. Gel filtration of L and H by isocratic elution with absolute methanol when concentrated give a single spot on the TLC plate led to the isolation of compounds BE1 and BE2 respectively. Both BE1 and BE2 gave a single homogenous spot with solvent systems of Hexane: ethylacetate 1:1 and Chloroform:ethylacetate 9:1 indicating their purity. The isolated compounds were subjected to physicochemical test as well as spectroscopic analysis to elucidate their chemical structures.

## RESULTS AND DISCUSSION

BE1 was isolated as white powder of melting point 275-277$^{o}$C. It showed a brown spot of $R_f$ value 0.80 (Hexane:ethylacetate 1:1) and showed positive Liebermann Burchard test indicating the presence of steroids or triterpenes nucleus [7]. The IR spectrum of BE1 revealed a broad band at 3403.1 cm$^{-1}$ typical of O-H stretching, 2922.2 cm$^{-1}$ and 2855.1 cm$^{-1}$ were due to aliphatic asymmetric and symmetric C-H stretching, respectively, 1461.1 cm$^{-1}$ was due to bending frequency for cyclic (C-C bending), 1271.0 cm$^{-1}$ and 1707.1 cm$^{-1}$ was due to C-O and C=O stretching of carboxylic acid respectively [8].

The $^{1}$H NMR spectrum of BE1 revealed the presence of seven singlet methylene protons signals at δH 0.90 (H-25), 0.87 (H-26), 0.92 (H-24), 0.97 (H-29), 1.24 (H-23), 1.27 (H-27), and 1.12(H-30) (3H each) typical of triterpenes and in close agreement with values reported for oleanolic acid [8]. It also showed a doublet signal at δH 5.27(1H, d, *J*=4) indicative of olefinic proton and a carbinol proton at δH3.66 (1H, d, *J*=8).

The structural assignment of BE1 was further substantiated by the $^{13}$C NMR experiment which revealed seven peaks at δc 29.35 (C-23), 16.98 (C-24), 15.54 (C-25), 18.25 (C-26), 25.91 (C-27), 33.04 (C-29), 22.68 (C-30) attributable to the seven methyl groups. The olefinic carbon signals appeared at δc 122.58 (C-12) and 143.53 (C-13) while the signal at δc 183.16 revealed the existence of the carbonyl group attributed to C-28. These values are in good agreement with those reported in the literature [9, 10]. Several phytochemicals like triterpenes, flavonoids, saponins, steroids and alkaloids have been found to possess antipyretic activity [11]. Oleanolic acid isolated from the stem bark extract of *Brachystegia eurycoma*, belongs to the class of triterpenes which could be responsible for the antipyretic activity. The forgoing spectral analysis and comparison with reported value led us to confirm the structure of BE1 as oleanolic acid as shown in Figure 1.

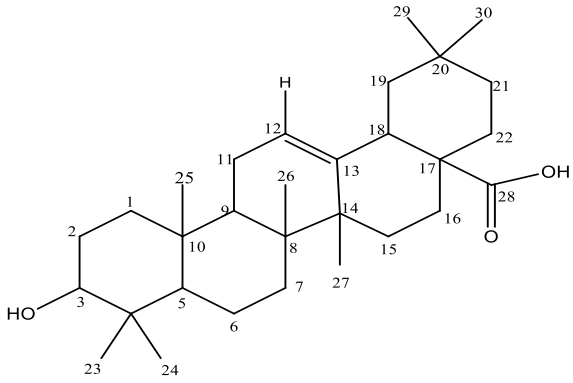


**Figure 1**: Structure of compound BE1 (Oleanolic acid)

BE2 was isolated as white amorphous powder of melting point 318-320$^{o}$C. It showed a pink spot of $R_f$ value 0.68 with solvent system CF:EA (9:1) and showed positive Liebermann Burchard test indicating the presence of steroids or triterpenes nucleus [12]. The IR spectrum of BE2 revealed a broad band at 3071.3 cm$^{-1}$ typical of O-H stretching, 2922.2 cm$^{-1}$ and 2855.1 cm$^{-1}$ were due to aliphatic asymmetric and symmetric C-H stretching, respectively, 1461.1 cm$^{-1}$ was due to bending frequency for cyclic $(CH_2)_n$, 1379.1 cm$^{-1}$ was due to C-H bending of a symmetrical $CH_3$, 1241.2 cm$^{-1}$ and 1707.1 cm$^{-1}$ was due to C-O and C=O stretching of carboxylic acid respectively [13, 14]. The $^{1}$H NMR spectrum of BE2 revealed the presence of five singlet methyl protons signals at δH 0.96, 0.68, 0.74, 0.92, and 0.87 (3H each) are in close agreement with values reported betulinic acid [15]. It also showed an allylic methyl group at δH1.67 (1H, s) and terminal methylene proton at δH 4.6 (IH, s).

The structural assignment of BE2 was further justify by the $^{13}$C NMR experiment which revealed

five peaks at δc 27.96 (C-23), 15.34 (C-24), 16.11 (C-25), 15.99 (C-26), 14.67 (C-27) attributable to the five methyl groups. The carboxylic (COOH) signals appeared at δc 180.25(C-28). These values are in good agreement with those reported in the literature (13, 14, 16, 17]. Several phytochemicals like triterpenes, flavonoids, saponins, steroids and alkaloids have been found to possess antipyretic activity [11]. Betulinic acid isolated from the stem bark extract of *Brachystegia eurycoma*, belongs to the class of triterpenes which could be responsible for the antipyretic activity. On the basis of IR and 1D spectra and in comparison, with literature, compound BE2 was confirmed to be betulinic acid (Figure 2).

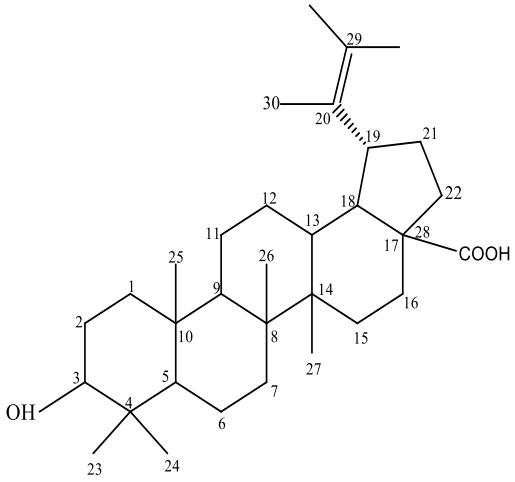


**Figure 2**: Structure of compound BE2 (Betulinic acid)

## CONCLUSION

The study reported the isolation and characterization of oleanolic and betulinic acid coded as compound BE1 and BE2 respectively from the chloroform fraction of the methanol stem bark of *B. eurycoma* for the first time to the best of our literature search.

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**Table 1**: Summary of $\delta^1$H and $\delta^{13}$C- NMR data BEI and BE2 compared with those of literature (1H* and 13C*)

| Position | BE1 | | | | BE2 | | | |
|---|---|---|---|---|---|---|---|---|
| | $\delta^{13}$C | $\delta^{13}$C* | $\delta^1$H | $\delta^1$H* | $\delta^{13}$C | $\delta^{13}$C* | $\delta^1$H | $\delta^1$H* |
| **1** | 38.74 | 39.0 | 1.06 | 1.02 | 38.67 | 39.1 | 1.60 | 1.52dd |
| **2** | 28.07 | 28.1 | 1.86 | 1.82 | 27.36 | 28.0 | 1.40 s | 1.43m |
| **3** | 79.02 | 78.2 | 3.66 d | 3.44 d | 79 | 77.7 | 3.19 | 2.98m |
| **4** | 39.24 | 39.4 | - | - | - | 39.4 | - | - |
| **5** | 55.17 | 55.9 | 0.87 d | 0.88 d | 55.29 | 55.8 | 0.67 | 0.63m |
| **6** | 19.00 | 18.8 | 1.58 | 1.58 | 18.26 | 18.8 | 1.48 | 1.50m |
| **7** | 33.04 | 33.4 | 1.55 d | 1.53 | 34.28 | 34.8 | 1.36 | 1.33 |
| **8** | 39.61 | 39.8 | - | - | 40.66 | 40.9 | - | - |
| **9** | 47.59 | 48.2 | 1.61 d | 1.71tr | 50.47 | 49.5 | 1.57 | 1.60m |
| **10** | 37.03 | 37.4 | - | - | 37.1 | 37.6 | - | - |
| **11** | 23.55 | 23.8 | 1.98 | 1.96 | 20.82 | 21.3 | 1.24 | 1.23s |
| **12** | 122.58 | 122.6 | 5.27 d | 5.49 s | 25.40 | 25.9 | 0.96 | 0.99m |
| **13** | 143.53 | 144.8 | - | - | 38.33 | 38.5 | 2.24 | 2.24m |
| **14** | 41.59 | 42.2 | - | - | 42.40 | 42.9 | - | - |
| **15** | 29.24 | 28.4 | 1.16 | 1.22 | 31.91 | 30.1 | 1.10 s | 1.19m |
| **16** | 23.55 | 23.8 | 2.01 s | 2.12tr | 32.11 | 32.6 | 1.36 | 1.38m |
| **`17** | 46.50 | 46.7 | - | - | 56.28 | 56.3 | - | - |
| **18** | 40.97 | 42.1 | 3.23 d | 3.30 d | 46.85 | 50.8 | 1.51 | 1.54m |
| **19** | 46.50 | 46.6 | - | 1.83 | 49.20 | 47.5 | 2.99 | 2.95m |
| **20** | 31.91 | 31.0 | - | - | 150.51 | 151.2 | - | - |
| **21** | 33.77 | 34.3 | 1.40 | 1.46 | 30.51 | 31.0 | 1.37 | 1.37 d |
| **22** | 32.59 | 33.2 | 1.86 | 1.82 | 37.03 | 37.2 | 1.98 | 1.83 d |
| **23** | 29.35 | 28.8 | 1.24 | 1.24 s | 27.96 | 28.9 | 0.96 s | 0.87 s |
| **24** | 16.98 | 16.5 | 0.92 s | 1.02 s | 15.34 | 16.8 | 0.68 s | 0.65 s |
| **25** | 15.54 | 15.6 | 0.90 s | 0.93 s | 16.11 | 16.6 | 0.74 s | 0.76 s |
| **26** | 18.25 | 17.5 | 0.87 | 1.04 | 15.99 | 15.3 | 0.92 s | 0.93 s |
| **27** | 25.91 | 26.2 | 1.27 s | 1.30 s | 14.67 | 16.6 | 0.87 s | 0.87 s |
| **28** | 183.16 | 180.0 | - | - | 180.42 | 178.1 | - | - |
| **29** | 33.04 | 33.4 | 0.97 s | 0.97 s | 109.68 | 110.4 | 4.73 s | 4.69 s |
| **30** | 22.68 | 23.8 | 1.12 s | 1.02 s | 19.34 | 19.8 | 1.67 s | 1.64 s |
| **OH** | - | | - | | 38.84 | 39.1 | 4.60 s | 4.26 s |