# ISOLATION OF LUPEOL FROM METHANOL EXTRACT OF THE ROOT BARK OF *Bombax costatum*

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Abstract: *Bombax costatum* is a member of the family Bombacaceae and is commonly called red-flowered silk-cotton tree or red kapok tree. In Nigeria, it is locally known as Kurya or Gujjiya in Hausa, Joohi or Kuruhi in Fulfulde and Kutupkaci in Nupe. Phytochemical studies conducted on the chloroform fraction from the methanol crude extract lead to the isolation of a pentacyclictriterpenoid of the Lupane group (lupeol). The isolation and purification of the compound was carried out by silica gel column chromatographic technique and the structure was elucidated by spectroscopic methods including IR, 1D and 2D NMR. This is the first report of the isolation of this compound from the root bark of this plant. Thus, the isolated compound may be responsible for the various medicinal action of the plant in the management of various diseases.

**Keywords:** *Bombax costatum*, phytochemical, chromatographic technique, IR, 1D, 2D NMR

## Introduction

*Bombax costatum* is a member of the family Bombacaceae (Burkill, 1985) and is commonly called red-flowered silk-cotton tree or red kapok tree. In Nigeria, it is locally known as Kurya or Gujjiya in Hausa, Joohi or Kuruhi in Fulfulde and Kutupkaci in Nupe (Burkill, 1985). It is a deciduous tree growing straight up to about 30 m tall and 100 cm in diameter. The genus is best known for the species *BombaxCeiba*, which is widely cultivated throughout tropical and sub-tropical regions of the world. *Bombax coxtatum* is used ethnomedicinally for the treatment of epilepsy, malaria, skin diseases, yellow fever, headaches, haemorrhages, wound healing, blennorrhoea and diarrhoea (Ngwuluka *et al.*, 2012; Orwa *et al.*, 2009; Burkil, 1985).

Previous preliminary phytochemical screening of the methanol extracts of the stem bark revealed the presence of saponins, carbohydrate, cardiac glycoside, flavonoid, triterpenes alkaloid and steroid (Apinega *et al.*, 2018; Meshack, 2016). This study therefore, report the isolation of a pentacyclictriterpenoid of the Lupane group from the chloroform soluble fraction of a methanol extract of the root-bark of *Bombax costatum.*

## Material and Method

### *Collection and identification of plant material*

The root bark of *Bombax costatum* was collected from Basawa in Zaria; it was identified and authenticated at the herbarium unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria. A voucher number 1749 was collected for reference purpose.

### *Preparation of the extract*

The root-bark was dried at room temperature under shade for three weeks and size reduced

Manually using mortar and pestle. The size reduced root-bark (2 kg) was subjected to extraction with Methanol using cooled maceration method and after evaporation of the solvent, 200 g of the extract was obtained. The crude methanol extract was subjected to a liquid – liquid fractionation using various organic solvents in order of increasing polarity starting from n-Hexane, chloroform, ethyl acetate and n-butranol to yield n-Hexane fraction (nHxF), chloroform fraction (CF), ethyl acetate fraction (EAF), and n-butanol fraction, respectively.

### *Phytochemical screening*

The presence of phytochemicals constituents in the crude extract were tested using simple qualitative methods as described by Trease and Evans (2002).

### *Column chromatography of chloroform fraction*

The chloroform fraction was subjected to column chromatography over a silica gel-packed column of dimension 75 x 3.5 cm; and was gradiently eluted using n-hexane: ethyl acetate mixtures (Gibbons and Gray, 1998). Eluents of 20 mL aliquots were collected and monitored on TLC for similar fractions. A total of 24 collections were made and pooled together into 4 major fractions coded C1-C4 based on their similarity on TLC (Cannell, 1998). Recrystallisation of chloroform column fraction C1 led to the isolation of compound coded HB3 (4.8 mg). Compound HB3 was isolated as white crystal, it gave a single spot on TLC using two different solvent system: hexane:ethylacetate (7:3) and hexane:ethylacetate (9:1) indicating its purity (Plate 1). The isolated compound was also subjected to some physical and chemical tests. Infrared spectroscopy (IR) recorded on Agilent Technologies Cary 6030 Fourier Transform Infrared Spectrophotometer available at the Multi-User Research Science Laboratory, Ahmadu Bello University, Zaria-Nigeria and nuclear magnetic resonance (NMR) obtained on a Bruker AVANCE 500 MHz Machine were used to established the structure of the isolated compound.

Hexane:ethylacetate (7:3) Hexane:ethylacetate (9:1)

**Plate 1: TLC profile of the isolated compound using two different solvent systems**

**Result and Discussion**

The results of the study are shown in Table 1 and Fig. 1, respectively. Recrystallisation of chloroform column fraction C4 led to the isolation of compound HB3. HB3 was isolated as a white crystalline solid. It was soluble in chloroform and gave a single spot on TLC developed in two different solvent systems. The compound tested positive to both Lieberman Burchad and Salkowski test suggesting that compound is a triterpenes. The melting point of HB3 was found to be 214 – 215$^{o}$C which is in agreement with the report of (Jamal *et al.,* 2008). IR frequencies at 3269, 2937, 2853, 1355 and 1471 cm$^{-1}$ suggested vibrations for OH, C–H (asymmetric and symmetric), C=C and C–O, respectively, and the vibration observed at 874 cm$^{-1}$ was due to an unsaturated out of plane C-H vibration. The $^{1}$H-NMR (500 MHz, $CDCl_3$) revealed olefinic protons at δ 4.70 (1H, s, J=4.0Hz) and δ 4.59 (1H, s, J=4.0Hz), hydroxymethines proton at δ 3.21 (1H, dd, J = 8.0, 4.0, 12.0 Hz,), vinyl methyl proton singlet at δ 1.68 (3H, s,), six singlets assigned to the tertiary methyl protons at δ 1.01 (3H, s, J=4.0 Hz), δ 0.96 (3H, s,), δ 0.87 (3H, s,), δ 0.81 (3H, s, J=8.0 Hz), δ 0.78 (3H, s, J=12.0 Hz) and δ 0.71 (3H, s, J =12.0 Hz,), δ 2.36 (1H, m, J = 4.0, 8.0, 12.0 Hz,), δ 1.62 (2H, m, J = 4.0, 12.0 Hz,), δ 1.60 ( t, J= 12.0 Hz,), δ 1.54 (2H, m,), δ 1.46 (2H, m, J=4.0 Hz), δ 1.45 (1H, m, J=4.0 Hz), δ1.41 (2H, m, J=4.0, 8.0 Hz), δ 1.36 (1H, m, J=8.0 Hz), δ 1.34 (1H, m, J=12.0 Hz), δ 1.28 (2H, m, J=8.0 Hz), δ 1.27 (1H, m, J=12.0 Hz), δ 1.17 (1H, m, J=12.0 Hz), δ 0.99 (2H, m, J=8.0, 12.0 Hz), δ 0.94 (2H, m, J=4.0, 8.0 Hz) and δ 0.69 (1H, m, J=8.0 Hz) (Table 1).

**Table 1: Comparison of 1D spectral data for compound HB3 (500MHz, $CDCl_3$) with literature (400MHz, $CDCl_3$)**

| Position | δ H | δ H, Haruna *et al.* (2017) | δ $^{13}$C | δ $^{13}$C, Haruna *et al.* (2017) |
|---|---|---|---|---|
| 1 | 0.87, m | 0.93, m | 38.92, $CH_2$ | 38.20, $CH_2$ |
| 2 | 1.54, m | 1.60, m | 27.66, $CH_2$ | 25.30, $CH_2$ |
| 3 | 3.21, dd | 3.21, dd | 79.22, CH | 79.10, CH |
| 4 | - | - | 39.07, C | 38.9, C |
| 5 | 0.69, m | 0.68, m | 55.51, CH | 55.50, CH |
| 6 | 1.49, m | 1.40, m | 18.53, $CH_2$ | 18.50, $CH_2$ |
| | 1.14, m | 1.19, m | | |
| 7 | 1.36 m | 1.38, m | 34.49, $CH_2$ | 34.50, $CH_2$ |
| 8 | - | - | 41.04, C | 41.00, C |
| 9 | 1.27, m | 1.28, m | 50.65, CH | 50.60, CH |
| 10 | - | - | 37.38, C | 37.30, C |
| 11 | 1.46, m | 1.40, m | 21.14, $CH_2$ | 21.10, $CH_2$ |
| 12 | 1.62, m | 1.40, m | 25.35, $CH_2$ | 27.50, $CH_2$ |
| | 1.68, m | 1.68, m | | |
| 13 | 1.65, t | 1.63, t | 38.26, CH | 39.00, CH |
| 14 | - | - | 43.04, C | 43.00, C |
| 15 | 0.96, m | 0.97, m | 27.63, $CH_2$ | 27.60, $CH_2$ |
| | 1.54, m | 1.60, m | | |
| 16 | 1.45, m | 1.45, m | 35.80, $CH_2$ | 35.80, $CH_2$ |
| 17 | - | - | 43.22, C | 43.20, C |
| 18 | 1.36, m | 1.38, m | 48.52, CH | 48.50, CH |
| 19 | 2.36, m | 2.36, m | 48.20, CH | 48.10, CH |
| 20 | - | - | 151.20, C | 151.10, C |
| 21 | 1.28, m | 1.28, m | 30.06, $CH_2$ | 30.00, $CH_2$ |
| 22 | 1.17, m | 1.17, m | 43.14, $CH_2$ | 40.20, $CH_2$ |
| 23 | 0.99, s | 0.99, s | 28.20, $CH_3$ | 28.20, $CH_3$ |
| 24 | 0.78, s | 0.75, s | 15.58, $CH_3$ | 15.60, $CH_3$ |
| 25 | 0.81, s | 0.80, s | 16.33, $CH_3$ | 16.30, $CH_3$ |
| 26 | 0.98, s | 1.02, s | 16.19, $CH_3$ | 16.20, $CH_3$ |
| 27 | 0.94, s | 0.91, s | 14.76, $CH_3$ | 14.70, $CH_3$ |
| 28 | 0.68, s | 0.75, s | 18.21, $CH_3$ | 18.20, $CH_3$ |
| 29 | 4.70, br s | 4.67, br s | 109.54, $CH_2$ | 109.50, $CH_2$ |
| | 4.59, br s | 4.56 br s | | |
| 30 | 1.68, s | 1.68, s | 19.52, $CH_3$ | 19.50, $CH_3$ |

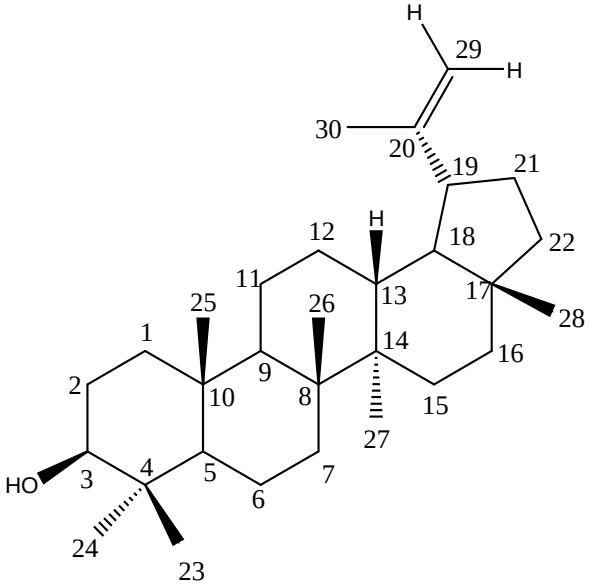


**Fig. 1: Structure of compound HB3**

The $^{13}$C-NMR (100 MHz, $CDCl_3$) spectrum of compound HB3 reveals the presence of thirty (30) carbon signals at δ 151.20 (C, C-20) and δ 109.54 ($CH_2$, C-29) for olefinic carbons signals, hydroxymethine carbon signal at δ 79.22 (CH, C-3), δ 14.76 ($CH_3$, C-27), δ 15.58 ($CH_3$, C-24), δ 16.19 ($CH_3$, C-26), δ 16.33 ($CH_3$, C-25), δ 18.21 ($CH_3$, C-28), δ 18.53 ($CH_2$, C-6), δ 19.52 ($CH_3$, C-30), δ 21.14 ($CH_2$, C-11), δ 25.35 ($CH_2$, C-12), δ 27.63 ($CH_2$, C-15), δ 27.66 ($CH_2$, C-2), δ 28.20 ($CH_3$, C-23), δ 30.06 ($CH_2$, C-21), δ 34.49 ($CH_2$,C-7), δ 35.80 ($CH_2$, C-16), δ 37.38 (C, C-10), δ 38.26 (CH, C-13), δ 38.92 ($CH_2$, C-1), δ 39.07 (C, C-4), δ 40.22 (C, C-22), δ 41.04 ($CH_2$, C-8), δ 43.04 (C, C-14), δ 43.22 (C, C-17), δ 48.20 (CH, C-19), δ 48.52 (CH, C-18), δ 50.65 (CH, C-9), δ 55.14 (CH, C-5), respectively (Table 1). The DEPT experiment revealed the presence of seven (7) Methine carbon atom at C-2, C-3, C-6, C-7, C-2', C-5' and C-6'. A methylene carbon at C-4 and seven (7) quarternary carbons at C-5, C-7, C-9, C-10, C-1', C-3' and C-4'.

The heteronuclear spin quantum correlation (HSQC) spectrum of HB3 was used in the assignment of protons to their respective carbon atoms. The HMBC spectrum revealed the correlations between protons and the neighboring carbons, up to 3 bonds. Through which the connectivity between the various atoms and units in the molecule was Established. Correlations were established between proton H-29 with C-30, C-19, C-30 and C-19, and proton H-30 with C-20, C-29 and C-18. Correlations were also established between H-26 with C-9 and C-14 and H-15 with C-3, C-5, and C-4, correlation was also observed between proton H-27 with C-28 and C-14. proton at H-25 with C-9 and C-5 and proton H-28 with C-3 and C-15 (Fig. 1).

The spectral data for compound HB3 agreed with those reported previously for lupeol (Jamal *et al.,* 2008; Haruna *et al.,* 2017).

**Conclusion**

A white crystalline solid was isolated from the chloroform fraction of *Bombaxcostatum* and was coded HB-3. It was found to be soluble in chlorofoam and tested positive to Salkowski and Liebermann-Burchard test confirming the presence of sterol and triterpenoid compound. It was found to melt between 186 – 191$^{o}$C. The structure of the isolated compound was established using 1D and 2D NMR, IR and by comparison with literature data.

**Conflict of Interest**

Authors have declared that there is no conflict of interest reported in this work.

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