Trop J Nat Prod Res, June 2018; 2(6):290-292 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Isolation and Characterisation of Lup-20(29)-en-3*β*-ol from the Methanol Stem Bark Extract of *Bombax costatum* pv (Bombacacea)

Levi A. Apinega[1]*, Stephen Dlama[1], Zakari Ladan[2], Kunkadma B. Inusa [1], Adejumobi S. Adejoke[1], Garba Dauda [1], Aliyu M. Musa [1]

[1]*Department of pharmaceutical and medicinal chemistry, Ahmadu Bello University, Zaria, Nigeria.*
[2]*Department of chemistry, Kaduna state university, Kaduna State, Nigeria.*

ARTICLE INFO

Article history:
Received 17 May 2018
Revised 02 June 2018
Accepted 06 June 2018
Published online 07 June 2018

ABSTRACT

Bombax costatum, a plant widely distributed in the Sahel savannah of West Africa is well known for its traditional use as a diuretic. This study is aimed at isolation, purification and characterization of phytoconstituents from the stem bark of this plant. The powdered stem bark was extracted with methanol by maceration at room temperature. Phytochemical screening of the crude methanol extract was done according to standard procedure. The crude methanol extract was subjected to solvent-solvent fractionation to obtain the hexane, chloroform and ethylacetate fractions. Repeated column chromatographic separation of the chloroform fraction resulted in the isolation of white amorphous solid (compound **B3**). Phytochemical screening of the stem bark of *B. costatum* revealed the presence of flavonoids, saponins, glycosides, tannins, steroids and terpenoids. On the basis of spectroscopic analyses (1D and 2D NMR) and comparison of spectra data with literature, compound **B3** was identified as a lupeol a triterpenoid of the lupane class.

Keywords: *Bombax costatum*, lupeol, NMR, phytoconstituents, chromatography.

Introduction

Bombax costatum belongs to the family Bombacaceae and is locally called red-flowered silk-cotton tree or red kapok tree. It is a deciduous tree growing straight up to about 30 m tall and 100 cm in diameter. It is a characteristic fire resisting tree of the savannas and dry woodlands from Senegal to Central Africa, and Guinea across Ghana and Nigeria to southern Chad.[1] This plant has been used in ethno-medicine for the treatment of various ailments which includes, epilepsy, leucorrhoea, diarrhea, trichomoniasis, amoebiasis dysentery and severe cases of oedema.[2] It has been reported to possess high level of anti-inflammatory activity in laboratory animal models[3] which may be due to the various bioactive secondary metabolites present in the plant.
Lupeol a phytosterol well distributed in plants has been associated with various biological activity including anti-oxidant and anti-cataractogenic.[4] the most important benefit of lupeol and other phytosterols like stigmasterol and beta sitosterol is their enrolment amongst the health promoting constituents of natural foods which contains them. In fact, the European Foods Safety Authority recommends consuming about 1.5 - 2.4 g/day of phytosterols and/or stanols in order to reduce blood cholesterol.[5] Furthermore, the Food and Drug Administration (FDA) has approved the role of foods containing phytosterol esters inside a low saturated fat and cholesterol diet in reducing the risk of heart disease, especially consumption of at least 1.3 g of sterols, twice a day.[6] In this study we reported the extraction, purification and characterization of lupeol from the chloroform fraction of *B. costatum.*

*Corresponding author. E mail: **apinegalevi@gmail.com**
Tel: + 234 8101111551

Citation: Apinega LA, Dlama S, Ladan Z, Inusa BK, Adejoke SA, Dauda G, Musa AM. Isolation and Characterisation of Lup-20(29)-en-3*β*-ol from the Methanol Stem Bark Extract of *Bombax costatum* pv (Bombacacea). Trop J Nat Prod Res. 2018; 2(6):290-292. doi.org/10.26538/tjnpr/v2i6.6

Materials and Methods

General experimental procedures
NMR spectra were recorded on a Bruker DRX500 spectrophotometer (Bruker BioSpin, Rheinstetten, Germany) at 400 MHz (^{1}H) and 100 MHz (^{13}C). Samples were prepared in $CDCl_3$ with tetramethylsilane (TMS) as an internal standard. The chemical shift (δ) values were measured relative to the internal standard in ppm. The IR spectrum was recorded on KBr disc on a Happ-Genzel 4000-650 spectrophotometer. The UV spectrum was recorded on a THERMO ELECTRON-VISIONpro V3.00 spectrophotometer. TLCs were performed on pre-coated TLC plates Si 60 F_{254} (Sigma-Aldrich). The developed plates were visualized under UV light (254/366 nm). Column chromatography (CC) was performed by gravity using glass columns of appropriate sizes with Silica gel 60 Å, 230-400 mesh (Sigma – Aldrich Co.ST Louis, MO USA).

Plant collection and identification
The plant material was collected from Federal Housing Estate North Bank Makurdi, Benue State Nigeria on the 7th December 2016. It was authenticated by Mallam Namadi Sunusi of the Herbarium unit, Department of Botany, Ahmadu Bello University, Zaria. A voucher number (No:1211) was deposited at the herbarium of the University.

Phytochemical screening
The presence of saponins, phytosterols, tannins, alkaloids, terpenoids, flavonoids and glycosides in the crude methanol stem bark extract were tested using simple qualitative methods as previously reported by Trease and Evans.[7]

Extraction and isolation
Stem bark of the plant was cleaned and air dried under shade and the size was reduced into semi powdered material using wooden mortar and pestle. The semi powdered material (2.5 kg) was extracted with about 12 L of methanol by cold maceration to obtain the crude methanol extract. The crude methanol extract (170 g) was subjected to a liquid – liquid fractionation using various organic solvents in order of increasing polarity beginning with hexane, then chloroform, ethylacetate and

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

butanol. The chloroform fraction was subjected to column chromatography using silica gel and eluted with 100% chloroform, then a step-wise gradient of increasing polarity with ethyl acetate up to chloroform:ethyl acetate (95:5). A total of 32 column fractions were collected. Fraction coded B3 was re-chromatographed on silica gel column, eluted with a solvent mixture of chloroform:ethyl acetate (90:10). The eluate on drying gave a white amorphous solid (compound B3, 50 mg).

Results and Discussion

The methanol stem bark extract of *B. costatum* was screened for the presence of the following secondary metabolites, alkaloids, glycoside flavonoids, carbohydrates tannins terpenoids and saponins. The phytochemical content was found to be similar to that reported by Mesheck.[3] The presence of these phytochemical constituents in the methanol extract is quite instructive as it lends credence to the use of the plant for medicinal purposes, although a lot of plants have non-toxic glycosides, when hydrolyzed to phenolic compounds, they may be toxic to microbial pathogens.[8] Saponins are known to possess the property of precipitating and coagulating red blood cells.[9] In aqueous solution, saponins are also known to have hemolytic effect and can likewise bind to cholesterol sites, making it possible for them to exhibit pharmacological properties.[9]

Alkaloids were found to be present in the methanol extract and this can be corroborated with established literature, that naturally occurring alkaloids and their synthetic derivatives have analgesic, antiplasmodial and bactericidal activities.[10] Hence the use of the plant in traditional medicine for the treatment of malaria, oedema, diarrhea and dysentery. The presence of flavonoids was evident. Plants containing flavonoids have been used as diuretics and laxatives.[11] Furthermore, anti-viral activity has been associated with tannins found in some medicinal plants.[12] Hence *B. costatum* with tannin content could be a source of phytochemical for the treatment of viral and bacterial infections. Therefore, these phytochemicals detected in this study may be responsible for the anti-inflammatory effect of the stem bark of this plant as reported by Meshack,[3] and also give credibility to the various claims of traditional application of the plant as remedies for various ailments.

Isolation and characterisation

The compound B3 (50 mg) was isolated as a white amorphous solid with a melting point of 158 – 160ºC. UV λmax 324 nm, The IR spectrum of B3 showed characteristic absorption frequencies at 3291 and 1241 cm^{-1} typical of the O-H and C-O bond vibrations, respectively; the absorption observed at 879 cm^{-1} was due to an unsaturated out of plane C-H vibration; the C=C vibrations was shown around 1636 cm^{-1} as weakly intense band; stretching and bending vibrations due to methyl groups were represented by the bands at 2922 cm^{-1} and the signal at 1453 cm^{-1} was due to methylenic vibration.

The ^{1}H-NMR spectrum revealed the presence of seven tertiary methyl protons at δ 0.77, 0.79, 0.8, 0.94, 0.99, 1.03 and 1.68 (integrated for 3H-each). A sextet of one proton at δ 2.37 ascribable to 19*β*–H is characteristic of lupeol. The H-3 proton showed a multiplet at δ 3.19 while a pair of broad singlets at δ 4.56 and δ 4.69 (IH, each) was indicative of olefinic protons at H-29a and H-29b. These assignments are in agreement with the structure of lupeol.[13] The structural assignment of B3 was further substantiated by the ^{13}C-NMR experiments which showed seven methyl groups at δc: 28.14 (C-23), 18.17 (C-28), 16.12 (C-25), 16.27 (C-26), 15.52 (C-24), 14.69 (C-27) and 19.45 (C-30)]; the signals due to an exomethylene group at δc: 109.67 (C-29) and 151.12 (C-20)]; ten methylene, five methine and five quaternary carbons were assigned with the aid of DEPT experiment. The deshielded signal at δc 79.15 was due to C-3 with a hydroxyl group attached to it. The confirmation of the structure of B3 was accomplished through the use of 2D NMR experiments.

In the HMBC spectrum, the methine proton signal at δH 1.68 (H-30) showed cross peaks with a quaternary carbon signal (δc 151.12, C-20) by *J2* correlation, a methine carbon signal (δc 109, C-29) by *J*3 correlation and a methane carbon signal δc 48.44 (C18). The methyl signal at δH 1.03 (H-26) showed cross peaks with quarternary carbon signals δc 43.15 (C-14) and a methine carbon δc 50.57 (C-9)], a methylene proton at δH 0.95 (H-15) showed cross peaks with two methine carbon δc 79.15 (C-3),55.43 (C-5) and a quarternary carbon at δc 39.01 (C-4). The pair of broad singlets of olefinic proton at δH 4.56 and 4.69 showed cross peaks with a methylene carbon signal [δc 48.13 (C-19) and δc 19.45 (C-30)] by *J3* correlation. The forgoing spectral analysis and, comparison with reported data, led us to propose the structure of B3 as lupeol, a pentacylic triterpenoid (Figure 1).

Table 1: Qualitative phytochemical analysis of the methanol stem bark extract of *B. costatum.*

Phytochemicals	Inference
Saponins	present
Tannins	present
Alkaloids	present
Flavonoids	present
Carbohydrates	present
Glycosides	present
Steroids	present
Terpenoids	present

Table 2: Spectra data for compound B3 ($CDCl_3$, 400 MHz) with TMS as the reference standard.

Position	δ ^{1}H	δ ^{13}C	DEPT 135
1	0.87	38.91	CH2
2	1.52	27.46	CH2
3	3.19	79.15	CH
4	-	39.01	C
5	0.68	55.43	CH
6	-	18.46	CH2
7	1.37	34.32	CH2
8	-	40.87	C
9	1.26	50.57	CH
10	-	37.31	C
11	1.42	21.07	CH2
12	1.66	25.35	CH2
13	-	38.19	CH
14	-	42.97	C
15	0.95	28.18/27.59	CH2
16	1.47	35.73	CH2
17	-	43.15	C
18	1.37	48.44	CH
19	2.37	48.13	CH
20	-	151.12	C
21	1.91	29.77	CH2
22	1.18	40.02	CH2
23	0.99	28.14	CH3
24	0.77	15.52	CH3
25	0.79	16.12	CH3
26	1.03	16.27	CH3
27	0.93	14.69	CH3
28	0.77	18.17	CH3
29	4.69, 4.56	109.67	CH2
30	1.68	19.45	CH3

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

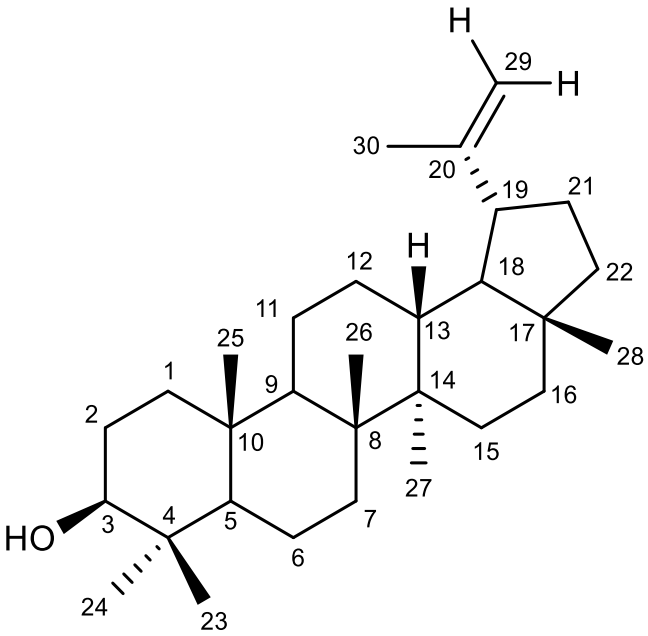

Figure 1: Proposed structure of B3: Lupeol.

Conclusion

Chromatographic separation of the chloroform fraction of the aqueous methanol extract of *B. costatum* led to the isolation of a pentacyclic triterpene characterized as lupeol on the basis of spectra data and comparison with literature.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

The authors are grateful to Dr. Y.M Sani of the Department of Medicinal Chemistry, Ahmadu Bello University, Zaria, and Dr. J.V Anyam of the Department of Chemistry, University of Agriculture, Makurdi, Benue state, Nigeria.

References

1. Oyen LPA. *Bombax costatum* pellegr. and vuillet (internet) Record form PROTA4U. Bank, M. and Achigan-Dako, E. G (Editors) PROTA (Plant Resources of tropical Africa/ Resources vegetales de l' Afrique tropicale) Wageningen Netherland [online].2011[cited 2018 March 26] Available from http//www.prota4u.org/search.asp>.
2. Orwa C, Mutua A, Kindt R, Jamnadass R, Anthony S. Agroforest Database: a tree reference and selection guide version 4.0[online].2009[cited 2018 Feb. 16] Available from (http://www.worldagroforestry.org/sites/treedbs/treedatabases.asp).
3. Meshack AA. Studies on the Anti-inflammatory properties of the Aqeous Ethanol Extract of the stem bark of *Bombax costatum p.v* M.phil. Thesis, pharmacology. kwame Nkrumah University of science and technology, Kumasi, Ghana. 2016. 49 – 50 p.
4. Annie A. Lupeol, a pentacyclic triterpenoid isolated from *vernonia cinerea* attenuate selenite induced cataract formation in spraque dawley rat pups. Chemico-Biological Interaction 2015; 245(2016):20-29.
5. The European Food and Safety Authority. Plant Sterols and Blood Cholesterol. European Food and Safety Authority Journal 2008; 781:1-12.
6. FDA. Phytosterols and Risk of Coronary Heart Disease; Proposed Rule. Federal Register 2010; 75:76526-76571.
7. Trease K and Evans WC. Text book of pharmacognosy, 14th edition, balliere, tindall, London,1996. 251-293 p.
8. Abaoba OO and Efuwape BM. Antibacterial Properties of some Nigerian Spices. Biol Res Commun. 2001; 13:183-188.
9. Sodipo OA, Akanji MA, Kolawole FB, Adetuga OO. Saponins is the active antifungal principle in *Garcinia kola*, heckle seed. Biol Sci Res Commun. 1991; 3:171.
10. Okwu DE and Okwu ME. Chemical composition of *Spondias mombin* Linn plants. J Sus Agric Environ. 2004; 6(2):140.
11. Baba-Mousa F, Akpagana K, Bouchet P. Antifungal activities of seven west African combrataceace used in traditional medicine. J Ethnopharm 1999; 66:335-338.
12. De-Ruiz REL, Fusco RMD, Angela S, Sohar O.R. Isolation of flavonoids and anthraquinones of *Amaranthus muricatus* (Moquin) ex Hicken Amarathaceae) Acta Farm Bon. 2001; 20:9-12.
13. Abdullahi SM, Musa AM, Abdullahi MI, Sule MI, Sani YM. Isolation of Lupeol from the stem bark of *lonchocarpus sericeus* (paplionaceae). Scholars Acad J Biosci. 2013; 1(1):18-19.