

**Phaeophytin a and Triterpenoids from *Brachystelma Togoense* Schltr, a Nigerian Medicinal Herb**

**ABSTRACT**

The medicinal herb, *Brachystelma togoense* schltr (Apocynaceae) was used traditionally for treatment of ailments. From the MeOH and CH<sub>2</sub>Cl<sub>2</sub> extracts of *Brachystelma togoense*, phaeophytin a (1),  $\alpha$ -amyrin (2) and lupeol (3) respectively were isolated and identified as the secondary metabolites from this plant. The structures were elucidated using <sup>1</sup>H, <sup>13</sup>C and 2D NMR. These phytochemicals have shown to possess various biological activities such as anti-inflammatory, anti-fungal, anti-inflammatory and anti-cancer. Therefore, the uses of *Brachystelma togoense* for medicinal purpose in Nigeria were due to the presence of these compounds.

**Keywords:** Secondary metabolites; phaeophytin a;  $\alpha$ -amyrin; lupeol; *Brachystelma togoense* schltr

**1. INTRODUCTION**

*Brachystelma* was first described by Robert Brown in 1822. The genus *Brachystelma* R. Br. (Apocynaceae: Asclepiadoideae) is represented by about 100-120 species (1). The genus *Brachystelma* is chiefly distributed in South Africa, South-East Asia and Australasia (2). A total of 18 species are known in India (3) and out of them, 3 species in Maharashtra. It is an erect perennial herb, growing up to 30 cm, recorded from Ghana to Nigeria, in lowlands to montane situations. The tuber is said to be edible raw (4). Many of the tuberous *Brachystelma*

are known to be used medicinally for the treatment of headache, stomach ache and colds in children.

## 2.MATERIAL AND METHOD

### 2.1 Collection

The aerial parts of *Brachystelma togoense* was collected in April 2018 from Ugbokolo forest, in Okpokwu local government area of Benue State-Nigeria. The plant was authenticated by Mr. Namadi Sanusi and a voucher specimen 25856 had been retained at the Department of Biological Sciences, Ahmadu Bello University, Zaria-Nigeria (Figure 1).

### 2.2 General experimental procedure

NMR spectra were recorded in CD<sub>3</sub>OD or CDCl<sub>3</sub> on a 400MHz or 500 MHz Bruker AVANCE III NMR instrument at room temperature. HREIMS were recorded on an Agilent Technologies 6550 iFunnel Q-TOF LC/MS with samples dissolved in CH<sub>2</sub>Cl<sub>2</sub>. Optical rotations were determined in CH<sub>2</sub>Cl<sub>2</sub> on a JASCO P-1020 polarimeter and the infrared spectra were recorded using a Perkin-Elmer (2000 FTIR) spectrometer on NaCl plates.

### 2.3 Extraction and isolation

Air dried *B. togoense* (1000 g) was extracted on a shaker at room temperature successively with 100 % CH<sub>2</sub>Cl<sub>2</sub> and 100 % CH<sub>3</sub>OH for 72 h with each solvent. The extracts were concentrated using rotary evaporator at 40 °C to a yield brown gum (32 g) The CH<sub>2</sub>Cl<sub>2</sub> extract (32 g) was separated by flash chromatography (Biotage) over silica gel using three solvents, first with a hexane/ CH<sub>2</sub>Cl<sub>2</sub> step gradient starting with 100 % hexane and gradually increasing the polarity to 100 % CH<sub>2</sub>Cl<sub>2</sub>. Secondly, CH<sub>2</sub>Cl<sub>2</sub>/EtOAc from the last concentration (100 % CH<sub>2</sub>Cl<sub>2</sub>) to 50 % EtOAc and to 100 % EtOAc to yield compounds **1** (51.0 mg), **2** (32.0 mg) and **3** (28.0 mg).

## 4. Results and Discussion

50 The air dried aerial parts *B. togoense* (1000 g) collected at Ugbokolo forest, Okpokwu local  
51 government area of Benue State-Nigeria, were extracted with dichloromethane and methanol.  
52 A combination of flash chromatography (biotage system), column chromatography and thin-  
53 layer chromatography of these extracts yielded 1 (51.0 mg; 0.16 %), 2 (32.0 mg; 0.10 %) and  
54 3 (28.0 mg; 0.09 %). The known compounds 1-3 (Figure 2) were elucidated based on  
55 comparison of previous data (5–9). In the past, pheophytin a has been reported to possess  
56 strong antimicrobial activity against *C. albicans* (ATCC 90028) and *C. albicans* (ATCC  
57 76615) (10) as well as antioxidant activity (11).  $\alpha$ -amyrin has been reported to exhibit  
58 antimicrobial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*,  
59 *Staphylococcus aureus* and *Trichophyton mentagrophytes* (12). Antiprotozoal, anti-  
60 inflammatory, antitumor and antimicrobial activity had been reported for lupeol (13).

## 61 **Conclusion**

62 In conclusion, this was first record about phytochemical analysis of *B. togoense*. These  
63 secondary metabolites pheophytin a (**1**),  $\alpha$ -amyrin (**2**) and lupeol (**3**) were reported to show  
64 various biological activities. Therefore, the results of chemical component analysis for *B.*  
65 *togoense* suggested the ethnomedicinal uses of this plant in Nigeria.

66 .

## 67 **Competing Interests**

68 Authors have declared that no competing interests exist.

69



Figure 1: *Brachystelma togoense* in its natural habitat (14)

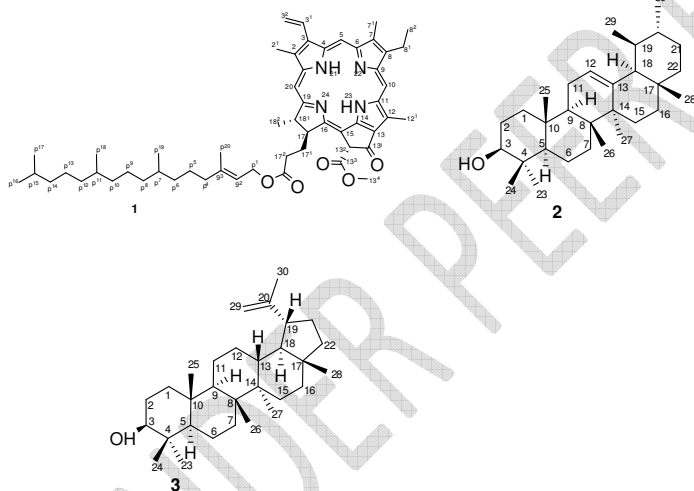


Fig.2: structures of isolated compounds **1-3** from *B.togoense* schltr

1. phaeophytin a

2.  $\alpha$ -amyrin

3. lupeol

## References

1. Bruyns P V. Three New Species of *Brachystelma* (Apocynaceae, Asclepiadoideae,

- Ceropegieae) from South Tropical and Southern Africa. Vol. 19. SPIE; 2009. 5 p.
2. Ollerton J, Masinde S, Meve U, Picker M, Whittington A. Fly pollination in Ceropegia (Apocynaceae: Asclepiadoideae): biogeographic and phylogenetic perspectives. Ann Bot. 2009;103(9):1501–14.
  3. Britto SJ, Bruyns P V. Three new species of *Brachystelma* from Tamil Nadu, India. Haseltonia. 2016;(22):48–54.
  4. Kew Royal Botanical Gardens. Electronic Plant Information Centre (ePIC) [Internet]. 2019 [cited 2019 Feb 7]. Available from: <http://epic.kew.org/index.htm>
  5. Xueyan R, Jia Y, Xuefeng Y, Lidan T, Qingjun K. Isolation and purification of five phenolic compounds from the *Xinjiang* wine grape (*Vitis Vinifera*) and determination of their antioxidant mechanism at cellular level. Eur Food Res Technol [Internet]. 2018;244(9):1569–79. Available from: <https://doi.org/10.1007/s00217-018-3070-z>
  6. De Britto J, Soosai Manickam V, Gopalakrishnan S, Ushioda T, Tanaka N. Chemical and chemotaxonomical studies of ferns. Determination of aglycone chirality in dihydroflavonol 3-O-.ALPHA.-L-rhamnosides by 1H-NMR spectroscopy [Internet]. Vol. 43. 1995. 338-339 p. Available from: [https://www.jstage.jst.go.jp/article/cpb1958/43/2/43\\_2\\_338/\\_pdf](https://www.jstage.jst.go.jp/article/cpb1958/43/2/43_2_338/_pdf)
  7. Silva ATM e, Magalhães CG, Duarte LP, Mussel W da N, Ruiz ALTG, Shiozawa L, et al. Lupeol and its esters: NMR, powder XRD data and in vitro evaluation of cancer cell growth. Brazilian J Pharm Sci [Internet]. 2018 Feb 1 [cited 2019 Feb 19];53(3). Available from: [http://www.scielo.br/scielo.php?script=sci\\_arttext&pid=S1984-82502017000300621&lng=en&tlng=en](http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1984-82502017000300621&lng=en&tlng=en)
  8. Abreu VG da C, Corrêa GM, Lagos IA dos S, Silva RR, Alcântara AF de C. Pentacyclic triterpenes and steroids from the stem bark of *uchi* (*Sacoglottis uchi*, Humiriaceae). Acta Amaz. 2013;43:525–8.

9. Schwikkard SL, Mulholland DA, Hutchings A. Phaeophytins from *Tapura fischeri*.  
Phytochemistry. 1998;49(8):2391–4.
10. Gomes RA, Teles YCF, Pereira F de O, Rodrigues LA de S, Lima E de O, Agra M de  
F, et al. Phytoconstituents from *Sidastrum micranthum* (A. St.-Hil.) Fryxell  
(Malvaceae) and antimicrobial activity of pheophytin a. Brazilian J Pharm Sci  
[Internet]. 2015;51:861–7. Available from:  
[http://www.scielo.br/scielo.php?script=sci\\_arttext&pid=S1984-  
82502015000400861&nrm=iso](http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1984-82502015000400861&nrm=iso)
11. Kusmita L, Puspitaningrum I, Limantara L. Identification, isolation and antioxidant  
activity of pheophytin from green tea (*Camellia sinensis* (L.) Kuntze). Procedia Chem  
[Internet]. 2015;14:232–8. Available from:  
<https://dx.doi.org/10.1016/j.proche.2015.03.033>
12. Ragasa CY, Puno MRA, Sengson JMAP, Shen C-C, Rideout JA, Raga DD. Bioactive  
triterpenes from *Diospyros blancoi*. 2009;23(13):1252–8. Available from:  
<https://dx.doi.org/10.1080/14786410902951054>
13. Gallo M, Miranda bullet, Sarachine J. Biological activities of lupeol. In: International  
Journal of Biomedical and Pharmaceutical Sciences. 2009. p. 46–66.
14. Erpenbach A. West African Plants - A Photo Guide - *Brachystelma togoense* Schltr.  
[Internet]. 2009 [cited 2019 Feb 18]. Available from:  
[http://www.westafricanplants.senckenberg.de/root/index.php?page\\_id=14&id=4246](http://www.westafricanplants.senckenberg.de/root/index.php?page_id=14&id=4246)