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

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Authors’ contributions

This work was carried out in collaboration among all authors. Author AE designed the study, performed the experimental study and wrote the protocol and the first draft of the manuscript. Authors RGOA, JDH and IH supervised the work. All authors read and approved the final manuscript.

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ABSTRACT

The medicinal herb *Brachystelma togoense* Schltr (Apocynaceae) is used traditionally for treatment of ailments. The secondary metabolites, phaeophytin a, \(\alpha\)-amyрин и лупеол were isolated from the \(\text{CH}_2\text{Cl}_2\) and MeOH extracts of *Brachystelma togoense*. The structures were elucidated using \(^1\text{H},\ \text{\(^{13}\text{C}\)}\) and 2D NMR. These phytochemicals have previously been reported to have various biological activities such as anti-inflammatory, anti-fungal and anti-cancer. The presence of phaeophytin a, \(\alpha\)-amyрин и лупеол in *Brachystelma togoense* justified the use of the plant for medicinal purpose in Nigeria.

Keywords: Secondary metabolites; phaeophytin a; \(\alpha\)-amyрин; лупеол; *Brachystelma togoense* Schltr.

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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]. It is an erect perennial herb, growing up to 30 cm high. 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. *Brachystelma* is found from Ghana to Nigeria, in lowlands to montane areas [4]. The raw tuber is said to be edible [4]. Many of the tuberous *Brachystelma* are known to be used medicinally for the treatment of headache, stomachache and colds in children [5]. *Brachystelma togoense* has being medicinally used for the treatment of dysentery, cough and cold, wounds, stomach ache, typhoid and erectile dysfunction.

2. MATERIALS AND METHODS

2.1 Collection

The aerial parts of *Brachystelma togoense* was collected during April 2018 from the Ugbokolo forest in Okpokwu local government area of Benue State-Nigeria. The plant was collect and stored in a plastic container before it was air-dried. The collected specimen was positively identified by Mr. Namadi Sanusi, a botanist at Ahmadu Bello University, Zaria as *Brachystelma togoense*. A specimen (no. 25856) had been retained at the Department of Biological Sciences, Ahmadu Bello University, Zaria-Nigeria (Fig. 1).

2.2 Extraction and Isolation

The air-dried *B. togoense* was manually reduced to powder using mortar and pestil. Exactly 1000 g of the powdered plant material was extracted on a shaker at room temperature using 100% dichloromethane (CH$_2$Cl$_2$) for 72 h. The extracts were concentrated using a rotary evaporator at 40˚C resulting in a brown gum-like texture (32 g). The same procedure was used for methanol (MeOH) which yielded a brown gum-like texture (36 g). The CH$_2$Cl$_2$ and MeOH extracts were separated by flash chromatography (Biotage system) over silica gel using three solvents. Firstly, a hexane/ CH$_2$Cl$_2$ gradient starting with 100% hexane and gradually increasing the polarity to 100% CH$_2$Cl$_2$. Secondly, CH$_2$Cl$_2$/EtOH/Ac from a 100% CH$_2$Cl$_2$ to 50% EtOH/Ac and to 100% EtOH/Ac to yield various fractions (fr. 1-100). Fr.20 was spotted on the TLC plate using 100% CH$_2$Cl$_2$ and appeared a pure compound 1 (51.0 mg). The same procedure was repeated for the MeOH extract yielding compounds 2 (32.0 mg) and 3 (28.0 mg) which were spotted as pure compounds using CH$_2$Cl$_2$/EtOH/Ac (7:3) from fr.30.

2.3 General Experimental Procedure

NMR spectra were recorded in CDCl$_3$ 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$_2$Cl$_2$. Infrared spectra were recorded using a Perkin-Elmar (2000 FTIR) spectrometer on NaCl plates.

3. RESULTS AND DISCUSSION

The following following compounds phaophytin a (51.0 mg; 0.16%), α-amyrin (32.0 mg; 0.10%) and lupeol (28.0 mg; 0.09%) were isolated from *Brachystelma togoense* using flash chromatography (biotage system). These compounds (Fig. 2) were elucidated based on comparison of previous data [6–8].

Phaeophytin-a was isolated as a dark green solid from the CH$_2$Cl$_2$ extract of the aerial parts of *B.
**Fig. 2. Structures of isolated compounds 1-3 from B. togoense schtlr**

1. Phaeophytin a; 2. α-Amyrin; 3. Lupeol

togoense that was previously described [6]. The IR spectrum showed absorbance bands for vinyl proton (3056 cm⁻¹) and sp³ CH (2987, 2932 cm⁻¹) and carbonyl (1736 cm⁻¹) groups. A molecular ion could not be seen in the HRMS spectrometer despite repeated attempts.

From the ¹H and ¹³C NMR spectra, it was evident that phaeophytin-a belonged to the phaeophytin class. This was particularly evident by the downfield shifts at δ_H 9.32 s, 9.48 s and 8.56 s which could be assigned as H-5, H-10 and H-20 respectively. The deshielded methyl groups proton resonances occurred at δ_H 3.19 (3H-6), δ_H 3.3 (3H-7') and δ_H 3.38 (3H-12') and a methoxy group proton resonance occurred at δ_H 3.89 (3H-13'). The presence of a C-20 phytol tail was evident from the presence of four methyl protons (δ_H 0.80 d, J = 7.3, δ_H 0.82 d, J = 7.3, δ_H 0.79 s, δ_H 1.61 s) and ester carbonyl resonance at δ_C 173.8 (C-13'). A comparison of the NMR data of phaeophytin-a against literature values for phaeophytin a showed the enabled assignment of a keto group carbon resonances at δ_C 189.9 to C-13'[6,9]. The ¹H and ¹³C NMR spectra for compound 1 were assigned using HSQC and HMBC as given in Table S1.

Amyrin (α) was isolated as a brown solid from the CH₂Cl₂ extract of the aerial parts of B. togoense, which had been isolated previously from the methanol extract of Sacoglossis uchi [7]. The IR spectrum showed absorbance bands for hydroxyl (3055 cm⁻¹) and sp³ CH (2987 cm⁻¹) in conjugation and unsymmetrical ethylenic double bond (1733 cm⁻¹) and olefinic carbon (1422 cm⁻¹) groups.

The molecular ion was not observed in the HRMS spectrum, however 30 carbons could be counted in the ¹³C NMR spectrum, indicating the compound was a triterpenoid.

The ¹H and ¹³C NMR spectra (spectrum 2.2 and 2.3) showed the presence of one trisubstituted double bond. A hydroxyl group was placed on C-3 confirmed by the C-3 (δ_C 79.3) resonance correlating with both the 3H-23 (δ_H 0.99 s), 3H-24 (δ_H 0.78 s) and H-5 (δ_H 0.73 d, J = 11.5) resonances. A further singlet (δ_H 0.79, 0.93,
inflammatory, antitumor and antimicrobial activity against 
Staphylococcus aureus, Pseudomonas aeruginosa 
and C. albicans possess antimicrobial activity against 
Amyri 76615) (ATCC 90028) and C. albicans (ATCC 76615) [12]. Amyrin (α) has been reported to exhibit antimicrobial activity against Escherichia coli, Pseudomonas aeruginosa, C. albicans, Staphylococcus aureus and Trichophyton mentagrophytes [14]. Antiprotzoal, anti-inflammatory, antitumor and antimicrobial activity had been reported for lupeol [15]. Ref [16] gives the picture of B. togoense in its natural habitat.

4. CONCLUSION

Phaeophytin a, α-amyrin and lupeol are reported here for the first time from B. togoense. This was also the first report of the phytochemical quantification in B. togoense in Nigeria. However, these secondary metabolites, i.e phaeophytin a, α-amyrin and lupeol were reported previously to show various biological activities. Therefore, the results of chemical compound analysis of B. togoense justified the ethnomedicinal uses of this plant in Nigeria.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


0.99, 0.78 and 1.24) and two doublet (δH 0.86 d, J= 6.2 and δH 0.95 d, J= 6.2) methyl group proton resonances were present and the typical 12-oleanene double bond (δC 5.25, δC 126.1, δC 138.2) was seen. A comparison against literature data [7] confirmed that this compound was α-amyrin which has been isolated previously from the stem bark of Sacoglottis uchi (Humiriaceae) [7].

The configuration of the hydroxyl group at C-3 was confirmed as β by the coupling constant of H-3 (J = 5.1, 11.3 Hz). The configurations at the chiral centres were confirmed using the NOESY spectrum. The 1H and 13C NMR spectra for compound 2 were assigned using HSQC and HMBC as given in Table S2.

Lupeol was isolated as a brown solid from the MeOH extract of the aerial parts of B. togoense which had been isolated previously from the hexane extract of Magnolia salicifilia [10] as well as synthesised [8]. The IR spectrum showed an absorbance band for hydroxyl (3363 cm⁻¹). The molecular ion was not seen in the HRMS spectrum, however 30 carbons could be counted in the 13C NMR spectrum indicating the compound was a triterpenoid.

The NMR spectra of lupeol showed the presence of an iso-propenyl group typical of the lupenetype of pentacyclic triterpenoids. Coupled 2H-29 methylene protons (δH 4.69 d, J = 2.1, δH 4.57 d, J = 2.4) and 13C NMR resonances (δC 105.9, δC 151.2, δC 19.5) could be assigned to two H-29 and C-29, C-20 and C-30 respectively [11].

Compound 3 was identified as the known 3β-hydroxy-lup-20(29)-ene, commonly referred to as lupeol. A literature search revealed that the 13C NMR chemical shifts were similar to those of lupeol. The configurations at the chiral centres were confirmed using the NOESY spectrum. The 1H and 13C NMR spectra for compound 3 were assigned using HSQC and HMBC as given in Table S3.

Previously, phaeophytin a has been reported to possess antimicrobial activity against Candida albicans (ATCC 90028) and C. albicans (ATCC 76615) [12] as well as antioxidant activity [13]. Amyrin (α) has been reported to exhibit antimicrobial activity against Escherichia coli, Pseudomonas aeruginosa, C. albicans, Staphylococcus aureus and Trichophyton mentagrophytes [14]. Antiprotzoal, anti-inflammatory, antitumor and antimicrobial activity had been reported for lupeol [15]. Ref [16] gives the picture of B. togoense in its natural habitat.

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