Original Research Article

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

ABSTRACT

The medicinal herb *Brachystelma togoense* schtlr (Apocynaceae) is used traditionally for treatment of ailments. The secondary metabolites, phaeophytin *a*, α-amyrin and lupeol were isolated from the CH_2Cl_2 and MeOH extracts of *Brachystelma togoense.* The structures were elucidated using ^{1}H, ^{13}C and 2D NMR. These phytochemicals have previously being reported to have various biological activities such as anti-inflammatory, anti-fungal and anti-cancer. The presence of phaeophytin *a*, α-amyrin and lupeol in *Brachystelma togoense* justified the use of the plant for medicinal purpose in Nigeria.

Keywords: Secondary metabolites; phaeophytin *a*; α-amyrin; lupeol; *Brachystelma togoense* schtlr

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. MATERIAL AND METHOD

2.1 Collection

The aerial parts of *Brachystelma togoense* was collected during April 2018 from the Ugbokolo forest in Okpokwu, which is the local government area of Benue State-Nigeria. The plant was collect around 10:00 (correct it am or pm) (why specific? Remove it if not necessary) and stored in a plastic container before it was air-dried. The collected specimen was positively identified by Mr. Namadi Sanusi, a botanistat 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 (Figure 1).

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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_2Cl_2) for 72 h. The extracts were concentrated using a rotary evaporator at 40^{o} 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_2Cl_2 and MeOH extracts were separated by flash chromatography (Biotage system) over silica gel using three solvents. Firstly, a hexane/ CH_2Cl_2, gradient starting with 100 % hexane and gradually increasing the polarity to 100 % CH_2Cl_2. Secondly, CH_2Cl_2/EtOH/Ac from a 100 % CH_2Cl_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_2Cl_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_2Cl_2 /EtOH/Ac (7:3) from fr.30.

2.2 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_2Cl_2. Infrared spectra were recorded using a Perkin-Elmar (2000 FTIR) spectrometer on NaCl plates.

3. Results and Discussion

The 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 (Figure 2) were elucidated based on comparison of previous data (6–8).

Phaeophytin-*a* was isolated as a dark green solid from the CH_2Cl_2 extract of the aerial parts of *B. togoense* that was previously described (6). The IR spectrum showed absorbance bands for vinyl proton (3056 cm^{-1}) and sp^3 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 ^{13}C 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-2'), 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-13^4). 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-13^3). 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-13^1 (6,9).

Amyrin (α) was isolated as a brown solid from the CH_2Cl_2 extract of the aerial parts of *B. togoense*, which had been isolated previously from the methanol extract of *Sacoglottis uchi* (7). The IR spectrum showed absorbance bands for hydroxyl (3055 cm^{-1}) and sp^3 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 ^{13}C NMR spectrum, indicating the compound was a triterpenoid.

The ^{1}H and ^{13}C NMR spectra (spectrum 3.2 and 3.3) (where 3.2 and 3.3 given and not showed in the manuscript) 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, 0.99, 0.78 and 1.24) and two doublet (δ_H 0.86 d, J= 6.2 and δ_H 0.95 d, J= 6.2) methyl group proton resonances were present and the typical 12-olaenene double bond (δ_H 5.25, δ_C 126.1, δ_C 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).

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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). The configurations at the chiral centres were confirmed using the NOESY spectrum.

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 no seen in the HRMS spectrum, however 30 carbons could be counted in the ^{13}C NMR spectrum indicating the compound was a triterpenoid.

The NMR spectra of lupeol showed the presence of an *iso*-propenyl group typical of the lupene-type of pentacyclic triterpenoids. Coupled 2H-29 methylene protons (δ_H 4.69 d, J = 2.1, δ_H 4.57 d, J = 2.4) and ^{13}C NMR resonances (δ_C 105.9, δ_C 151.2, δ_C 19.5) could be assigned to two H-29 and C-29, C-20 and C-30 respectively (11).

Lupeol was identified as the known 3β-hydroxylup-20(29)-ene, commonly referred to as lupeol. A literature search revealed that the ^{13}C NMR chemical shifts similar to those of lupeol ~~had been reported for lupeol~~. The configurations at the chiral centres were confirmed using the NOESY spectrum.

Previously, pheophytin *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). Antiprotozoal, anti-inflammatory, antitumor and antimicrobial activity had been reported for lupeol (15).

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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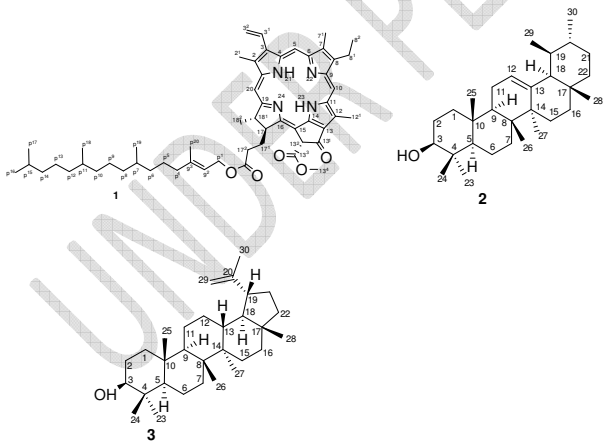
Competing Interests

Authors have declared that no competing interests exist.

Figure 1: *Brachystelma togoense* in its natural habitat (16)



(Need clear images of the chemical structures)

Fig.2: Structures of isolated compounds **1-3** from *B.togoense* schtlr

1. Phaeophytin *a*

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2. α-Amyrin

3. Lupeol

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