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 ~~previously~~ reported to have ~~shown~~ 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~~is~~ use of the plant for medicinal purpose in Nigeria.

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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,

stomach ache 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~~h~~ and stored in a plastic container before it was ~~air dried~~air-dried. The collected specimen was positively identified by Mr. Namadi Sanusi, a botanistat Ahmadu Bello University, Zaria as B~~b~~*rachystelma togoense*. A specimen (no. 25856) had been retained at the Department of Biological Sciences, Ahmadu Bello University, Zaria-Nigeria (Figure 1).

2.2 Extraction and isolation

The ~~air dried~~air-dried *B. togoense* was manually reduced to powder using mortar and pestle~~istil~~. Exactly ~~(~~1000 g~~)~~ of the powdered plant material was ~~successfully~~ 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.

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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.

4. Results and Discussion

The air-dried aerial parts *B. togoense* (1000 g) collected at Ugbokolo forest (Okpokwu local flash chromatography (biotage system), this extract phaophytin *a* (51.0 mg; 0.16 %), α-amyrin (32.0 mg; 0.10 %) and lupeol (28.0 mg; 0.09 %). The compounds (Figure 2) were elucidated based on comparison of previous data (6–8).

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Phaeophytin- *a* was isolated as a dark green solid from the CH_2Cl_2 extract of the aerial parts of *B. ~~rachystelma~~ togoense* that was~~had~~ previously described (7). 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.

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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 (7,9).

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α Amyrin (α) was isolated as a brown solid from the CH_2Cl_2 extract of the aerial parts of *B. rachystelma togoense*, which had been isolated previously from the methanol extract of *Sacoglottis uchi* (6). 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 present/observed? ~~seen~~ 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) 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 (6) confirmed that this compound was α-amyrin, which has been isolated previously from the stem bark of *Sacoglottis uchi* (Humiriaceae)(6).

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. rachystelma 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,

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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~~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.~~andida~~ 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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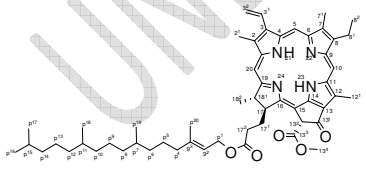
Acknowledgments

The author wishes to thank the natural product group, university of Surrey, UK for the research work.

Competing Interests

Authors have declared that no competing interests exist.

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



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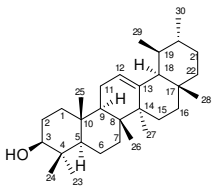

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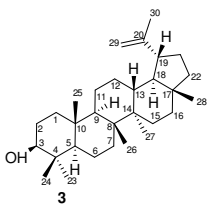

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Comment [PM2]: I am concerned about this statement! Did the university of Surrey do the research? What role did the author play in this investigation? Is the author part of the Surrey research group? Is the author a registered student at university of Surrey? This statements has op be rewritten and clarified. This statement puts doubt on the authenticity of the work as far as the author is concerned.

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

1. Phaeophytin *a*

2. α-Amyrin

3. Lupeol

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Comment [PM3]: In lines 158-159, 169, 189, 192 the journal names are abbreviated, yet the other journal names are written out in full. Lines 171 and 204. Be consistent.

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