Original Research Article

Phaeophytin *a* 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 and identified from MeOH and CH_2Cl_2 extracts of *Brachystelma togoense.* The structures were elucidated using ^{1}H, ^{13}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 is because of the presence of phaeophytin *a*, α-amyrin and lupeol.

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 (Reference needed). The raw tuber is said to be edible (4).

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Comment [PM2]: The introduction could include more information regarding the use of Brachystelma as a herb.

Many of the tuberous *Brachystelma* are known to be used medicinally for the treatment of headache, stomach ache and colds in children (Reference needed).

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 collected specimen was positively identified by Mr. Namadi Sanusi as *Brachystelma 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

Air dried *B. togoense* (1000 g) was successfully extracted on a shaker at room temperature using a 100 % dichloromethane (CH_2Cl_2) and 100 % methanol (CH_3OH) for 72 h. The extracts were concentrated using a rotary evaporator at 40°C resulting in a brown gum-like texture (32 g). The CH_2Cl_2 extract (32 g) was 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 compounds **1** (51.0 mg), **2** (32.0 mg) and **3** (28.0 mg).

2.3 General experimental procedure

Nuclear magnetic resonance (NMR) spectra were recorded in CD_3OD or $CDCl_3$ on a 400MHz or 500 MHz Bruker AVANCE III NMR instrument at room temperature. High-resolution electron impact mass spectrometry (HREIMS) was recorded on an Agilent Technologies 6550 iFunnel Q-TOF LC/MS with samples dissolved in CH_2Cl_2. Optical rotations were determined in CH_2Cl_2 on a JASCO P-1020 polarimeter and the infrared spectra were recorded using a Perkin-Elmer (2000 FTIR) spectrometer on NaCl plates.

Comment [PM3]: At what time of the day was the material collected? How was the material stored until required for preparation?

Comment [PM4]: Who is Mr Sanusi? Is he a botanist? Where does he work?

Comment [PM5]: How was the air-dried plant material prepared for extraction? Ground, chopped, powder, etc.

Comment [PM6]: What do you mean by "successfully extracted"?

Comment [PM7]: What was the ration between plant material and extraction solvent? What was the ratio between dichloromethane and methanol? Was it two separate extractions, one using methanol and one using dichloromethane or a mixture of the two solvents?

Comment [PM8]: How many replicates? If any

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4. Results and Discussion

The air-dried aerial parts *B. togoense* (1000 g) collected at Ugbokolo forest (Okpokwu local government area of Benue State-Nigeria) were extracted with dichloromethane and methanol. A combination of flash chromatography (biotage system), column chromatography and thin-layer chromatography of these extracts yielded phaeophytin *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 (5–9). Previously, pheophytin *a* has been reported to possess ~~strong~~ antimicrobial activity against *Candida albicans* (ATCC 90028) and *C. lbicans* (ATCC 76615) (10) as well as antioxidant activity (11). Amyrin (α) has been reported to exhibit antimicrobial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *C. albicans*, *Staphylococcus aureus* and *Trichophyton mentagrophytes* (12). Antiprotozoal, anti-inflammatory, antitumor and antimicrobial activity had been reported for lupeol (13).

Conclusion

This was a first report of the phytochemical compound quantification in *B. togoense* in Nigeria. These secondary metabolites, i.e. phaeophytin *a*, α-amyrin, and lupeol were reported to show various biological activities. Therefore, the results of chemical compound analysis of *B. togoense* suggested the ethnomedicinal uses of this plant in Nigeria.

Comment [PM10]: Where are these results? This is work reported by Gallo et al. If you are the author of this paper (Gallo) you should say previous work done by the author regarding the biological activity of B. togoense etc

.Competing Interests

Authors have declared that no competing interests exist.

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

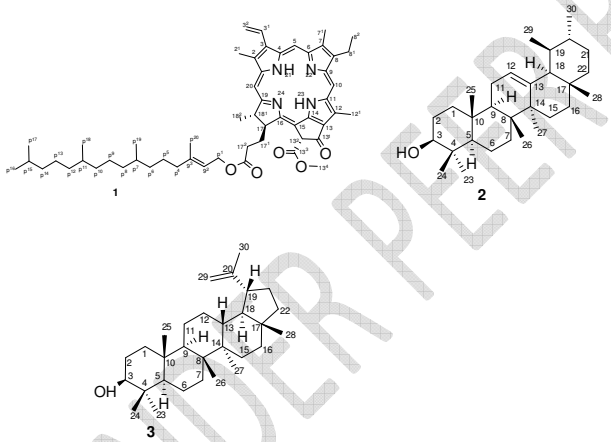

Fig.2: Structures of isolated compounds **1-3** from *B. togoense* schtlr (Please acknowledge source of the structures)

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

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Comment [PM11]: You are not consistent in writing of the references. Should the journal names be abbreviated or not? Some are abbreviated and some are not abbreviated. Space between author initials or not?

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