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Bioactivities of Phaeophytin *a*, α-Amyrin, and lupeol from *Brachystelma togoense* Schltr

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Abstract: Phaeophytin *a*, α-amyrin and lupeol isolated from *Brachystelma togoense* were screened against *S. aureus, E. coli, S. pneumoniae, S. typhi,* and *C. albicans* using Ciprofloxacin and Terbinafine as standards. The results showed that these phytochemicals displayed antimicrobial activity against the tested organisms with the zone of inhibition from 12 – 27 mm. The result of minimum inhibitory concentration (MIC) showed that phaeophytin *a* was most active against *C. albicans* (0.09 mg/mL). The minimum bactericidal concentration (MBC) showed that phaeophytin *a* and lupeol were most active against *S. aureus, S. pneumoniae* and *S. typhi* (0.37 mg/mL). The result of minimum fungicidal concentration (MFC) showed that phaeophytin *a* was most active against *C. albicans* (0.1875 mg/mL). The activities of these phytoconstituents in *B. togoense* justified ethnomedicinal uses of the plant to treat various ailments.

Keywords: Bioactivity, Phaeophytin *a*, α-amyrin and lupeol, *Brachystelma togoense* Schtlr

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INTRODUCTION

Medicinal plants are used in developing countries as alternative medical treatments and phytochemicals isolated from these plants have shown *in vitro* and *in vivo* biological activity (1). Previously, humans have used natural products, such as plants, animals, microorganisms, and marine organisms, in medicines to alleviate and treat various diseases.

These natural products have a unique chemical diversity, which results in diversity in their biological activities and drug-like properties (2). The use of medicinal plants for the treatment of diseases dates back to the history of human life, as the use of plants was their only choice of treatment (3). The World Health Organization (WHO) defines traditional medicinal plants as natural plant materials which are used at least or in the absence industrial processing for the treatment of diseases at a local or regional scale. Traditional herbal medicine has been used for thousands of years because it is natural and causes relatively fewer complications (3). There is a promising future of

medicinal plants as many plants around the world, and most of them are not investigated yet for their medical activities and their hidden potential of medical activities could be useful in the treatment of present and future studies (4).

Brachystelma was first described by Robert Brown in 1822. The genus *Brachystelma* R. Br. (Apocynaceae: Asclepiadoideae) is represented by about 100-120 species (5). *Brachystelma* is chiefly distributed in South Africa, South-East Asia, and Australasia (6). A total of 18 species are known in India (7) and out of them, 3 species are in Maharashtra. *Brachystelma togoense* is distributed on partially degraded hill slopes and open hill tops among grasses. 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 (8). The leaves are green in color, opposite, decussate, sessile or subsessile, linear to lanceolate to narrowly elliptic, 3-8 x 0.4-1 cm, acute, and margin ciliate. Tubers of *Brachystelma* species are known to be eaten as a food supplement in rural areas when there is a food shortage. Many of the tuberous *Brachystelma* are known to be used medicinally for the treatment of headache, stomach ache, and colds in children. In China, *Brachystelma edule* tubers are edible and the plant is used for the treatment of coughs and reduction of phlegm (9).

The secondary metabolites, phaeophytin *a*, α-amyrin, and lupeol, were previously isolated from the CH_2Cl_2 and MeOH extracts of *B. togoense.* The structures were elucidated using ^{1}H, ^{13}C and 2D NMR. The presence of phaeophytin *a*, α-amyrin, and lupeol in *B. togoense* justified the use of the plant for medicinal purpose in Nigeria (10). The aim of this study is to carry out antimicrobial studies of the phaeophytin *a*, α-amyrin, and lupeol isolated from the aerial parts of *B. togoense.*

MATERIALS AND METHODS

Chemicals

The chemicals used for this study are CH_2Cl_2, MeOH, EtOH, DMSO, and EtOAc. All the chemicals were of analytical grade.

Material

Collection of plant

The stem bark of *Brachystelma togoense* was collected in April 2018 from Benue State, Nigeria. The plant was identified by the plant taxonomist Mallam Sanusi Namadi and a voucher specimen (no. 25856) is retained in the Biological Science Department, Ahmadu Bello University, Zaria-Nigeria.

Extraction and isolation

The air-dried *B. togoense* was manually reduced to powder using a mortar and pestle. Exactly 1000 g of the powdered plant material was extracted on a shaker (labcon sw39, 136 rpm) at room temperature (at 25 °C) using 100% dichloromethane (CH_2Cl_2) for 72 h. The extracts were concentrated using a rotary evaporator (Rotavapor R-215) at 40 °C resulting in a brown gum-like texture (32.0 g). The same procedure was used for methanol (MeOH) which yielded a brown gum-like texture (36.0 g). The CH_2Cl_2 and MeOH extracts were then separated by flash chromatography (Biotage SP1) over silica gel using three solvents. Firstly, an n-hexane/ CH_2Cl_2, gradient starting with 100% hexane and gradually increasing the polarity to 100% CH_2Cl_2. Secondly, CH_2Cl_2/ EtOH/EtOAc from a 100% CH_2Cl_2 to 50% EtOH/ EtOAc and to 100% EtOH/ EtOAc 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/ EtOAc (7:3) from fr.30. The structures of phaeophytin *a* (1), α-amyrin (2) and lupeol (3) are shown in Figure 1.

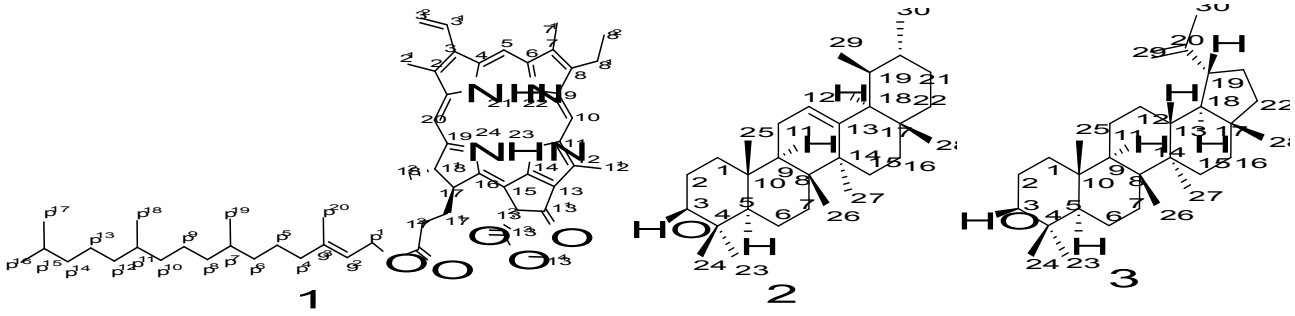

Figure 1. Structures of phaeophytin *a* (**1**), α-amyrin (**2**) and lupeol (**3**).

Antimicrobial screening of the isolates

The antimicrobial activities of the isolates were determined using some pathogenic microorganisms. The microorganisms were obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria. All isolates were checked for purity and maintained in slants of blood agar. The antimicrobial activities of the isolates were evaluated by the broth dilution assay as described previously for the bacteria and Mueller Hinton broth assay for the fungi (11). The microorganisms tested were as follows: *Staphylococcus aureus, Streptococcus pneumoniae, Escherichia coli, Salmonella typhi, and Candida albicans.*

Determination of Zone of Inhibition

Solutions of 6.0 mg of the compounds were prepared using 10.0 mL dimethyl sulfoxide (DMSO). This solution was used to check the antimicrobial activity of the compound. A control experiment was also set up using DMSO.

The standardized inocula of the isolates were uniformly streaked onto freshly prepared Mueller Hinton agar plates with the aid of a sterile swab stick. Using a sterile cork borer (6 mm in diameter), 5 appropriately labelled wells were punched into each agar plate. An aliquot of 0.3 mL of the appropriate isolate concentration was placed in each well and then allowed to diffuse into the agar. An extra plate was streaked with the isolate and Ciprofloxacin (10 µg/disc) was placed on it. The plates were incubated at 37 °C for 24 h. While for the fungi, Sabouraud dextrose broth was used and the incubation period was 30 °C and 48 h. The antimicrobial activities were expressed as diameter (mm) of inhibition zones produced by the plant extracts.

Minimum Inhibition Concentration (MIC)

The minimum inhibition concentrations of the isolates were carried out on the test microbes using the broth dilution method as outlined by the Clinical and Laboratory Standards Institute (CLSI) (12). Mueller Hinton broth was prepared as follows: 10.0 mL was dispensed into tubes and was sterilized at 121 °C for 15 minutes and allowed to cool. The McFarland turbidity standard scale 0.5 was prepared to give turbidity of the solution. Normal saline was prepared, 10.0 mL was dispensed into a sterile test tube and the test microbes were inoculated and incubated at 37 °C for 24 hours. Dilution of the test microbes was done in the normal saline until the turbidity matched that of the McFarland turbidity scale by visual comparison at this point the test microbe has a concentration of about $1.5x10^8$ cfu/mL. Two-fold serial dilution of the extracts in the sterilized broth was made to obtain the concentrations of 3.00 mg/mL, 1.50 mg/mL, 0.75 mg/mL, 0.37 mg/mL, 0.18 mg/mL and 0.09 mg/mL. The initial concentration was obtained by dissolving 6.0 mg of the isolate in 10 mL of the sterile broth. Having obtained the different concentrations of the extracts in the sterile broth, 0.3 mL of the standard inoculum of the test microbe in the normal saline was then inoculated into the different concentrations. Incubation was made at 37 °C for 24 h, after which each test tube of the broth was observed for turbidity (growth). The lowest concentration of the isolate in which the broth shows no turbidity was recorded as the Minimum Inhibition Concentration (MIC).

Determination of Minimum Bactericidal Concentration/Minimum Fungicidal Concentration (MBC/MFC)

This was carried out to determine whether the microorganisms could be completely killed or their growth could only be inhibited. The minimum

bactericidal concentration of the isolates was determined as outlined by the CLSI on the nutrient agar plates. Minimum bactericidal concentrations were determined by assaying the test tube contents of the MIC determinations. A loopful of the content of each tube was inoculated by streaking on a solidified nutrient agar plate and then incubated at 37 °C for 24 h for bacterial and 30 °C for 48 h for fungi. The lowest concentration of the subculture with no growth was considered as minimum bactericidal concentration/ minimum fungicidal concentration.

Table 1. Diameter of Zone Of Inhibition (mm) of Phaeophytin *a.*

	Concentration (mg/mL)					
Microorganisms	3	1.5	0.75	0.375	Ciprofloxacin ($10x10^{-6}$)	Terbinafine ($30x\ 10^{-6}$)
S. aureus	21	19	16	14	25	
E. coli	20	17	14	12	33	
S. pneumoniae	20	17	14	12	20	
S. typhi	19	17	15	13	19	
C. albicans	27	24	21	20		33

Table 2. Diameter of Zone Of Inhibition (mm) of α-amyrin.

	Concentration (mg/mL)					
Microorganisms	3	1.5	0.75	0.375	Ciprofloxacin ($10x10^{-6}$)	Terbinafine ($30x\ 10^{-6}$)
S. aureus	18	16	15	14	25	
E. coli	20	18	16	13	34	
S. pneumoniae	18	14	13	12	20	
S. typhi	19	16	15	14	18	
C. albicans	22	19	16	14		33

Table 3. Diameter of Zone Of Inhibition (mm) of lupeol.

	Concentration (mg/mL)					
Microorganisms	3.0	1.5	0.75	0.375	Ciprofloxacin ($10x10^{-6}$)	Terbinafine ($30x\ 10^{-6}$)
S. aureus	19	16	14	13	25	
E. coli	20	18	16	16	35	
S. pneumoniae	22	18	15	13	20	
S. typhi	20	18	16	15	20	
C. albicans	21	18	15	13		33

Table 4. Minimum Inhibitory Concentration (MIC)

	Concentration(mg/mL)		
Microorganisms	Phaeophytin *a*	α-amyrin	lupeol
S. aureus	0.18	0.37	0.37
E. coli	0.18	0.37	0.37
S. pneumoniae	0.18	0.37	0.18
S. typhi	0.18	0.37	0.18
C. albicans	0.09	0.37	0.18

Table 5. Minimum Bactericidal Concentration (MBC).

	Concentration(mg/mL)		
Microorganisms	Phaeophytin a	α-amyrin	lupeol
S. aureus	0.37	0.75	0.75
E. coli	0.75	0.75	0.75
S. pneumoniae	0.75	0.75	0.37
S. typhi	0.75	0.75	0.37

Table 6. Minimum Fungicidal Concentration (MFC).

	Concentration(mg/mL)		
Microorganism	Phaeophytin a	α -amyrin	lupeol
C. albicans	0.18	0.75	0.37

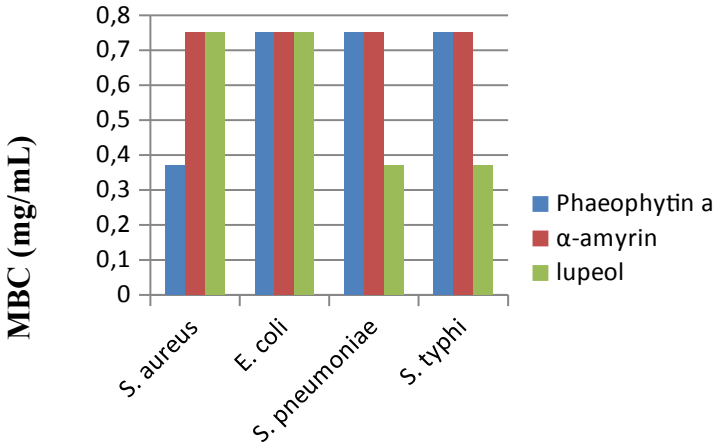

Figure 2. Graphical representation of Minimum Inhibitory Concentration (MIC) of the isolates against the microoganisms.

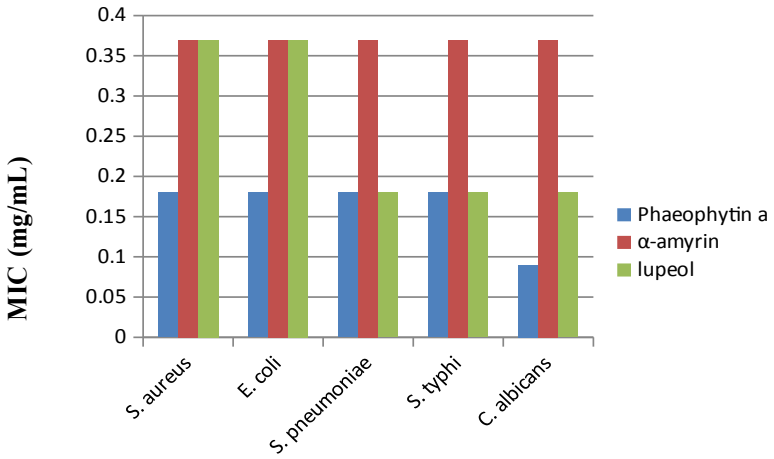

Figure 3. Graphical representation of the Minimum Bactericidal Concentration (MBC) of the isolates against the microorganisms.

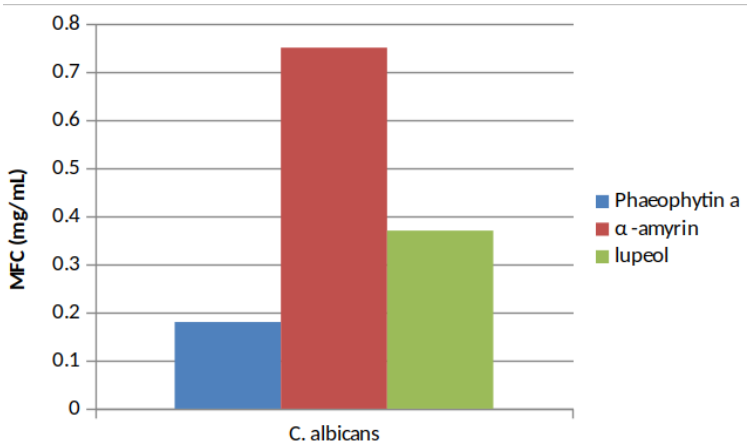

Figure 4. Graphical representation of the Minimum Fungicidal Concentration (MFC) of the isolates against the fungus.

RESULTS AND DISCUSSION

This study was carried out to investigate the in vitro antimicrobial activities of the phytoconstituents in *B. togoense* so as to justify its ethnomedicinal uses in the treatment of various ailments. The results of the antimicrobial studies showed that the compounds exhibited remarkable activity at 3.0 mg/mL against the five microorganisms tested. These phytochemicals displayed antimicrobial activity against the tested organisms with the zone of inhibition at various concentrations used ranging between 12 – 27 mm (Tables 1, 2 and 3). The MIC (Table 4 and Figure 2) showed that Phaeophytin *a* inhibited the growth of the microorganisms between 0.09- 0.18 mg/mL. That of α-amyrin was observed at 0.37 mg/mL while lupeol showed MIC between 0.37-0.75 mg/mL. The MBC (Table 5 and Figure 3) showed that Phaeophytin *a* killed the microbes between 0.37-0.75 mg/mL. That of α-amyrin was observed at 0.75 mg/mL while lupeol showed MBC between 0.37-0.75 mg/mL against these microbes. The result of MFC (Table 6 and Figure 4) showed that phaeophytin *a*, α-amyrin and lupeol stopped the growth of the microbes at 0.18 mg/mL, 0.75 mg/mL and 0.37mg/mL, 0.18 mg/mL respectively.

Phaeophytin *a* was most potent against *C. albicans* (0.09 mg/mL). The MBC showed that Phaeophytin *a* and lupeol were the most potent against *S. aureus, S. pneumoniae* and *S. typhi* (0.37 mg/mL) which confirmed the antimicrobial activity of lupeol (13). Pheophytin had exhibited *in vitro* antimicrobial action against fungal and bacterial standard strains (14). This confirmed the potency of Phaeophytin *a* in this study against the microorganisms tested. The result of MFC showed that Phaeophytin *a* was most potent against *C. albicans* (0.1875 mg/mL) confirmed that pheophytin *a* possess strong antimicrobial activity against *C. albicans* (ATCC 90028) and *C. albicans* (ATCC 76615) (14). Pheophytin *a* had also exhibited antimicrobial action against various microorganisms tested (15). α- and β-amyrin and amyrin acetate derivatives have exhibited potential antifungal activity against *Candida* spp (16). The pentacyclic triterpenoids, α -amyrin and β -amyrin, and lupeol have showed antibacterial activity against *S. aureus* and *E. coli* (17). In general, α-, β-amyrin have proved to be active by reducing bacterial viability < 20 % (18). α- and β-amyrin had inhibited the growth of *Bacillus subtilis, Staphylococcus aureus*, *Enterococcus faecium* and *Staphylococcus saprophyticus* (19).

CONCLUSION

Phaeophytin *a*, α-amyrin, and lupeol isolated from *Brachystelma togoense* were screened against *S. aureus, E. coli, S. pneumoniae, S. typhi,* and *C. albicans* using Ciprofloxacin and Terbinafine as standards. The results of activity of these isolates supported the ethnomedicinal uses of this plant in the treatment of skin infections, abdominal

disorders, gonorrhea, cough, cold, and typhoid fever. Though isolation and characterization of these compounds have been reported (10), this is the first report of antimicrobial activity of this genus. The activities of these phytoconstituents in *B. togoense* justified ethnomedicinal uses of the plant.

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