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Research Article

NEW DERIVATIVE OF LOPHIRONE A FROM THE ROOTS OF *ALLEXIS BATANGAE* (VIOLACEAE) AND EVALUATION OF ANTIBACTERIAL, ANTIOXIDANT AND ANTIPLASMODIAL ACTIVITIES

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ABSTRACT

In the search for new compounds endowed with antibacterial, antioxidant and antiplasmodial properties in the Cameroonian pharmacopoeia, new was established using spectroscopic analysis techniques, isolated from the ethyl acetate fraction of the antibacterial, antioxidant and antiplasmodial activities of this compound was assessed in this study very responsive to the chloroquine-sensitive *Plasmodium falciparum* strain 3D7, but a weak antioxydant activity.

INTRODUCTION

Allexis batangae belonging to the family *Violaceae*, is commonly Cameroon [1]. Violaceae plants have been constantly used in traditional medicine to treat many diseases caused by pathogenic agents. The bark of *Allexis cauliflora* used to treat fever and syphilis [2,3]. *Allexis* genera has been reported as matrix metalloproteinase inhibitors [4], antioxidants [5] and antiplasmodials [6]. No phytochemical study has been published on *Allexis batangae* to date despite the ethnobotanical use of some plants of the genus allexis. In the present study, we report the isolation and structural elucidation of a new named 4, 4'''-dimethoxylophirone A (**1**) which was extracted from the roots of We also the antibacterial, antiplasmodial and antioxidant activities of this compound.

RESULTS AND DISCUSSION

The fraction of roots was purified by column chromatography on silica gel. A flavonoid and a mixture of compounds were obtained. flavonoid was obtained white powder in the solvent mixture CH_2Cl_2 / MeOH (30/1). The compound presented the base peak of the molecular ion at m/z 561.1356 $[M + Na]^+$, (calcd. For 561.1360) in HRESI/MS, corresponding to the formula $C_{32}H_{26}O_8Na$. The UV spectrum showed absorption bands at ƛmax 243, 247 and, suggesting an isoflavonoid nucleus [7,8,9]. The ^{13}C NMR spectrum associated with DEPT showed a total of 32 carbons, including 2 carbonyl groups

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(δ=206.4 and 173.2), 11 quaternary sp^2 carbons of which 5 were attached to oxygen, 15 sp^2 tertiary carbons and 4 sp^3 hybridized carbons highlighting the skeleton of a biflavonoid. In the ^1H NMR spectrum, 14 aromatic protons, 1 vinyl proton, 2 methynic protons linked to sp^3 carbons and two methoxy proton groups were observed. The COSY spectrum ^1H-^1H, analysis suggested the presence of 1,4-disubstitutedaromatic chains and two 1,2,4-trisubstituted aromatic rings. Furthermore, the singlet δ_H = 8.27., was characteristic of the proton H-2 of isoflavones [13]. The protons of methoxy groups were observed at 3.65 and 3.90, as well as the protons of the AB system at 5.98 and 4.65 (d, J = 12.5 Hz). The above information coupled with the presence of the 2 methynes at δ=4.3 and, and the two carbonyls at 175.6 and 203.3, suggested that the isolated flavonoid could be a derivative of lophirone A [13]. From the HMBC spectrum, correlations were detected between carbonyl at (C-c_1) and protons at 7.91 (H-6), (H-b_1) and 6. (H-a_2); between the same protons and the carbon atoms at δc = 206.4 (C-c_2), 54. (C-b_2), and (C-1 " and C-1 '"). Moreover, the carbonyl at 206.4 (C-c_2) correlates with the protons at 4.70 (H-b_2) and 6.11 (H-a_2), as well as with the aromatic protons H-6 (δH =). Proton H-b_2 correlates with carbons (C-2'' and C-6'') and carbons (C-2'"and C-6'"). Additionally, correlations observed between C-4'"(158.6) and the protons of the aromatic ring at δ_H = 7. and 6. (H-2'", H-6 '", H-3" and H-5'") respectively, as well as with the protons of the methoxy group at δ_H = 3., between the protons of MeO at 3. and C-4 (δC=164.1) suggested that the structure of biflavonoid could correspond to that of 4,4 '"- dimethoxylophirone (fig1).

Position	δCmult	δH m, J(Hz)	HMBC
B_1-1	109.1	-	-
2	158.0	-	-
3		6.72 (d ; 2.5)	C-1 ; C-5
4	164.1	-	-
5		6.86 (dd ; 2.5 ; 9)	C-1 ; C-3
6	127.6	7. (d ; 9)	C-2 ; C-4 ; c_1
c_1	175.6	-	-
a_1	121.5	-	-
b_1	157.0	(s)	C-2 ; c_1 ; a_1 ; a_2
B_2-1'	118.1	-	-
2'	165.5	-	-
3'		6,14 (d ; 2)	C-1' ; C-2' ; C-4' ; C-5'
4'	164.4	-	-
5'	103.2	6, (dd ; 2 ; 9)	C-1' ; C-3'
6'	134.0	6.17	
c_2	203.3	-	-
a_2		(d ; 12.5)	b_1 ; b_2 ; c_2 ; a_1 ; C-1'' ; C-1'''
b_2		4. (d ; 12.5)	C-1'' ; C-2'' ; C-1''' ; C-2''' ; c_2
A_1-1''	134.3	-	-
2''	129.0	7.27 (d ; 8.5)	C-3''' ; C-4'' ; b_2
3''	114.7	6.63 (d ; 8.5)	C-1'' ; C''-4''
4''	156.6	-	-
5''	114.6	6.63(d ; 8.5)	C-1'' ; C-3'' ; C'-4''
6''	128.0	7.29(d ; 8.5)	C-2'' ; C-4'' ; b_2
A_2-1'''	134.5	-	-
2'''	131.3	7. (d ; 8.5)	C-3''' ; C-4''' ; b_2
3'''	120.0	6.60 (d ; 8.5)	C-1''' ; C-4'''
4'''	158.6	-	-
5'''	117.9	6.60 (d ; 8.5)	C-1''' ; C-3''' ; C-4'''
6'''	130.0	6. (d ; 8.5)	C-2''' ; C-4''' ; b_2
4'''-OCH_3		3. (s)	-
4-OCH_3		3.	-
OH	-	-	-
OH	-	-	-

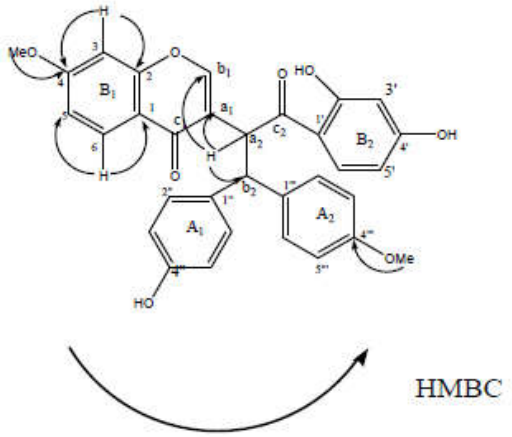

Figure 1 4,4 '"- dimethoxylophirone

Nine strains of Gram-negative bacteria including reference strains (ATCC) and multi-resistant clinical isolates were used. These strains belonging to different bacterial species are distributed as follows:

- A strain of Escherichia coli (ATCC8739)
- - A strain and three clinical isolates of Enterobacter aerogenes (ATCC13048; CM64; EA289; EA294)
- Two clinical isolates of Enterobacter cloacae (BM67, K2)
- A clinical isolate from Providencia stuartii (PS299645)
- A strain of Klebsiella pneumoniae (ATCC11296)

Table 2. Results of Antibacterial activities of compound I and EA extract

When the Minimum bactericidal concentration (MBC) of an antibiotic on a given strain is close to the Minimum Inhibitory Concentration (MBC/ MIC = 1 or 2), the antibiotic is said to be bactericidal, in contrast, if these values are relatively distant (4 <MBC / CMI> 16), the antibiotic is said to be bacteriostatic. Finally, if MBC / CMI> 32, we speak of "tolerance" of the microbial strain.

Results indicated in Table 2 shows that the MBC / CMI ratio of the root extract EA varies from 1 to 2 for strains CM64, K2, ATCC13048, EA289, ATCC11296. While, the new biflavonoid shows that the MBC / CMI ratio varies from 1 to 2 for strains CM64, EA289 and ATCC11296, with the same values that the EA extract on these strains; we can therefore suspect that this biflavonoid is the active substance contained in the acetate extract with respect to these pathogens.

In order to evaluate the antioxidant activities, two tests were implemented, DPPH assay to evaluate radical- scavenging activity and the Ferric reducing-antioxidant power (FRAP) assay for assessing reducing power.

Table3 Results Antioxidant activities of compound I

	Radical activity	**scavenging**	**Reducing power**
	SC_{50}()	EC_{50}()	FRAPµg EAA/mgdw
1	06.50±0.48	181.50	121.65±3.5421
AA	04.50±0.28	112.50	206.48±12.33

AA= Ascorbic Acid

This table that compound 1 (06.50±0.48) has a weaker DPPH radical scavenging activity compared to the standard reference ascorbic acid (SC_{50} = 4.50 mM). The principle used for the reductive power test is based on the reduction of the Fe^{3+} ions in potassium ferricyanide to Fe^{2+}, leading to the formation of a blue color complex. analytes with high reducing power, the concentration of the complex formed is and absorbance values high, and vice versa. The high antioxidant activity of phenolic substances is often attributed to their -OH moieties [10]. They are good proton donors because of the delocalization of electrons (phenols which) along the molecule. This fact can explain why this biflavonoid has a weak antioxydant activity, because some -OH groups are methylated.

We evaluated antiplasmodial activity against the chloroquine-sensitive Plasmodium falciparum strain 3D7 for compound 1 and EA extract.

Table 4 Results of antiplasmodial activities of compound.

Compounds	IC_{50} (3D7 strain P. falciparum)
1	10.57±1.44 µM
Chloroquine	0.006±0.3 µM

The antiplasmodial activity of compound 1 could be explained by the presence of a methoxy group on C-4 and C-4'''. This group enhances the lipophilicity of the substance, improving its incorporation into cells [10].

In conclusion, one new compound was isolated from the ethyl acetate extract of Allexis batangae roots. In this study, we evaluated three types of activities namely, antibacterial, antioxidant and antiplasmodial on compound . It exhibited good antiplasmodial activity against the chloroquine-sensitive falciparum strain 3D7 (IC_{50} =10.57±1.44 µM) but, did not show strong antioxidant. The antiplasmodial properties of compound 1 support the ethno-medicinal use of roots in the treatment of malaria.

Experimental

General experimental procedures

The UV spectra were recorded on UV-570/VIS/NIP. 1D (1H, 13C, DEPT) and 2D (COSY, NOESY, HSQC and HMBC) NMR spectra were recorded in DMSO-d6 and MeOH-d4 using a Bruker 600 (600 MHz for 1H NMR, 150 MHz for 13C NMR) spec TMS as internal standard at room temperature. The EIMS spectra were obtained at 70 eV in a VG Autospec apparatus. Flash column chromatography was performed using silica gel 60 (Merck, 0.040–0.063 mm). TLC was conducted on pre-coated Merck Kieselgel 60 F254 plates (20 _ 20 cm, 0.25 mm). Visualization of the compounds on the chromatographic plates was made under ultraviolet light, exposure to iodine vapor or spraying with 50% diluted sulphuric acid in water, followed by gentle heating.

Plant material

was collected on 7th June 2014 at Bidou II, 20 km from the town of Kribi (South Cameroon) under the leadership of M. NANA (Botanist). The identification was carried out at the National Herbarium of Cameroon by M. NANA in comparison with specimen number 31839 / HNC. Identified in Gabon and southern Cameroon (Kribi) in the Kienke forest, it is a small Shrub up to 6 m tall, with a pale brown smooth stem and small leaves. The flowering is done on the stem. It has pedicel fifteen millimeters long (Achoundong and Onana, 1998).

Extraction and isolation

Dried and powdered of Allexis batangae (1 kg) extracted with MeOH (3L) at room temperature and evaporated to yield a crude extract (124 g). 100g of crude extract was dissolved in MeOH-H_2O (8/2) and partitioned with n-hexane (3×200 mL) and ethyl acetate (3×200 mL). Ethyl acetate portion (22g) was column chromatography over silica gel eluting with gradients of CH_2Cl_2/MeOH to produce 95 fractions of 250 mL each. These fractions were grouped according to their TLC profiles into 3 major fractions: A (1.68 g, 1–67); B (1.46 g, 68–80); C (1.44 g, 80–95). Fractions A (CH_2Cl_2/MeOH 50:1); B (CH_2Cl_2/MeOH 40:1); C (CH_2Cl_2/MeOH 30:1). Fraction C (CH_2Cl_2/MeOH 30:1) was purified by silica gel column chromatography with a gradient of CH_2Cl_2/EtOAC (20/1) to yield compound 1 (32 mg).

4,4'''-dimethoxylophorone A

White powder; IR (KBr) ymax3320, 1620, 1656, 1570, 1514, 1456 and 1231 cm^{-1}; ^1H and ^{13}C NMR data (, 600 and 150 MHz) see Table1; positive HRESI/MS m/z 561.1356 $[M+ Na]^+$ (calculated for $C_{32}H_{26}O_9Na$).

In vitro evaluation of the antibacterial activity of the crude extracts

7.6 g of Mueller Hinton Agar (MHA) was dissolved in 200 mL of distilled water and then heated on autoclave at 121 ° C for 30 min. After cooling the mixture was poured into the petri dishes near the beak of Bunsen.

Liquid medium

*13.65 g of Mueller Hinton Broth (MHB) were dissolved in 650 ml of distilled water. A part of this medium was distributed in tubes of (10.*per tube which will be used for inocula). Another part was distributed in the 2 mL tubes (1.7 mL per tube for the dilution of the extracts). These tubes and the rest ofmedium were heated in an autoclaveat 121°C for 30 min.

Culture of bacterial strains

The different bacterial strains were subcultured by the method of the streaks on MHA agar medium poured into the Petri dishes. The petri dishes were introduced into the incubator at 37 ° C. for 18 hours in order to obtain a young culture and isolated colonies. The isolated colonies were used to prepare the inoculum.

Preparation of the inoculums

Using a sterile platinum loop, a few colonies of bacteria from each strain were taken from the activation medium and each introduced into a tube containing a sterile physiological solution (0.9% NaCl). The contents of each tube were homogenized using the vortex in order to obtain a turbidity comparable to the standard scale of Mc Farland (Table 1) corresponding to the concentration of 1.5. 108 CFU / mL. Subsequently, 147 µl of the resulting suspension was removed and introduced into 10.85 mL of MHB for a volume of 11000 mL of an inoculated medium at 2.10^6 CFU / mL.

Evaluation of antioxidant Activity and Antiplasmodial Activity

DPPH Radical-Scavenging Activity

Evaluation of the ability of 1 to scavenge DPPH free radicals was according to the method of Brand-williams *et al*., 1995. 5mL aliquot of the sample solution was mixed with 2 mL of DPPH (0.04 mM in methanol). Thereafter, the mixture was homogenized for 30 minutes. In order to evaluate the activity, the absorbance of the mixture was measured according to the principle, low absorbency implying efficient free radical scavenging and vice versa. As a standard, we used ascorbic acid in the same concentration. Radical scavenging activity was evaluated as the percentage of inhibition according to the following equation: % inhibition = [((absorbance of control– absorbance of sample)/absorbance of control)] 100.

Evaluation of Reducing Power

The reducing powers of compounds 1 was evaluated according to the method of Benzie and Strain (1999). In a test tube we introduce1.0 mL of sample solution, 2.5 mL of phosphate buffer (2 M, pH 6.6) and 2.5 mL of 1% (m/v) $K_3[Fe(CN)_6]$. The whole was incubated for 20 min. Thereafter, 2.5 mL of trichloroacetic acid (10% m/v) was added, and the solution was centrifuged at 4000 rpm for 20 min. A 2.5 mL aliquot of the supernatant was mixed with 2.5 mL of ultra-pure water and 0.5 mL of ferric chloride (0.1%). The absorbance of this mixture was measured at 700 nm. The activity of an ascorbic acid solution was used for normalisation.

Antiplasmodial Assays

The culture of *P. falciparum*and antiplasmodial test were carried out as previously described (Frédérich and Prosperi, 2001; Jonville *et al*., 2008). The host cells were human red blood cells (A or Rh+). The culture medium was RPMI 1640 (Gibco) containing 32 mM $NaHCO_3$, 25 mM HEPES and 2.05 mM L-glutamine. The medium was supplemented with 1.76 g/l of glucose (Sigma-Aldrich), 44 mg/ml of hypoxanthin (Sigma-Aldrich), 100 mg/l of gentamycin (Gibco), and 10% human pooled serum (A or O Rh+). Parasites were subcultured every 3–4 days with initial conditions of 0.5% para-sitaemia and 1% hematocrit. The EtOAC extract and pure compound were evaluated in vitro for their activity against *P. falciparum*(3D7).
Abbreviations

MBC: Minimum bactericidal concentration
MIC: Minimum Inhibitory Concentration
SC50= Scavenging concentration 50, indicate the concentration of a given extract which stabilize 50% DPPH available in test tubes given their instability.
MBC/MIC> 4: the extract or compound is bacteriostatic
MBC/MIC <4: the extract or compound is bactericidal
EC50: Effective concentration 50, which indicates the concentration of a given extract which reduces by 50% the initial concentration of DPPH in the reaction mix.
EAA/mgdw= Equivalent ascordic acid per milligram weight of tested sample

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