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Research Article

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Determination of the Antimycobacterial Activity of the Ethyl Acetate Fractions and Isolated Compounds from the Roots of *Allexis batangae* and *Allexis obanensis*

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ABSTRACT

The ethyl acetate extracts (AcOEt) obtained from the fractionation of the methanol (MeOH) extract of Allexis obanensis, Allexis batangae and two compounds, 7-hydroxy-3-(3-hydroxy-4-methoxyphenyl)-5-methoxy-4H-chromen-4-one and 4,4'''-dimethoxylophirone A, respectively isolated from these plants were tested against two species of Mycobacterium. The minimum inhibitory concentration (MIC) of the Allexis obanensis ethyl acetate fractions and Allexis batangae ranged from 0.78 to 1.3 mg/mL against a non-pathogenic strain of mycobacteria, M. smegmatis. Allexis batangae showed the best activity, with a MIC of 0.78 mg/mL and a minimal bactericidal concentration (MBC) of 1.560 mg/mL against M. smegmatis. A drug susceptible M. tuberculosis strain was found to be sensitive to Allexis obanensis extracts of Allexis batangae. (MIC 0.78 and 0.80 mg/mL respectively) when using the BACTEC rapid radiometric method. The MIC of 4,4 '"- dimethoxylophirone A against M. tuberculosis was 100 µg/mL, whereas 7-hydroxy-3-(3-hydroxy-4-methoxyphenyl)-5-methoxy-4H-chromen-4-one had a MIC of 50 µg/mL.
Keywords: *Allexis obanensis; Allexis batangae; M. smegmatis; M. tuberculosis*

INTRODUCTION

Outbreaks of M. tuberculosis infections including those caused by MDR-TB strains, are a major global concern. In addition, we have not seen any new anti-TB drugs in the last 40 years, and there is a clear need for new and effective anti-tuberculosis drugs. As the number of patients suffering from tuberculosis (TB) is increasing daily, with the emergence of multidrug-resistant cases (MDR) has led to the search for new anti-tuberculosis drugs. Phytochemical analyses of essential oils, glycolipids, sesquiterpenoids, terpene triterpenoids, steroids, tannins and triterpenoids with potential antimycobacterial activity have been reported [1]. Previous studies of isolated compounds such as naphthoquinones (diospyrin, isodiospyrin, mamegakinone, 7-methyljuglone, neodiospyrin and shinanolone) have been shown to have antimycobacterial properties [2,3]. Mycobacterium tuberculosis is a complex and resilient organism. It is worth mentioning that new drugs can reduce the six-month duration of TB treatment and can be effective against drug-resistant strains of mycobacteria. Several bioactive compounds that have shown antimicrobial activity come from plants. The search for biologically active extracts based on traditional plant use is relevant because of the emergence of microbial resistance to many antibiotics and the occurrence of life threatening opportunistic infections.

In this study, the antimycobacterial activity of the compounds isolated from the ethyl acetate fractions of the roots of *Allexis batangae* and *Allexis obanensis* was evaluated using two species of Mycobacterium [4-7].

MATERIALS AND METHODS

Plant Material:

Allexis batangae and *Allexis obanensis* were collected on 7th June 2014 at Bidou II, 20 km from the town of Kribi (South Cameroon) under the leadership of Mr. NANA (Botanist). NANA in the National Herbarium of Cameroon did a comparison with specimen number 31839/HNC and 49778/HNC for *Allexis batangae* and *Allexis obanensis* respectively.

Extraction and Isolation

Dried and powdered roots of *Allexis batangae* and *Allexis obanensis* (1 kg) were extracted with MeOH (3 L) and are available in MeOH-H_2O and partitioned with *n*-hexane (3×150 mL) and ethyl acetate (3×200 mL). From the ethyl acetate fraction of each plant, we isolated respectively 4,4’’’-dimethoxylophirone A and 7-hydroxy-3-(3-hydroxy-4-methoxyphenyl)-5-methoxy-4H-chromen-4-one. Ciprofloxacin and isoniazid (INH) were provided by the "Centre Pasteur" as well as the drug-susceptible *M. tuberculosis* strain, H37Rv (ATCC 27264) and *M. smegmatis (Mc2155)* [7-10].

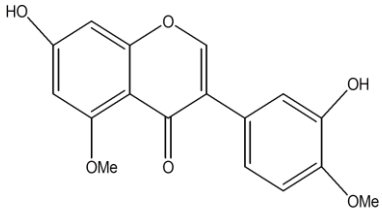

Figure 1: 7-hydroxy-3-(3-hydroxy-4methoxyphenyl)-5-methoxy-4H chromen-4-one.

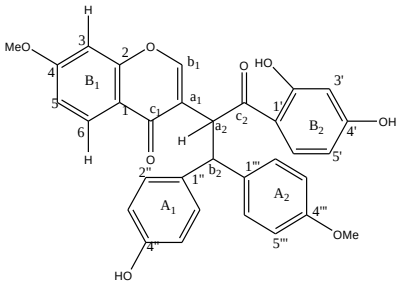

Figure 2: 4,4’’’-dimethoxylophirone A.

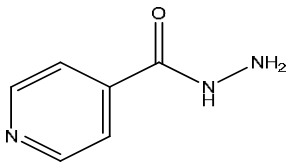

Figure 3: Isoniazid (INH).

Table 1: Spectroscopic data of 4,4'''-dimethoxylophirone A

Position	δC mult	δH m, J(Hz)
B_1-1	109.1	-
2	158	-
3	102.3	6.72 (d; 2.5)
4	164.1	-
5	117.7	6.86 (dd; 2.5; 9)
6	127.6	7.94 (d; 9)
c_1	173.2	-
a_1	121.5	-
b_1	157	8.14(s)
B_2-1'	118.1	-
2'	165.5	-
3'	102.3	6,14 (d; 2)
4'	164.4	-
5'	103.2	6,38 (dd; 2; 9)
6'	134	6.17
c_2	206.4	-
a_2	44.4	6.11 (d; 12.2)
b_2	53.4	4.70 (d; 12.2)
A_1-1''	134.3	-
2''	129	7.27 (d; 8.5)
3''	114.7	6.63 (d; 8.5)
4''	156.6	-
5''	114.6	6.63(d; 8.5)
6''	128	7.29(d; 8.5)
A_2-1'''	134.5	-
2'''	131.3	7.32 (d; 8.5)
3'''	120	6.60 (d; 8.5)
4'''	158.6	-
5'''	117.9	6.60 (d; 8.5)
6'''	130	6.65 (d; 8.5)
4'''-OCH_3	53.6	3.67 (s)
4-OCH_3	53.9	3.71
OH	-	-
OH	-	-

Table 2: Spectroscopic data of 7-hydroxy-3-(3-hydroxy-4methoxyphenyl)-5-méthoxy-4H.

Position	^{13}C ppm	^{1}H ppm
2	148,8	8,19 s
3	125,1	
4	182,6	
4a	106,7	
5-OCH_3	164,9	
6	100,9	6,42 s
7-OH	163,8	
8	95,5	6,28 s
8a	160,0	
2’	148,8	6,93 d (1H, *J*=1,9)
3’-OH	146,6	
4’-OMe	149,0	
5’	146,6	6,88 d (1H, *J*=7,9)
6’	115,4	7,5 dd (1H, *J*=8,2-1,9)
OCH_3	55,1	3,9 s (3H)
OCH_3	55,0	3,91 s (3H)

Microplate Susceptibility Test against *M. smegmatis*

To test for the antimycobacterial activity, the ethyl acetate fractions and the isolated compounds were tested against *M. smegmatis* using the microplate dilution method. Each sample was dissolved in 10 % DMSO in a sterile Middlebrook 7H9 broth base to obtain a basal concentration of 100.0 mg/mL and 0.5 mg/mL respectively. Dilution series of the fractions and isolated compounds to be evaluated were performed with 7H9 broth to give volumes of 100.0 µL with final concentrations ranging from 25.0 to 0.195 mg/mL. Ciprofloxacin at a final concentration of 0.156 mg/mL served as a positive drug control [11-13].

Rapid radiometric anti-tuberculosis assay using *M. tuberculosis*

The radiometric respiratory system with the BACTEC device was used for *M. tuberculosis* susceptibility testing. The bacterial cultures used in this study were obtained from specimens received from the "Centre Pasteur". A drug-susceptible strain of *M. tuberculosis*, H37Rv, collection (ATCC), 27294, was used in the screening procedure. Each sample was dissolved at 10.0 mg/mL in DMSO and added to 4.0 mL of BACTEC 12B broth to reach final concentrations of 5.0-0.05 mg/mL (in triplicate, one with PANTA and two without PANTA, an antimicrobial supplement). The BACTEC drug susceptibility test was also performed for the primary INH drug at a concentration of 0.2 µg/mL against the H37Rv sensitive strain (Figures 1-3).

Direct biological test

The isolated compounds were evaluated on TLC plates by applying a small spot of 20.0 µL on silica gel 60 PF254 plates. The plates were developed in the Hex/EtOAc system (7:2) and dried thoroughly. The 24 hours of *M. smegmatis* (1.26 x 10 8 CFU/mL) in 7H9 broth was centrifuged at 1000 rpm for 15 minutes. The supernatant was discarded and the pellet was resuspended in fresh 7H9 broth. A fine spray was then used to apply the bacterial suspension to the TLC plates [4]. The plates were then incubated at 37 °C for 24 hours under humid conditions. After incubation, the plates were sprayed with 2.0 mg/mL INT and inhibition zones were noted.

RESULT AND DISCUSSION

Table 3: Antimycobacterial activity of the Ethyl acetate fractions and isolated compounds from roots of *A. batangae* and *A. obanensis* against *M. smegmatis* and *M. Tuberculosis.*

Tested samples	*M. smegmatis*		*M. tuberculosis*	
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	ΔGI
AcOEt fraction of *A. batangae*	0.78	1.56	1.20(S)	0.0 ± 0.0
AcOEt fraction of *A. obanensis*	0.8	1.62	1.3 (S)	0.0 ± 0.0
4,4'''-dimethoxylophirone A	0.03	0.12	0.10 (S)	8.0 ± 2.8
7-hydroxy-3-(3-hydroxy-4-méthoxyphenyl)-5-methoxy-4H-chromen-4-one	0.12	0.06	0.05 (S)	2.0 ± 1.4
Ciprofloxacin (positive drug control for *M. smegmatis*)	0.15	0.31	nd	nd
Isoniazid (positive drug control for *M. tuberculosis*)	nd	nd	2 x 10^{-4} (S)	13.0 ± 0.7

MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration; ΔGI: value (mean ± SD) of the control vial (10), 38.0 ± 3.8 for the sensitive strain; S: Susceptible; nd: Not determined.

In the present study, acetate fractions and compounds exhibited inhibitory activity. The *Allexis batangae* acetate fraction was found to be active with MIC values of 0.78 mg/mL and 1.2 mg/mL respectively against *M. smegmatis* and M. tuberculosis and a MBC of 1.56 mg/mL against *M. smegmatis*. The *Allexis obanensis* acetate fraction was found to be active with MIC values of 0.80 mg/mL and 1.3 mg/mL respectively against *M. smegmatis* and M. tuberculosis and a MBC of 1.62 mg/mL against *M. smegmatis*. The antituberculous activity of 4,4 '"-dimethoxylophirone A showed an activity at 0.03 and 0.10 mg/mL against M. tuberculosis and a MBC value of 0.10 against *M. smegmatis*, respectively. 7-hydroxy-3-(3-hydroxy-4-methoxyphenyl)-5-methoxy-4H-chromen-4-one had bactericidal effects at 0.06 mg/mL against *M. smegmatis* and MIC values of 0.12 and 0.05 respectively against *M. smegmatis* and M. tuberculosis respectively (Tables 1-3).

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