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(4-METHOXY-BENZO[1,3]DIOXOL-5-YL)-PHENYLMETHANONE: AN  
ANTIBACTERIAL BENZOPHENONE FROM *SECURIDACA*  
*LONGEPEDUNCULATA*

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## Abstract

The dichloromethane extract of *Securidaca longepedunculata* Fresen yielded a benzophenone, (4-methoxy-benzo[1,3]dioxol-5-yl)-phenylmethanone (**1**), together with other three known compounds, 1,7-dihydroxy-4-methoxyxanthone (**2**), benzyl 2-hydroxy-6-methoxybenzoate (**3**), and methyl 2-hydroxy-6-methoxybenzoate (**4**). Compound (**1**) exhibited antibacterial activity against *Pseudomonas aeruginosa*, and antifungal activity against *Aspergillus fumigatus*. Compound **2** exhibited antibacterial activity against *Staphylococcus aureus*, and antifungal activity against *Aspergillus niger*, *Aspergillus fumigatus* and a *Penicillium* species. Compounds **3** and **4** were inactive against all the tested bacteria and fungi. These results provide proof of efficacy of the extracts of *S. longepedunculata* in treating bacterial infections, but further studies should be carried out to ascertain these results with respect to the widely reported traditional use.

**Key words:** *Securidaca longepedunculata*, Antimicrobial, Benzophenone, (4-Methoxy-benzo[1,3]dioxol-5-yl)-phenylmethanone

## Introduction

*Securidaca longepedunculata* Fresen (Polygalaceae) is a tree or shrub, which occurs, predominantly, in tropical Africa where it is widely used for medicinal purposes. In Tanzania, the plant is known as *Mlyangabako* by the Hehe of Iringa, and *Masukemengi* by the Zigua of Tanga. Information obtained during interviews with healers in the two regions shows that in Tanga it is used for the management of some manifestations of non-insulin dependent diabetes, while in Iringa it is used for the treatment of gonorrhea and malaria. This plant is also used in combination with other plants for the management of opportunistic infections among HIV patients. Information

from the literature indicates that a decoction of the dried bark is used to treat bacterial infections (de Tommasi et al., 1993; Arnold and Gulumian, 1984), inflammation (de Tommasi et al., 1993), insanity and epilepsy (Mathias, 1982). The leaves are used for treating wounds and sores, cough, venereal diseases, snake bite and as a purgative (Chhabra et al., 1991; Hedberg et al., 1983). They are also used to treat tuberculosis (Asres et al., 2001), bilharzia (Kamwendo et al., 1985), rheumatism (Akah and Nwambie, 1994; Asres et al., 2001), skin diseases (Odebiyi, 1978), headache and mental illness (Msonthi and Magombo, 1983), including convulsions in children (Sofowora, 1980). The root decoction is used to hasten labour (Kokwaro, 1976; Yu, 1982), to treat malaria (Chhabra et al., 1991), rheumatism (Kloos et al., 1978), gonorrhea, palpitations, pneumonia, syphilis (Desta, 1993), and asthma (Akah et al., 1997).

This work was done as part of ongoing initiatives to validate claims on plants that are being used by traditional healers for the management of opportunistic infections among HIV/AIDS patients.

## **Materials and Methods**

### **Plant preparation**

The fresh root barks of *S. longipedunculata* were collected from Iringa district, in April 1999. Identification and authentication was done by Mr. F. Mbago of the Department of Botany, Faculty of Science, University of Dar es Salaam, and the voucher specimen was deposited at the Herbarium of the Institute of Traditional Medicine, Muhimbili University College of Health Sciences.

### **Phytochemical screening**

The powdered, air dried root bark (500 g) was extracted consecutively for 48 h at room temperature with petroleum ether, dichloromethane and ethanol and the extracts dried *in vacuo*. The dichloromethane extract (8.81 g) was further fractionated by vacuum liquid chromatography (VLC) over silica gel, eluting with n-hexane containing increasing amounts of ethyl acetate yielding 11 fractions. The first two fractions (ethyl acetate:n-hexane 2.5:97.5; 10:90) contained less polar compounds that could not be separated using the available chromatographic materials. VLC fraction 3 was subjected to column chromatography eluting with 2.5% ethyl acetate in petroleum ether to give compound **3** (13.3 mg) as a yellow oil and compound **4** (17.7 mg) as a pale yellow oil. TLC analysis indicated that VLC fractions 4 and 5 contained a similar compound as a major constituent. Thus, column chromatography on each fraction separately eluting with 2.5% ethyl acetate in petroleum ether yielded compound **1** (143 mg). Compound **2** (29.5 mg) was obtained by column chromatography from VLC fraction 7 eluting with petroleum ether and from fraction 8, eluting with 10% ethyl acetate in petroleum ether. Purification was achieved by recrystallization from petroleum ether.

### **Identification of compounds**

Infrared spectra were recorded on a Shimadzu IR-435 spectrophotometer. Samples were prepared as chloroform solutions. Mass spectra were recorded under

chemical ionization conditions at 70 eV.  $^1\text{H}$  NMR spectra were recorded on a Bruker spectrometer operating at 300 MHz using  $\text{CDCl}_3$ ,  $\text{CD}_3\text{OD}$  or DMSO as solvents. Chemical shifts are given in  $\delta$ -values relative to internal standard TMS ( $\delta = 0$ ).  $^{13}\text{C}$  NMR spectra were recorded on a spectrometer operating at 75 MHz and  $\text{CDCl}_3$ ,  $\text{CD}_3\text{OD}$ .

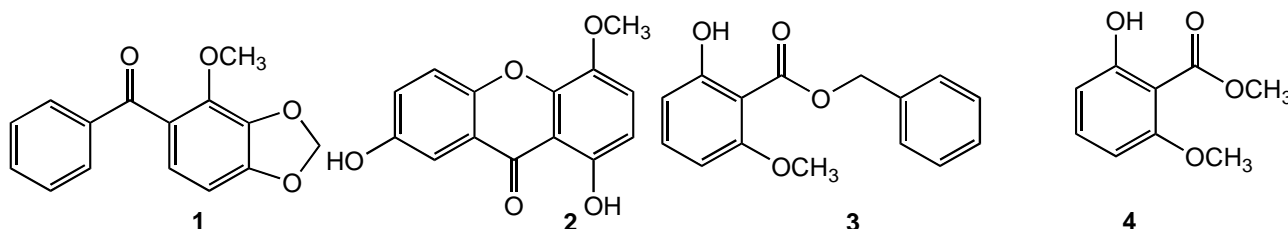
### Antimicrobial tests

*Staphylococcus aureus* (NCTC 6571), *Escherichia coli* (NCTC 10418), *Pseudomonas aeruginosa* (NCTC 0031), and 4 fungi, *Candida albicans* (Strain HG 392), local strains of *Aspergillus niger*, *Aspergillus fumigatus* and a *Penicillium* species were generously supplied by the Department of Microbiology and Immunology, Muhimbili University College of Health Sciences. Testing was done using the disk diffusion method (Singh et al., 2002). Thus, filter paper discs (Whatman No. 1; 5 mm diameter) were impregnated with crude extracts (10 mg/disc) or standard drugs (20  $\mu\text{g}$ /disc ampicillin, 10  $\mu\text{g}$ /disc gentamicin; for bacteria) and ketoconazole (20  $\mu\text{g}$ /disc; for fungi), and the discs overlayed on Mueller Hinton agar plates (for bacteria) or Saboraud's dextrose agar plates (for fungi). The plates were incubated at 37 °C, for 24 h in the case of bacteria and *Candida* and for 48 h in the case of the other fungi. The discs were tested in triplicate, including one with a solvent blank and 3 for the standard drugs. Inhibition zones were calculated as the difference between disc diameter (5 mm) and the diameters of inhibition (Hewitt and Vincent, 1989). The mean inhibition zones were used to calculate the Activity Index (AI). The Activity index was calculated as the mean inhibition zone for test sample divided by the mean inhibition zone for the standard drug (Singh et al., 2002).

### Results

The Petroleum ether extract exhibited antifungal activity against *Candida albicans*, *Aspergillus fumigatus* and *Aspergillus niger*, while the dichloromethane extract was active against *Aspergillus fumigatus*, *Aspergillus niger* and the *Penicillium* spp. (Table 1). Repeated column chromatography of the dichloromethane extract yielded a new compound **1**, as a creamy oil at room temperature that crystallized on standing in cold conditions. Structural determination was achieved by employing MS,  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR Spectra, DEPT, HMQC and HMBC experiments and by comparison with structures of similar compounds reported in the literature (Hesse et al., 1997). Thus, the  $^1\text{H}$  NMR spectrum of **1** consisted of three signals in the low field region at positions  $\delta$  7.81 (d,  $J = 7.4$  Hz), 7.57 (t,  $J = 7.7$  Hz) and 7.45 (t,  $J = 7.7\text{Hz}$ ) due to protons H-2'/H-6', H-4' and H-3'/H-5', respectively. The low field positions being attributed by the anisotropic effect of the carbonyl group. It also showed signals at positions  $\delta$  3.86 (s) and 6.05 (s) due to  $\text{CH}_3\text{O}$ -4 and H-2, respectively. The  $^{13}\text{C}$  NMR spectrum of **1** showed absorptions at  $\delta$  60.4 and 102 due to  $\text{CH}_3\text{O}$ -4 and C-2 respectively. It also showed four signals of the monosubstituted benzene ring, which are comparable to the ones reported in the literature for a benzophenone (Hesse et al., 1997). The HMBC experiment showed long range correlations between carbon C-1 and the dioxymethylene protons (O- $\text{CH}_2$ -O) which appeared at  $\delta$  6.05 (s). A signal due to protons of the methoxy group ( $\text{CH}_3\text{O}$ -4)  $\delta$  3.86 showed long range correlations with C-4,  $\delta$  142.7 while signals due to protons H-6 and H-2' exhibited long range correlations

with the carbonyl carbon (CO),  $\delta$  195.6. The Mass Spectrum of **1** consisted a molecular ion peak at  $m/z$  256 which corresponds to the formula  $C_{15}H_{12}O_4$ . The other significant peak in the spectrum is at  $m/z$  179 amu which corresponds to  $[M-C_7H_5O]$  followed by the loss of 15 amu ( $[M-C_7H_5O]-CH_3$  at  $m/z$  164. Hence the structure was concluded to be (4-Methoxy-benzo[1,3]dioxol-5-yl)-phenylmethanone (**1**). Cream oil. 143 mg. Unreactive with anisaldehyde spraying agent followed by charring at  $\sim 150^\circ C$ . IR, ( $CHCl_3$ )  $\nu_{max}$ : 2917, 1653, 1465, 1341, 1278, 1070  $cm^{-1}$ .  $^1H$ -NMR ( $CDCl_3$ ):  $\delta$  7.81 (2H, d,  $J = 7.4$  Hz, H-2' and H-6'), 7.57 (1H, t,  $J = 7.3$ , H-4'), 7.45 (2H, t,  $J = 7.7$  Hz, H-3' and H-5'), 6.98 (1H, d,  $J = 8.1$ , H-6), 6.63 (1H, d,  $J = 8.1$  Hz, H-7), 6.05 (2H, s, H-2), 3.86 (3H, s,  $CH_3O$ -);  $^{13}C$  NMR ( $CDCl_3$ ):  $\delta$ ; 195.6 (C=O), 152.1 (C-1), 142.74 (C-4), 138.8 (C-1'), 137.3 (C-5), 133.1 (C-4'), 130.1 (C-2', 6'), 128.5 (C-3', 5'), 126.6 (C-3), 124.8 (C-6), 103.2 (C-7), 102.0 (C-2), 60.4 ( $-CH_3O$ -); EIMS,  $m/z$  (% rel. abundance) 256 ( $M^+$ , 100), 228 (26), 197 (18), 179 (24), 164 (4), 139 (6), 105 (4).



The dichloromethane extract also yielded 1,7-dihydroxy-4-methoxyxanthone (**2**), benzyl-2-hydroxy-6-methoxybenzoate (**3**) and methyl-2-hydroxy-6-methoxybenzoate (**4**). The physical and spectral properties of compounds **2**, **3** and **4** compared well with those reported in literature for the compounds (Yang et al., 1982; Castillo et al., 1988; Marston et al., 1993). Compound (**1**) exhibited minimal antibacterial activity against *Pseudomonas aeruginosa* and a much higher activity against *Aspergillus fumigatus*. It did not show activity against the other test organisms. Compound (**2**) was active against *Staphylococcus aureus*, *Aspergillus fumigatus*, *Aspergillus niger* and the *Penicillium* species. It did not show activity against *Candida albicans*. Compounds (**3**) and (**4**) were inactive against all the test organisms.

## Discussion

Extracts of the stem bark (de Tommasi et al., 1993; Arnold and Gulumian, 1984) and roots (Chhabra et al., 1991; Desta, 1993) of *Securidaca longepedunculata* are used in traditional medicine for the treatment of bacterial infections. Among studies that have been done on the antimicrobial properties of this plant (El-Fatih, 1997; Taniguchi et al., 1978; Sanogo et al., 1998; Laurens et al., 1985; Odebiyi, 1978; Desta, 1993), the study by Desta (1993) was the only study which reported a strong antibacterial activity against *Pseudomonas aeruginosa*, *Staphylococcus albus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Salmonella gallinarum*. The activity was detected in a dichloromethane extract. The extract also exhibited a strong antifungal activity against *Candida albicans*. In this study the crude extract showed antifungal activity against *Aspergillus fumigatus*, *Aspergillus niger* and *Penicillium* species. It had no activity against the three bacteria used. However, the compounds which were subsequently isolated from the extract exhibited antibacterial and antifungal activity. Compound (**2**) showed activity against *Staphylococcus aureus*,

**Table 1:** Antimicrobial activity of extracts and pure compounds from *Securidaca longepedunculata*.

Test item	Test Organisms							
		Staph	E. Coli	Pseud	Cand	AF	AN	PEN
SLRT	IZ	0	0	0	15.0±0.7	17.0±1.6	16.0±1.0	0
	AI	0	0	0	0.8	0.9	0.9	0
SLRE	IZ	0	0	0	0	0	0	0
	AI	0	0	0	0	0	0	0
SLRD	IZ	0	0	0	0	6.0±1.7	5.0±1.1	8.0±0.7
	AI	0	0	0	0	0.4	0.3	0.5
1(83)	IZ	0	0	4±1.7	0	11.0±1.7	0	0
	AI	0	0			1.0	0	0
2(84)	IZ	15.0±0.7	0	0	0	13±1.0	13.0±2.3	18±1.7
	AI	0.8	0	0		0.8	0.7	1.1
3(79)	IZ	0	0	0	0	0	0	0
	AI	0	0	0	0	0	0	0
4(80)	IZ	0	0	0	0	0	0	0
	AI	0	0	0	0	0	0	0
Amp	IZ	20.0±1.0	18.0±1.7	-	-	-	-	-
	AI	1.0	1.0					
Gent	IZ	-	-	15.0	-	-	-	-
	AI			1.0	-	-	-	-
Keto	IZ	-	-	-	18.0±1.7	16.0±2.0	18±1.7	16±1.0
	AI				1.0	1.0	1.0	1.0

Results are represented as mean ±SD (n=3). IZ = inhibition zone

AI= activity index, represented by inhibition zone of test item/inhibition zone of standard drug

*A. fumigatus*, *A. niger* and the *Penicillium* species. This study also led to the isolation of a new compound (**1**) with both antibacterial and antifungal activity.

This study has confirmed previous claims on the antimicrobial properties of *S. longepedunculata* and has been the first study to identify active antimicrobial compounds from the plant. Further studies, using bioassay guided isolation are recommended as a way to identify more compounds that might be responsible for the antibacterial and antifungal properties of the plant.

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