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CYTOTOXIC COMPOUNDS FROM THE ROOT BARK OF *SECURIDAC ALONGIPEDUNCULATA*

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ABSTRACT. Chromatographic separation of the ethyl acetate extract of the root bark of *Securidaca longipedunculata* led to the isolation of one new xanthone derivative (**1**) along with nine known compounds (**2-10**). The structure of the isolated compounds were identified by NMR, mass spectrometric analyses, and comparison with the reported literature data. The isolates were evaluated for their cytotoxic activity with compounds **5** (IC_{50} = 0.38 µM) and **6** (IC_{50} = 52 µM) showed significant inhibitory activities against the human cervical cancer KB-3-1 cell line. Compound **5** displayed superior activity, which is even better than one of the reference drugs (griseofulvin, IC_{50} = 17 µM), whereas the rest showed little or none inhibitory activities.

KEY WORDS: *Securidaca longipedunculata*, Root bark, Xanthones, Cytotoxicity, Cervical cancer

INTRODUCTION

Securidaca longipedunculata Fresen (Polygalaceae) is a species that belongs to the genus *Securidaca*, mainly found in the tropical and subtropical parts of Africa [1, 2]. The species name, "*longipeduculata"* is believed to be derived from the long peduncle on which its flowers are born [3]. It is extensively used in Africa for the treatments of different ailments including syphilis, gonorrhoea, headache, and malaria [1]. In fact, it is known as a commercial plant in Africa for medicine [4], and in some parts of Ethiopia for cleaning purpose, as the lather from the bark of the root is used as soap for washing clothes. Phytochemically, the plant is a good source of bioactive secondary metabolites including xanthones [5, 6], steroids [7, 8], flavonoids [7, 9] and benzoic acid derivatives [10], which have been reported to possess antimicrobial [11-13], antimalarial [14], and cytotoxic [2] activities.

As part of our ongoing efforts to search for bioactive secondary metabolites from traditionally used medicinal plants, we report herein the isolation and characterization of one new and nine known compounds along with their cytotoxic activity from the root bark of *S. longipenduculata*, which has long been used in traditional medicines by the local healers.

EXPERIMENTAL

General

The solvents used for extraction and isolation purposes were of analytical grades. Column chromatography was carried out on silica gel (0.06-0.2 mm, Merck). Analytical TLC was performed on Merck pre-coated silica gel 60 F_{254} plates. UV spectra were recorded on UV-

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3100PC spectrophotometer, UWR international, China. Gel filtration was carried out on Sephadex LH-20. High Resolution ESIMS was done on Micromass AC-TOFmicro mass spectrometer (Micromass, Agilent Technologies 1200 series, Japan). IR spectra were recorded on a Nicolet 380 FT-IR spectrometer. 1D and 2D (H-H COSY, HSQC, HMBC, NOE) NMR spectra were recorded on a Bruker Avance 500 MHz spectrometer at 500 MHz (^{1}H) and 125 MHz (^{13}C) at 298 K using the residual solvent peaks as a reference.

Plant material

The root of *S. longipenduculata* was collected from Dongoro Muta Kebele, Ebantu District, East Wollega Zone, Oromia Reginal State, Ethiopia, about 520 km away from Addis Ababa in September, 2019. The plant material was identified and the specimen was deposited (voucher number tt1/2017) at Wollega University Herbarium [10].

Extraction and isolation

The air-dried and powdered root bark of *S. longipenduculata* (830 g) was extracted exhaustively with ethyl acetate four times each for 24 h at room temperature. The extract was then concentrated under reduced pressure by using rotary evaporator to yield 65 g. The extract (50 g) was subjected to column chromatography over silica gel (400 g) and eluted with petroleum ether with increasing gradient of ethyl acetate to give 40 fractions, each of *ca*. 250 mL.

Fractions 6-10 (eluted with 5% *v/v* ethyl acetate in petroleum ether) were combined and further purified by repetitive gel filtration chromatography on Sephadex LH-20 (eluent, CH_2Cl_2/MeOH, 1:1) to give 1-hydroxy-5,7-dimethoxyxanthone (**2**) (3.0 mg) and 1,7-dimethoxyxanthone (**4**) (2.1 mg). Fractions 12-25 (eluent of 10% *v/v* ethyl acetate in petroleum ether) showed four spots on TLC and were combined, and purified by column chromatography (column size: 60 cm length and 3 cm diameter) with an increasing gradient amounts of ethyl acetate in petroleum ether to afford methyl-2-hydroxy-6-methoxybenzoate (**7**) (8.0 mg), 2-methoxy-3,4-methylenedioxybenzophenone (**8**) (2.8 mg), 2,3-dimethoxy-4 hydroxybenzo-phenone (**9**) (2.3 mg) and methyl-2-hydroxy-6-methoxybenzoate (**10**) (6.1 mg). The post fractions, 28-40 (eluted at 35% *v/v* ethyl acetate in petroleum ether) were combined, and subsequently subjected to silica gel column chromatography (column size: 80 cm length and 4 cm diameter) with increasing gradient of ethyl acetate in petroleum ether. This was followed by gel filtration on Sephadex LH-20 (dichloromethane/methanol, 1:1) to give 1,7-dihydroxy-4-methoxyxanthone (**5**) (4.8 mg), 1,4-dihydroxy-7-methoxyxanthone (**6**) (3.9 mg), d 3,8-dihydroxy-1,2,4,5,6-pentamethoxyxanthone (**1**) (3.4 mg), and (1,7-dihydroxyxanthone (**3**) (4.0 mg).

*3,8-Dihydroxy-1,2,4,5,6-pentamethoxyxanthone (**1**)*. Yellow solid, UV (CH_3CN) λ_{max} [nm]: 234, 272, 306. IR (CH_2Cl_2) ν_{max} cm^{-1} 3412, 1704, 1611, 1561, 1348. ^{1}H and ^{13}C NMR (Table 1). HR-ESI-MS *m/z* = 379.10290, $[M+H]^+$; calculated m/*z* 379.1026 (consistent for $C_{18}H_{19}O_9$).

Cytotoxicity assay

The cytotoxic assay was performed on human cervical cancer, KB-3-1 cell line as previously described in Sammet *et al.* [15]. The cell lines were cultivated as a monolayer in DMEM (Dulbecco's modified Eagle medium) with glucose (4.5 g/L), L-glutamine, sodium pyruvate and phenol red, which were supplemented with 10% fatal bovine serum (FBS) and maintained at 5.3% CO_2 and 37 °C in humidified air. The cells (at 70% confluence) were detached with trypsin-ethylenediamine acetic acid solution (0.05%; 0.02% in DPBS) and placed in sterile 96-well plates in a density of 10^4 cells in 100 µL medium per well. The dilution series of the test compounds were prepared from stock solutions in DMSO of concentrations of 100, 50 or 25 mM and the

stock solutions were diluted with culture medium (10% FBS) down to picomolar (pM) concentrations. The dilution prepared from stock solution was added to the wells and each concentration was tested in at least six replicates. The control (cryptophycine-52 and griseofulvin) contained the same concentration of DMSO as the first dilution. After incubation for 72 h at 37 °C and 5.3% CO_2-humidified air, 30 µL of an aqueous resazurin solution (175 µM) was added to each well. The cells were incubated for 5 h at the same conditions. Subsequently, the fluorescence was recorded at a wavelength of 588 nm and then the IC_{50} values were calculated as a sigmoidal dose response curve using GRAPHPAD PRISM 4.03.

RESULTS AND DISCUSSION

Anticipated by the claim of the traditional healers for the managements of infectious diseases, the roots bark was phytochemically investigated to give a new compound (**1**) along with nine known compounds (**2**-**10**) (Figure 1). The known compounds are common denominators in the genus, and their structures were identified through analyses of their NMR spectral data and comparing with already reported literature [5, 9, 11, 16-20]. Thus, these compounds were identified as 1,7-dihydroxyxanthone (**3**) [5, 16], 1-hydroxy-5,7-dimethoxyxanthone (**2**) [17], 1,7-dimethoxy-xanthone (**4**) [16, 18], 1,7-dihydroxy-4-methoxyxanthone (**5**) [11], 1,4-dihydroxy-7-methoxyxanthone (**6**) [19], methyl-2-hydroxy-6-methoxybenzoate (**7**) [9], 2-methoxy-3,4-methylenedioxybenzophenone (**8**) [9], 2,3-dimethoxy-4-hydroxybenzophenone (**9**) [20], and methyl-2-hydroxy-6-methoxybenzoate (**10**) [9].

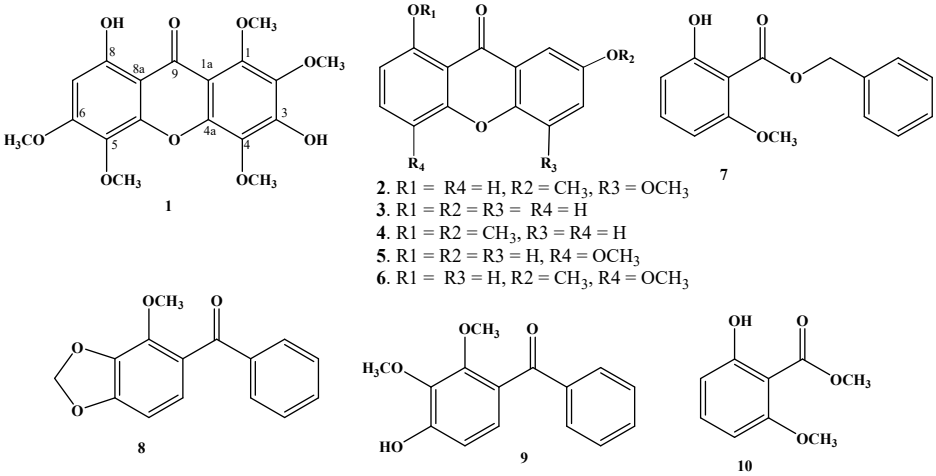

Figure 1. Structures of the isolated compounds from the root bark of *S. longipenduculata*.

Compounds **3**-**10** from *S. longipenduculata*; however, this is the first report of the isolation of compound **2** from *S. longipenduculata*, although it was previously reported from the roots of *Mesu aferrea* [17].

Compound **1** was isolated as yellow solid. The molecular formula, $C_{18}H_{18}O_9$ was assigned based on the protonated molecular ion peak (HRESIMS data ($[M+H]^+$) at *m/z* 379.10290; calculated *m/z* 379.1026), equivalent to ten indices of hydrogen deficiency. The UV (λ_{max} 234, 272, and 306 nm) and IR (ν_{max} 3412, 1704, 1611, 1561, 1348 cm^{-1}) spectra revealed absorptions for conjugated ketone and aromatic moieties [21]. In agreement with this, the ^{13}C NMR (Table 1) revealed thirteen sp^2 hybridized carbon atoms including a carbonyl carbon at δ_C 181.2 corresponding to xanthone moiety.

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz,) NMR data of compound **1** (in acetone-d_6).

Position	δ_H(*multiplicity*)	δ_C	HMBC
1		150.0	
1a		108.3	
2		139.8	
3		148.4	
4		133.1	
4a		149.3	
5		129.3	
5a		151.6	
6		160.4	
7	6.43 (s)	95.6	C-5, C-6, C-8, C-8a
8		160.0	
8a		103.6	
9		181.2	
1-OMe	3.91 (s)	62.2	C-1
2-OMe	3.90 (s)	61.7	C-2
4-OMe	4.03 (s)	61.7	C-4
5-OMe	3.89 (s)	61.5	C-5
6-OMe	3.98 (s)	56.8	C-6
8-OH	13.30 (s)		C-8, C-8a

The ^{1}H NMR spectrum showed a highly up field shifted singlet aromatic proton signal (δ_H 6.43) due to the mesomeric effect of the di-*ortho* oxygenation, otherwise fully substituted with two hydroxyl and five methoxy groups. The down field shifted signal at δ_H 13.30 for hydroxyl proton involved in hydrogen bonding was placed *peri*-to the carbonyl carbon at C-8 (δ_C 160.0). The position of the five methoxy groups were established at C-1, C-2, C-4, C-5 and C-5 based on the chemical shifts and HMBC correlations, and therefore the other hydroxyl group was placed at C-3. These placements were further confirmed by the NOE correlations observed between nearby methoxy protons. Therefore, based on the above spectroscopic evidences, the new compound was characterized as 3,8-dihydroxy-1,2,4,5,6-pentamethoxyxanthone (**1**).

The isolated compounds (**2**-**10**) were evaluated for their cytotoxic activities against human cervical cancer, KB-3-1 cell line, with both cryptophycine-52 (IC_{50} = 1.3 × 10^{-5} μM) and griseofulvin (IC_{50} = 17 μM) as positive control. Compounds **5** and **6** showed cytotoxic activities with IC_{50} values of 0.38 μM and 52 μM, respectively against the cell line. The activity value displayed by compound **5** is far better than one of the reference drug (griseofulvin). Otherwise, the rest of the tested compounds showed insignificant activities against the cell line.

CONCLUSION

A new xanthone (**1**) along with nine other known compounds were identified from the root bark of *S. longipenduculata*. Compound (**5**) showed strong cytotoxic activity against KB-3-1 human cervical cancer cell line, its IC_{50} value even greater than one of the reference drug, griseofulvin. Thus, the observed cytotoxic activities of the compound could give insight about potential of traditional medicinal plants as good source for lead compounds in development of cancer drugs.

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