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ORIGINAL ARTICLE

Coumarins from the roots of *Cleme Viscosa* (L.) antimicrobial and cytotoxic studies

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Antimicrobial activity

Abstract Two coumarins, 7-geranyloxycoumarin (auraptene) and 6′-hydroxy-β-cycloauraptene were isolated from the roots of *Cleme viscosa* (Capparidaceae). The second compound has never been reported previously from this plant. The isolation process involved extraction with various solvents and separation using column chromatography techniques. The structure of the compounds was assigned on the basis of spectroscopic data Such as IR, UV, ^{1}H NMR, ^{13}C NMR, COSY, HMQC, HMBC, DEPT and MS. However, bioactivity screening showed that the pure isolated compounds possessed no activity on two species of bacteria *Bacillus cereus* NRRLUI-1447 and *Pseudomonas aeruginosa* UI-60690 and four species of fungi (*Aspergillus* ochraceus NRRL 398, *Candida lipolytica* ATCC 2075, *Sacchromyces cereviseae* NRRL 2034 and *Sacchromyces lipolytica*). The cytotoxic test of the compounds against CEM-SS (T-cell lymphoblastic leukemia) cells were also carried out with IC_{50} values of 14 and 18 μg/ml ,respectively.

1. Introduction

Cleme viscosa, belongs to the Capparidaceae family, the plant is known locally as Fung kowdie (Ohashi et al., 2004). According to some authorities, Capparidaceae comprise of 24 genera and 48 species. Many species are valuable for their large pulpy fruits, some are useful for their timber and others are prized as ornamental. The family consists of trees, shrubs and woody climbers found mainly in the tropics, although American and Western Africa species have yellowish flowers. It bears smooth yellowish fruits, 3.5–7.5 cm long which are edible but of a poor flavor (Ohashi et al., 2004). It is not eaten fresh but it is used for making jellies.

The plant is being used in traditional medicine for the treatment of diabetic, after birth treatment and intermittent fever (Jamal and Mohamed, 2002; Leboeuf et al., 1998; Kosela et al., 2003). In South America and West Indies, the fruit is used to make corks (Wilzer et al., 2008). The *Cleme* species commonly found in Ethiopia, Saudi Arabia, Taiwan and Egypt are *Cleme iberica, Cleme dolichostyla, Cleme glabra* and *Cleme arrecta,* which were focussed on the isolation and

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identification of amino acids, diterpenes (Riyanto et al., 2003), sulfur compounds (Basu et al., 2004), sesquiterpenes (Chatterjee et al., 2004), alkaloids (Shoeb et al., 2002; Buckingham et al., 1994), and lipids (Chen et al., 2004). Other previous chemical studies on roots of *C*. viscosa have led to the isolation of psoralen, xanthotoxin, scopoletin, decursinol, haplopine and aegelinol (Kjare et al., 2003; Gupio and Dutt, 2001; Salleh and Ahmad, 2000). There are no studies done for the roots of Asudanese indigenous species, but there were studies conducted on the roots of other species in Taiwan (Murray et al., 1998) and 4 species in Egypt (Rashid et al., 2004). The aim of this work is to investigate the isolation and identification of coumarins present in roots of the plant species. The antimicrobial activity against some target microbes and IC_{50} values of cytotoxic test were also evaluated.

2. Experimental

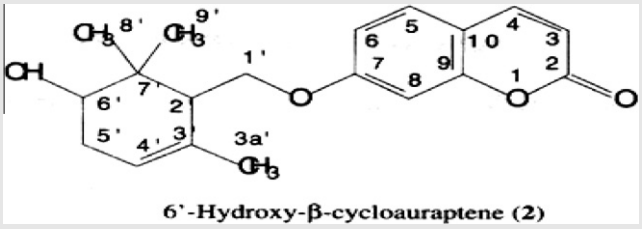
2.1. Materials and methods

Melting points (uncorrected) were determined on a Kofhler melting points apparatus. The IR spectra were recorded using KBr disc on Perkin–Elmer Lambda FTIR spectrophotometer model 1650, ultraviolet spectra were obtained on Perkin–Elmer lambda model 20 spectrometers. ^{1}H and ^{13}C NMR spectra were obtained on JEOL spectrometer at 500 and 125 MHz respectively, with tetramethylsilane (TMS) as internal standard. Mass spectra were recorded on a Finnigan MATSSQ 710 and GCMS-QP 5050 Ashimadzu mass spectrometers. The flash column and mini column chromatography were carried on silica gel Merck 1.07749 and 1.07734 respectively, whereas TLC analysis utilised Merck TLC plastic sheets silica gel 60 PF_{254}.

2.2. Plant material

The fresh roots of *C*. viscosa were collected from the central of Sudan (Maiurno Area-Sinar state) and identified by the taxonomy unit of the Department of Biological Sciences University of Khartoum, the roots collected were further shelled and the dried roots were ground to a fine powder using Thomas–Willey Milling Machine, a voucher specimens was deposited at the Herbarium of Biology Department, University of Khartoum.

Table 1 ^{1}H and ^{13}C NMR data of 6′-hydroxy-β-cycloauraptene (**2**).



6'-Hydroxy-β-cycloauraptene (2)

C	δ ^{1}H	δ ^{13}C	DEPT	COSY	HMQC	HMBC
1	–	–	–	–	–	–
2	–	162.40	C	–	–	H-3
3	6.19, d, *J* = 9.6 Hz	112.98	CH	H-4	H-3	H-4
4	7.59, d, *J* = 9.6 Hz	143.49	CH	H-3	H-4	H-5
5	7.32, d, *J* = 8.3 Hz	128.76	CH	H-6	H-5	H-4
6	6.79. d, *J* = 8.3, 2.4 Hz	113.16	CH	H-5	H-6	H-8
7	–	161.26	C	–	–	H-6, H-8
8	6.79, d, *J* = 2.4 Hz	101.54	CH	–	H-8	H-6
9	–	155.83	C	–	–	H-8
10	–	112.48	C	–	–	H-4, H-8
	4.56, d, *J* = 6.4 Hz	65.32	CH_2	–	H-1′	–
2′	2.67, t, *J* = 6.4 Hz	63.86	CH	–	H-2′	H-I′, H
5′	–	–	–	–	–	–
3′	–	141.44	C	–	–	H-3a′
4′	5.45, q, *J* = 6.4 Hz	119.03	CH	H-5′, H-6′	H-4′	H-3a′
5′	1.64, q, *J* = 7.6 Hz	27.07	CH_2	H-6′	H-5′	H-6′
6′	2.19, m	36.23	CH	H-5′, H-4′	H-6′	H-4′, H
5′	–	–	–	–	–	–
7′	–	58.41	C	–	–	H-8′, H
9′	–	–	–	–	–	–
8′	1.25, s	24.84	CH_3	-	H-8′	–
9′	1.23, s	18.78	CH_3	–	H-9′	–
3a′	1.74, s	16.79	CH_3	–	H-3a′	H-4′
OH	–	–	–	–	–	–

2.3. Isolation of the compounds

The powder (240.0 g) was extracted subsequently with petroleum ether, chloroform and methanol. The chloroform (22.0 mg) and methanol crude extracts (28.0 mg) were subjected to the gradient elution of flash column chromatography. The mixture of petroleum ether:chloroform:methanol was used as the eluant. A total of 18 and 24 fractions (24 ml) was obtained from the chloroform and methanol crude extracts respectively. Fractions 10 and 14–18 of the chloroform crude extract were further fractionated using mini column chromatography. Recrystallisation from acetone afforded compound (**1**) as pale yellow needles (8.0 mg). Whereas fractions 8, 12 and 18–22 of methanol crude extract were rechromatographed and eluted with methanol to yield 4 fractions. Fractions 2 and 4 gave solid material and on further purification and washing with acetone, yellow solid material was obtained. Further recrystallisation in acetone gave compound (**2**) (14.0 mg) as a white crystals.

Auraptene (**1**).MS *m*/*z* (% intensity): 298(M^+,2), 281(24), 267(16), 255(34), 187(5), 177(40), 162(78), 136(4), 105(10), 95(18), 9(30), 81(58), 69(100), 53(18), 41(71). λ_{max} (MeOH): 322 nm (log ε = 4.25), 220 nm (log ε = 4.11), 206 nm (log ε = 4.49). IR (cm^{-1}, KBr, disc): 3086, 3056, 2974, 2906, 1726, 1614, 1508, 1234, 1022. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.63 (d, J = 9.5 Hz, 1H, H-4), 7.36 (d, J = 8.5 Hz, 1H, H-5), 6.85 (dd, J = 8.5,2.4 Hz, 1H, H-8), 6.81 (dd, J = 8.5,2.4 Hz, 1H, H-6), 6.24 (d, J = 9.5 Hz, 1H, H-3), 5.46 (t, J = 6.5 Hz, 1H, H-2′), 5.09 (t, 1H, H-6), 4.60 (d, J = 6.5 Hz,1H, H-1), 2.09 (m, 4H, H-4′, H5′), 1.78 (s, 3H, H3a′), 1.66 (s, 3H, H-8′), 1.60 (s, 3H, H-9′). ^{13}C NMR (125 MHz,$CDCl_3$) δ: 162.1(C-2), 161.2(C-7), 155.9(C-9), 143.4(C-4), 142.3(C-3′), 131.9(C-7′), 128.6(C-5), 123.6(C-6′), 118.4(C-2′), 113.2(C-8), 112.9(C-3), 112.4 (C-10), 101.6(C-6), 65.5(C-1′), 39.5(C-5′), 26.2(C-4′), 25.6(C-3a′), 17.7(C-9′), 16.7(C-8′).

6′-hydroxy-βcycloauraptene (**2**). MS *m*/*z* (%intensity): 314(M^+,2), 281(44), 175(5), 162(34), 153(28), 134(2), 105(10), 93(15), 81(75), 7 l(100), 59(38). λ_{max} (MeOH): 322 nm (log ε = 4.25), 220 nm (log ε = 4.21), 206 nm (log ε = 4.49). IR (cm^{-1}, KBr, disc): 3434, 3083, 2959, 1709, 1608, 1460, 1282, 1235, 1127, 847. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.59 (d, J = 9.6 Hz, 1H, H-4), 7.32 (d, J = 8.3 Hz, 1H, H-5), 6.79 (dd, J = 8.3,2.4 Hz, 1H, H-6), 6.76 (d, J = 2.4 Hz, 1H, H-8), 6.19 (d, J = 9.6 Hz, 1H, H-3), 5.45 (t, J = 6.4 Hz, 1H, H-4′), 4.56 (d, J = 6.4 Hz,2H, H-l′), 2.67 (t, J = 6.4 Hz, 1H, H-2′), 2.19 (m, J = 7.2 Hz, 1H, H6′), 1.74 (s, 3H, H-3a′), 1.64 (q, J = 7.6 Hz, 2H, H-5′), 1.25 (s, 3H, H-8′), 1.23 (s, 3H, H-9′). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 162.4(C-2), 161.3(C-7), 155.8(C-9), 143.5(C-4), 141.4(C-3′), 128.8(C-5), 119.1(C-4′), 113.2(C-5), 113.0(C-3), 112.5(C-10), 101.5(C-8), 65.3(C-1′), 63.9(C-2′), 56.4(C-7′), 36.2(C-6′), 27.1(C-5′), 24.8(C-8′), 18.8(C-9′), 16.8(C-3a′).

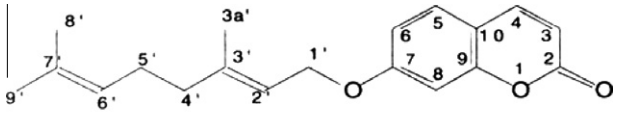
3. Bioassay

The microorganisms were obtained from the culture collection of the Department of Biology University of Juba, the stock cultures were grown on Potato Dextrose Agar (PDA) for 24 h at 28 °C at which time the cells were harvested by centrifugation (4 °C, 2000 rpm, 3 min.). The cells were washed and suspended in sterile 0.9% saline to give a final concentration of 10^5–10^6 CFU/ml using a haemocytometer (Bergeys, 1998). The target microbes used were Bacillus cereus NRRLUI-1447, Pseudomonas aeruginosa UI-60690 and four fungi (Aspergillus ochraceus NRRL 398, Candida lipolytica ATCC 2075, Sacchromyces cereviseae NRRL 2034 and Sacchromyces lipolytica).

Antibacterial activity of the isolated compounds was determined using disc diffusion method (Bauer et al., 2006). The discs were prepared by impregnating them in ethanolic solution of each sample (10 mg/ml) and evenly spaced out on the agar surface previously inoculated with the suspension of each microorganism to be tested. Standard discs of nystatin (50 g/discs) and streptomycin sulfate (25 g/discs) were used as positive controls. The plates were incubated at 37 °C for 24 h and the antimicrobial was recorded by measuring the width of the clear inhibition zones around each disc. Cytotoxicity test was determined against T-cell lymphoblastic leukemia (CEM-SS). The IC_{50} of the compounds was calculated based on the optical density measurement by using ELISA Reader Biotek EL 340 at 550 nm with a reference wavelength at 630 nm.

4. Results and discussion

Compound (**1**) was isolated as pale yellow needles (8.0 mg), m.p. 64–65 °C (lit. m.p. 68 °C)Basu et al., 2004. The UV spectrum showed bands at λ_{max} (MeOH) 322 nm (log ε = 4.25), 220 nm (log ε = 4.11) and 206 nm (log ε = 4.49) which are characteristic for coumarins 1. The IR spectrum revealed no peak of OH group. Peaks at 3086 and 3056 cm^{-1} show the existence of C–H aromatic, whereas peak at 2974 and 2906 cm^{-1} indicated the presence of C–H aliphatic group. Carbonyl group appears at 1726 cm^{-1}, while peaks at 1614 and 1508 cm^{-1} correspond to the conjugated C = C. Another peak at 1234 cm^{-1} was the signal of C–O–C group. Mass spectrum of the compound gave molecular ion at M^+ 298 assignable to the structure $C_{19}H_{22}O_3$. Peak at *m*/*z* 162 indicated the presence of coumarin unit. Peaks at *m*/*z* 281, 267, 255 and 177 were resulted from cleavage of bonds at the side chain. ^{1}H NMR spectrum showed peaks of methyl groups as singlets at δ 1.66 and δ 1.78.



7-Geranyloxycoumarin (**1**)

Two adjacent protons at the pyron ring were exhibited as doublets at δ 6.24 and δ 7.63 (J = 9.5 Hz). The doublet at δ 7.36, doublet of doublet at δ 6.85 and δ 6.81 were due to H-5, H-8 and H-6, respectively. ^{1}H-^{1}H COSY spectrum confirms the coupling interaction between protons H-3 and H-4; protons H-5, H-6 and H-8; protons H-l′; H-2′ and between protons H-5′, H-4′ and H-6′. The data was also compared with the previous study (Chatterjee et al., 2004). Based on above data, it can be concluded that compound (**1**) was 7-geranyloxycoumarin (auraptene).

Compound (**2**) was obtained as white crystals (14.0 mg), m.p. 40–42 °C (lit. m.p. 43–45 °C)Shoeb et al., 2002. The UV

spectrum showed bands at λ_{max} (MeOH) 322 nm ($\log \varepsilon = 4.25$), 220 nm ($\log \varepsilon = 4.21$) and 206 nm ($\log \varepsilon = 4.49$) typical to the other UV spectra of coumarin derivatives. IR spectrum of the compound gave broad peak at 3434 cm^{-1} which indicates the presence of OH group whereas the other peaks at 3082 cm^{-1} indicated the presence of C–H aromatic and peaks at 1608 cm^{-1} show the presence of the conjugated C=C. The expected molecular ion at m/z 314 corresponding to the molecular formula $C_{19}H_{22}O_4$ was observed. However, peak at m/z 281 which was probably formed through the cleavage of water molecule and a methyl group was present. The peak at m/z 162 was typical for umbeliferon unit (Buckingham et al., 1994).

^{13}C NMR spectrum indicates that there are 19 carbon atoms, which were further supported by DEPT spectrum that show 3 signals for CH_3, 3 signals for CH_2, 7 signals for CH and 6 signals for unprotonated carbon atom. ^1H NMR spectrum showed two doublets at δ 7.59 and δ 6.19 (J = 9.6 Hz) corresponding to H-4 and H-3, respectively. The coupling patterns of H-5, H-6 and H-8 (ABX type, J_{AB} = 8.3 Hz, J_{BX} = 2.4 Hz) were similar to compound (**1**). The COSY spectrum showed the cross peaks correspond to the interaction among H-4′, H-5,′ and H-6′. A triplet at δ 5.45 (J = 6.4 Hz) was the signal of an oleifenic proton (H-4′), while H-5′ appears as quartet at δ 1.64 (J = 7.6 Hz). Two geminal protons at H-l′ appeared as doublet at δ 4.56 (J = 6.4 Hz), whereas a triplet at δ 2.67 (J = 6.4 Hz) was due to H-2′. The methyl groups (H-3a′, H-8′ and H-9′) were represented as three singlets at δ 1.74, 1.25 and 1.23, respectively. The assignment of NMR data was further supported by HMQC and HMBC spectra and the correlated data was shown in Table 1. On the basis of above spectroscopic data, the compound was proposed as 6′-hydroxy-β-cycloauraptene (**2**). The results of the antimicrobial test indicated that the isolated compounds have no activity against the given micro-organisms. The cytotoxic test showed that each auraptene and 6′-hydroxy-β-cycloauraptene has weak cytotoxicity with IC_{50} values of 14 and 18 μg/ml, respectively.

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