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A Dihydroisocoumarin from the Rhizome of *Aloe pulcherrima*

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Abstract

Chromatographic separation of the dichloromethane/methanol (1:1) extract of the *Aloe pulcherrima* rhizome afforded a new dihydroisocoumarin derivative (1) along with six anthraquinone derivatives (2-7). The chemical structures of the compounds were established based on spectroscopic analyses including NMR (^{1}H and ^{13}C NMR, ^{1}H-^{1}H COSY, HMQC, HMBC, NOESY), MS and comparison with reported literature. The isolated compounds were evaluated for *in vitro* antibacterial and antiplasmodial activities. Almost all compounds showed antibacterial activity with the highest activity observed for compound 5 against *Enterococcus faecalis*. Whereas, only compound, 3, 4, 5 and 6 showed antiplasmodial activity against chloroquine-resistant (W2) strain of *Plasmodium falciparum*.

Keywords: Asphodelaceae; *Aloe pulcherrima*; Rhizome; Dihydroisocoumarin; Anthraquinone; Antibacterial; Antiplasmodial

Introduction

The genus *Aloe* (family Asphodelaceae, subfamily Alooideae) comprises of more than 400 species, ranging from diminutive shrubs to large tree-like mainly distributed in Africa and Madagascar with only a few species found in the Arabian Peninsula [1-3]. *Aloe* in Ethiopia is represented by 46 species, including 16 endemic species [4,5].

Aloe pulcherrima is one of the endemic species growing in Ethiopia. The name "*pulcherrima*" derived from "*pulcher*", refers to the beauty of the plant with bright red flowers and blue-like green leaves [5]. It has been traditionally used for the treatments of various ailments, particularly for wound healing, constipation and as insect repellents [6,7]. The phytochemical analysis of the leaf latex [7] and the roots of *A. pulcherrima* have resulted in the isolation of anthraquinones and pre-anthraquinones which have been reported to have antibacterial [7,8], antifungal [7] and antiplasmodial [8]. As part of our on-going program in search for new bioactive natural compounds from African traditional medicinal plants [8,9], now we report the isolation of one new compound (1) and six known compounds (2-7) along with their antibacterial and antiplasmodial activities from the rhizome of *A. pulcherrima*.

Materials and Methods

General

Column chromatography was carried out on silica gel (0.06-0.2 mm). Gel filtration was performed on Sephadex LH-20. Analytical TLC was performed on Merck pre-coated silica gel 60 F_{254} plates. Melting points were measured on B-540 melting point apparatus. UV spectra were recorded on a UV-3100 PC spectrophotometer (UWR international, Shanghai, China). IR spectra were recorded on a Nicolet 380 FT-IR spectrometer (Thermo Electron Corporation, Madison, WI, USA). High Resolution ESI-MS was done on a Micromass AC-TOF micro mass spectrometer (Micro mass, Agilent Technologies 1200 series, Tokyo, Japan). Optical rotations were measured on a P-1020 polarimeter. 1D (^{1}H, ^{13}C) NMR and 2D (COSY, HSQC, HMBC, NOESY) NMR spectra were recorded on an 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 rhizome of *A. pulcherrima* was collected from Guddo, Seka District, Jimma zone, Oromia regional state, Ethiopia in September 2016. The plant material was identified and the voucher specimen (voucher number AP001/2015) has been deposited in Jimma University Herbarium.

Extraction and isolation

The air-dried rhizome (320 g) of *A. pulcherrima* was milled into powder and then extracted using CH_2Cl_2/MeOH (1:1) four times for 24 hrs at room temperature. The extract was concentrated under vacuum using rotary evaporator to yield a dark brown residue (23 g, 7.2%). A 20 g portion of the extract was subjected to column chromatography on silica gel (300 g) eluting with petroleum ether containing increasing amount of ethyl acetate to afford 24 major fractions ca. 250 mL each. Fractions 2-10 (5% EtOAc in petroleum ether) were combined and purified by Sephadex LH-20 (eluting with CH_2Cl_2/MeOH; 1:1) to give chrysophanol (2, 3.8 mg) and aloesaponarin II (3, 4.2 mg). Fractions 11-17 (10% ethyl acetate in petroleum ether) showed mixtures of four compounds, which were combined and subjected to column chromatography (column size: 80 cm length and 4 cm diameter) on silica gel (250 g; eluent: increasing gradient of ethyl acetate in petroleum ether) followed by Sephadex LH-20 (eluting with CH_2Cl_2/MeOH; 1:1) yielding compound 1 (2.7 mg), aloesaponarin I (4, 3.1 mg), laccaic acid D-methyl ester (5, 3.6 mg) and aloesaponol I (6, 2.9 mg); while fractions 18-22 (20% EtOAc in petroleum ether) showed colourless blue fluorescing precipitate that was washed with 100% petroleum ether and further purified on Sephadex LH-20 (eluting with CH_2Cl_2/MeOH; 1:1) to give aloesaponol II (7, 4.3 mg).

4'-methoxyferalolide (1): Colorless solid. mp. 216-218°C. UV (CH_3CN): λ_{max} (logε)=264 (2.24), 294 (1.76) nm. IR (CH_2Cl_2) υ_{max} 3212, 1631, 1589 cm^{-1}. ^1H and ^{13}C NMR (Table 1). HR-ESI-MS m/z=381.0951, $[M+Na]^+$ (calculated for $C_{19}H_{18}O_7$).

Position	δ_H (m, J in Hz)	δ_C
1	-	170.4
3	4.82 (m)	80.6
4	2.94 (d, J=7.2 Hz)	33.2
4a	-	143
5	6.31 (d, J=2.1 Hz)	107.6
6	-	165.3
7	6.26 (d, J=2.1 Hz)	102
8	-	165.2
8a	-	101.8
1'α	3.09 (dd, J=13.9, 5.4 Hz)	39.4
1'β	3.23 (dd, J=13.9, 7.5 Hz)	
2'	-	139
3'	-	122.3
4'	-	162.8
5'	6.43 (d, J=2.3 Hz)	100.9
6'	-	159.8
7'	6.52 (d, J=2.3 Hz)	110.2
3'-$COCH_3$	2.58 (s)	32.8; 204.1
4'-OCH_3	3.80 (s)	55.7
8-OH	11.20 (s)	

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

Antibacterial assay (Agar disk diffusion method)

Four bacterial strains such as, *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) were used for *in vitro* evaluation of the antibacterial activity. An agar disk diffusion method was used to evaluate the antibacterial activity of the isolated compounds on nutrient agar. Briefly, the bacteria cultures were maintained on the nutrient agar slants which were stored at 40°C. The test solutions were prepared by dissolving 50 mg of the test samples to achieve final stock concentrations of 50 mg/ml in DMSO. Freshly grown liquid culture of the test pathogens solution of having similar turbidity with 0.5 McFarland were seeded over the Müeller-Hinton Agar medium with sterile swab. Sterile Whatman filter paper discs were soaked with 30 µL of the stock solution concentration of the samples and air dried to evaporate the solvent and then applied over the seeded plates at equidistance. The plates were then inverted and incubated at 37°C for 24 hr. After the incubation period, the plates were observed for a clearance zone around the disks and the clear zones formed around each disk were measured in millimeter. Each experiment was carried out in triplicates.

Antiplasmodial assay

A strain of *Plasmodium falciparum*, the Indochina W2 (chloroquine-resistant), was maintained in continuous culture to attain replication robustness prior to assays. Drug susceptibility was tested by the malaria SYBR Green I-based *in vitro* assay method [8]. he reference drug, chloroquine was tested along with the isolated compounds. A minimum of three separate determinations were carried out for each sample. Differential counts of relative luorescence units (RFUs) were used in calculating IC_{50} values using Prism 4.0 windows so tware.

Results and Discussion

The methanol/dichloromethane (1:1) extract of *A. pulcherrima* rhizome was subjected to repetitive column chromatography over silica gel yielded one dihydroisocoumarin (1), three anthraquinones (2-5) and two pre-anthraquinones (6 and 7) (Figure 1), of which the dihydroisocoumarin (1), was new compound. The known anthraquinones and pre-anthraquinones were identified as chrysophanol (2) [8,10], aloesaponarin II (3) [8,11], aloesaponarin I (4) [11,12], laccaic acid D-methyl ester (5) [12], aloesaponol I (6) [11,12] and aloesaponol II (7) [12] by comparison of their observed and reported spectroscopic data.

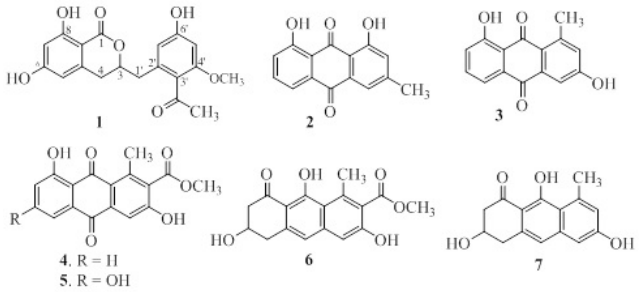

Figure 1: The structure of the compounds.

Compound 1 was isolated as a colorless amorphous solid. Its HRESIMS data showed a peak for a sodium adduct ion $[M+Na]^+$ at m/z 381.0951, corresponding to a molecular formula of $C_{19}H_{18}O_7$, which is consistent with eleven degrees of unsaturation. The UV-VIS absorption (λ_{max} 264, 294 nm), IR (ν_{max} 3212, 1631, 1589 cm^{-1}) and NMR spectra (Table 1) revealed the presence of hydroxyl and conjugated carbonyl moieties of the isocoumarin skeleton [13,14].

The ^1H NMR spectrum (Table 1; acetone-d_6, 500 MHz), showed the presence of one highly downfield shifted proton signals at δ_H 11.20 (s, ^1H) for phenolic hydroxyl group involved in hydrogen bonding indicating its peri-position to carbonyl group. The Presence of four meta-coupled aromatic protons at δ_H 6.26 (d, J=2.1 Hz, ^1H), 6.31 (d, J=2.1 Hz, ^1H), 6.43 (d, J=2.3 Hz, ^1H) and 6.52 (d, J=2.3 Hz, ^1H), is consistent with the presence of two tetra substituted aromatic moieties and were assigned to H-7, H-5, H-5' and H-6' respectively. The ^1H NMR spectrum further showed one oxygenated methine signal at δ_H 4.82 (m, ^1H) and two methylene groups at 2.94 (d, J=7.2 Hz, 2H) and 3.09- 3.23 (dd, *J*=13.9, 7.5/5.4 Hz, 2H) (deduced from DEPT and

HSQC experiments). The presence of other three proton singlet at δ_H 3.80 (s, 3H) and 2.58 (s, 3H) for a methoxyl and acetyl groups respectively were also evident.

The ^{13}C NMR spectrum showed carbon signals for 19 carbon atoms, representing two carbonyl (δ_C 204.1, 170.4), four oxygenated aromatic quaternary (δ_C 165.3, 165.2, 162.8, 159.8), four aromatic quaternary (δ_C 143.0, 139.0, 122.3, 101.8), four aromatic methine (δ_C 110.2, 107. 6, 102.0, 100.9), one oxygenated methine (δ_C 80.6), two methylene (δ_C 39.4, 33.2), a methoxyl (δ_C 55.7) and a methyl (δ_C 32.8) carbon atoms. The down-field shifted signal for one of the carbonyl carbon at δ_C 204.1 for the aryl ketone in the ^{13}C-NMR spectrum is consistent with a group being ortho-disubstituted, resulting in a deviation from planarity as a result of steric repulsion. The ^1H and ^{13}C NMR spectra of 1 are closely resemble those reported for feralolide, a dihydroisocoumarin previously reported from *A. ferox* [13]. The only notable difference is the presence of a signal at δ_H 3.80 (δ_C 55.7) for a methoxyl group in compound 1. The position of this methoxyl group was established at C-4' (δ_C 162.8) on the bases of HMBC experiment showing long range HMBC cross-coupling (Figure 2) with its neighboring carbons; C-3' (δ_C 122.3), C-4' (162.8) and C-5' (100.9). he optically activity ([α]20 D=-37° (c 0.5, acetonitrile)) data is in agreement with the compound being chiral having a center of chirality at C-3. he absolute configuration of the chiral center was then established to be R in compression with the related dihydroisocoumarin, feralolide [13]. herefore, based on the above spectroscopic evidence, the new compound was characterized as 3,4-dihydro-6,8-dihydroxy-3-(3'-acetyl-6'-hydroxy-4'-methoxy) methyl-1H- [2] benzopyran-1-one, for which the trivial name 4'-methoxyferalolide (1) was given. It is worth to point out that a similar dihydroisocoumarin with a methoxyl group at C-6' was previously reported from *A. vera* [15].

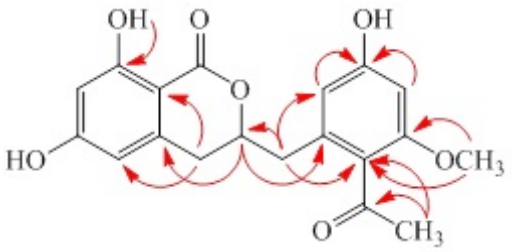

Figure 2: Key HMBC correlations of 1.

The isolation of anthraquinones and pre-anthraquinones, which appear to have been formed by two parallel routes differing by the way the octaketide chain was folded [16] from this plant are the common denominators of Alooideae [17] indicating chemical affinity of this plant to the related Aloe species and have limited chemotaxonomic importance at intrageneric level. However, it should be emphasized that dihydroisocoumarins are very rare from the genus *Aloe* and this is one of a few report of the occurrence of such compound from *Aloe* following the report from *A. ferox* [13], *A. hildebrandtii* [14], *A. vera* [15] and *A. hijazensis* [18,19]. It is worthwhile then to note that these compounds could serve as a taxonomic marker and therefore, the in-depth investigation of these compounds within the related *Aloe* species cannot be over emphasized so as to resolve the taxonomic instability among the species within the genus *Aloe*.

The isolated compounds were evaluated for their antibacterial activity against four bacterial strains including *Staphylococcus aureus, Enterococcus faecalis, Escherichia coli* and *Pseudomonas aeruginosa*. Disk diffusion method was employed to evaluate the antibacterial activities of the compounds and diameters of inhibition zones are reported in Table 2. The compounds showed antibacterial activity with variable degrees of potency. Compound 4 showed good activity against Gram-negative bacteria (*E. coli* and *P. aeruginosa*) while it has less inhibitory activity against Gram positive bacteria (*S. aureus* and *E. faecalis*). On the other hand, compound 5 exhibited highest zone of growth inhibition (26 mm) on Gram-positive bacteria, *E. faecalis*, which is almost comparable to that of reference drug, gentamycin (27 mm). This is may be related to the polar/none polar nature of the compounds, as the two compounds differ from one another by presence of free phenolic group in compound 5. It is therefore believed that compound 4 with no free phenolic group is less polar and can pass the outer lipid membrane of the Gram-negative bacteria. Whereas, gram positive bacterias which have no such preventive barrier were expected to be most susceptible to the more polar compound 5.

Bacterial Strains	Compounds							Controls	
	1	2	3	4	5	6	7	G (+)	DMSO (-)
E. coli	22	10	23	22	20	18	21	24	-
P. aeruginosa	23	14	21	23	21	20	23	26	-
E. faecalis	21	16	20	18	26	22	22	27	-
S. aureus	20	11	19	19	23	18	20	25	-

Key: These results are average results of three experiments. -: Not active; G: Gentamycin, DMSO: Dimethyl sulfoxide.

Table 2: *In vitro* antibacterial (Diameter of Zone of Growth Inhibition (mm)) activities of the compounds.

Chloroquine-resistant (W2) strain of *Plasmodium falciparum* was used for antiplasmodial activity test with chloroquine (IC_{50}=0.01 µg/mL) as positive controls, as described in previous reports. Compounds 3, 4, 5 and 6 showed antimalarial activity with IC_{50} values of 5.00, 0.92, 12.84 and 8.00 µg/mL respectively whereas the rest of the compounds showed little or no inhibitory activity.

Conclusion

A new dihydroisocoumarin was isolated along with six other known compounds from the rhizome of *A. pulcherrima*. Some of the compounds showed antibacterial activity with the highest activity observed for compound 5, whose activity is comparable to the reference drug (gentamycin) against *E. faecalis*, supports the traditional uses of the plant. This could give insight about the potentials of these compounds as lead structure in development of antibacterial drugs.

Acknowledgement

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Conflicts of Interest

The authors declare that there is no conflict of interest.

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