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Antimalarial Activity of *Aspilia pruliseta*, a Medicinal Plant from Uganda

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Abstract

Aspilia pruliseta Schweinf. (Asteraceae) is a medicinal plant indigenous to Uganda and the neighboring countries of East Africa. It has been used extensively by the rural population for the treatment of fevers and malaria. During the antimalarial evaluation of this plant, four nontoxic diterpenes were isolated that possessed moderate activity against chloroquine-sensitive (D6) and chloroquine-resistant (W2) clones of *Plasmodium falciparum*, with IC_{50} values ranging from 14 to 23 µM. These moderately active compounds included the previously undescribed diterpene, *ent*-15*β*-senecioyloxy-16,17-epoxy-kauran-18-oic acid that demonstrated an IC_{50} value of 23.4 µM against clone D6, but was devoid of activity against clone W2. Four additional diterpenes were obtained from the aerial parts of *A. pruliseta*, but these known compounds were essentially inactive. The moderate activities of select diterpenes of *A. pruliseta* could account collectively for the historical and enduring use of this plant in traditional African medicine.

Key words
malaria · *Plasmodium falciparum* · *Aspilia pruliseta* · diterpenes · Asteraceae · *ent*-15*β*-senecioyloxy-16,17-epoxy-kauran-18-oic acid

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The rural population in Central Uganda has historically embraced *Aspilia pruliseta* Schweinf. (Asteraceae), locally known as Makayi, for its ethnomedical use in the treatment of malaria and fever [1–3]. Other species of *Aspilia*, most notably *A. holstii* and *A. africana*, are also extensively utilized in African traditional medicine for the treatment of a wide range of afflictions [4, 5]. *Aspilia* spp. has reportedly been consumed by chimpanzees [6, 7], presumably for their beneficial pharmacological effects. Complement modulating and antiviral (HIV-1) activities have also been documented for extracts of *A. pruliseta* collected from Rwanda [8, 9]. Chemical constituents of *A. pruliseta* include the strong antibacterial compounds thiarubrin A and kaurenic acid [10, 11]. In an effort to

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Table 1 *In vitro* activities of pure compounds from *A. pruliseta* against *P. falciparum* clones D6 and W2.

	Cytotoxicity** EC_{50} (μM)	Antimalarial activity (*Plasmodium falciparum*) IC_{50} (μM)			
Compounds*	KB cell line	D6	SI#	W2	SI#
2	> 66.2	18.0 ± 0.5	> 4	17.6 ± 0.7	> 4
4	> 50.0	14.3 ± 0.2	> 4	18.4 ± 0.7	> 3
6	> 48.1	23.4 ± 0.6	> 2	17.5 ± 0.6	> 3
8	> 48.1	23.4 ± 0.8	> 2	Inactive	–
Positive control	(vinblastine sulfate) 0.042 ± 0.002	(chloroquine diphosphate) 0.033 ± 0.001	1486	(chloroquine diphosphate) 0.16 ± 0.01	236
Total chloroform extract	> 20 μg/mL	7.89 μg/mL	> 3	8.74 μg/mL	> 2

* Compounds **2, 4, 6**, and **8** were obtained with the following yields: 2.28%, 0.18%, 0.51%, and 0.027%, respectively; ** All compounds were tested at a maximum concentration of 20 μg/mL. # Selectivity Index (SI) = EC_{50} (KB)/IC_{50} (*Plasmodium falciparum*); Inactive: IC_{50} > 50 μM

Table 2 ^{1}H NMR spectral data for compounds **7** and **8** ($CDCl_3$, 300 MHz, TMS as internal standard).

Compound 7	Compound 8
δ 1.0 (3H, s, H-20)	δ 0.99 (3H, s, H-20)
δ 1.25 (3H, s, H-19)	δ 1.25 (3H, s, H-19)
δ 1.81 (3H, s, H-5′)	δ 1.90 (3H, d, *J* = 1.16 Hz, H-4′)
δ 1.84 (3H, s, H-4′)	δ 2.15 (3H, d, *J* = 1.17 Hz, H-5′)
δ 2.76 (1H, d, *J* = 5.65 Hz, H-17)	δ 2.7 (1H, d, *J* = 5.63 Hz, H-17)
δ 3.1 (1H, d, *J* = 5.65 Hz, H-17)	δ 3.1 (1H, d, *J* = 5.63 Hz, H-17)
δ 4.79 (1H, s, H-15)	δ 4.79 (1H, s, H-15)
δ 6.86 (1H, dd, *J* = 5.72 Hz, *J* = 5.69 Hz, 5.76 Hz, H-3′)	δ 5.73 (1H, m, *J* = 1.29 Hz, *J* = 1.30 Hz, H-2′)

characterize the active principles responsible for the reported antimalarial activity of *A. pruliseta*, the total chloroform extract of the aerial parts of this plant was subjected to detailed phytochemical and antimalarial evaluation. It demonstrated modest activity against the D6 and W2 clones of *P. falciparum* with IC_{50} values of 7.89 and 8.74 μg/mL, respectively (**Table 1**). A new diterpenoid, *ent*-15*β*-senecioyloxy-16,17-epoxy-kauran-18-oic acid (**8**) (**Fig. 1**), and seven known diterpenes (**1**-**7**) were isolated during bioactivity-guided fractionation of the total crude extract of *A. pruliseta*.

Connectivity between the ^{1}H NMR resonances of **8** was established from the combined results of ^{1}H-^{1}H COSY, HMQC, and HMBC experiments. In the ^{1}H NMR spectrum (**Table 2**), signals characteristic for protons at positions H-15, H-17, and H-2′ were observed at δ 4.79; both 2.7 and 3.1; and 5.73, respectively. Furthermore, the presence in the molecule of groups such as the 16–17 epoxide, 2′-3′ vinyl carbons, 1′ ketone, and a carboxylic acid moiety was supported by the UV absorption band at λ_{max} 222.5. The ^{13}C NMR studies involving proton decoupling and ^{135}DEPT experiments showed resonances consistent with the presence of vinyl carbons with two methyl protons attached at position 3′ of the senecioyloxy side chain at position 15, which differentiated this compound from compound **7** (**Fig. 1**). The detailed ^{13}C NMR assignments for compound **8** (**Table 3**) were comparable to those reported for closely related known compounds. The major long-range HMBC correlations that differentiated compound **8** from compound **7** were observed from the vinyl and methyl protons of the senecioyloxy chain, in particular, the vinyl proton H-2′ at δ 5.73 with C-4′ and C-5′, the methyl protons H-4′ at δ 1.90 with C-1′, C-2′, and C-5′, and the methyl protons H-5′ at δ 2.15 with C-1′, C-2′, and C-4′ (**Fig. 2**). Low resolution electron impact (LREI) MS of compound **8** gave an [M$^+$] at *m/z* 416.3 consistent with the molecular formula $C_{25}H_{36}O_5$. This molecular formula was further corroborated by positive HREI MS (*m/z* = 439.30451 [M + Na]$^+$; calcd. 439.30309). Absorptions at 1690 cm^{-1} (carboxylic acid), 1720 cm^{-1}, and 1650 cm^{-1} (unsaturated ester) were observed in the IR spectrum. Based on the foregoing spectral data, compound **8** was determined to be *ent*-15*β*-senecioyloxy-16,17-epoxy-kauran-18-oic acid.

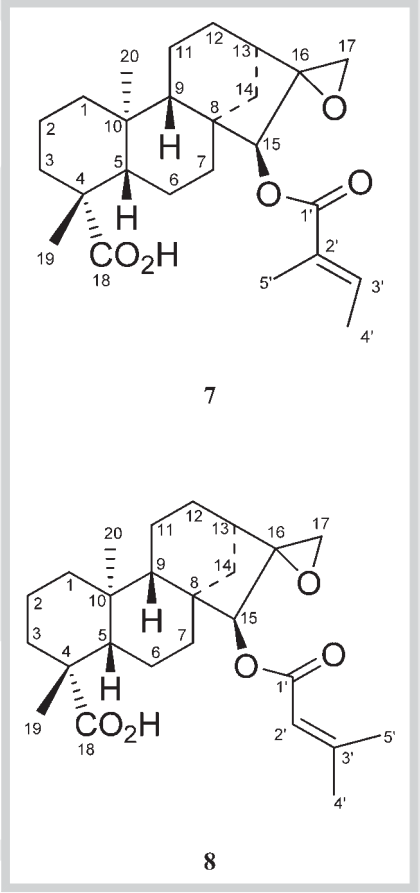

Fig. 1 Select compounds isolated from *A. pruliseta*.

Among the isolates, compounds **2, 4, 6**, and **8** were moderately active against *P. falciparum*, with IC_{50} values ranging from 14.3–

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Table 3 ^{13}C NMR signals of compounds **7** and **8** ($CDCl_3$, 75.5 MHz, TMS as internal standard).

Carbon	Compound 7	Compound 8
C-1	40.6	40.6
C-2	19.8	19.0
C-3	36.7	36.4
C-4	46.9	47.8
C-5	20.3	56.6
C-6	41.2	20.3
C-7	47.8	41.2
C-8	52.9	43.6
C-9	43.6	53.0
C-10	56.6	39.8
C-11	20.8	19.8
C-12	28.9	28.9
C-13	36.4	35.1
C-14	37.7	37.7
C-15	81.2	81.2
C-16	66.4	66.4
C-17	49.6	49.6
C-18	182.6	182.6
C-19	28.9	28.9
C-20	15.8	16.0
C-1′	166.5	166.5
C-2′	129.0	115.9
C-3′	137.1	156.8
C-4′	27.4	20.8
C-5′	20.8	27.4

23.4 µM for clone D6 and 17.5–18.4 µM for clone W2 (**Table 1**). Specifically, the new diterpene **8** exhibited an IC_{50} value of 23.4 µM against clone D6, but was inactive against clone W2 (IC_{50} > 50 µM). Compounds **1, 3, 5**, and **7** were inactive against both clones D6 and W2.

The total chloroform extract, fractions, and all pure compounds isolated were nontoxic to KB cells when tested at 20 µg/mL (**Table 1**). The lack of cytotoxicity enhanced the selectivity of these compounds for *Plasmodium falciparum*. Therefore, although the fractions and pure compounds of *A. pruliseta* demonstrated only moderate antimalarial activity, the present data, backed by the relatively high concentrations of these nontoxic antimalarial diterpenes in the plant, may possibly explain the continued and persistent use of *A. pruliseta* by the rural populations of Uganda and other countries of East Africa for the treatment of fever and malaria.

Materials and Methods

The aerial parts of *A. pruliseta* were collected from Bugiri near Kisubi (along Entebbe road) in Mpigi district, Uganda, in 2002. The plant material was identified by Olivia Wanyana Mangeni, and a voucher specimen (No. APS5) was deposited at the herbarium, Department of Botany, Makerere University.

The previously described diterpenes [12–18] isolated during the bioactivity-guided fractionation of the total chloroform extract of *A. pruliseta* are as follows: *ent*-16-kauren-19-ol (**1**), kaurenic acid (**2**), 15-[(2-methyl-1-oxo-2-butenyl)oxy]-4α,15β(E)-kaur-16-en-18-oic acid (**3**), 15-(2Z)-[(2-methyl-1-oxo-2-butenyl)oxy]-4α,15β-kaur-16-en-18-oic acid (**4**), *ent*-15β-senecioyloxy-kaur-16-en-19-oic acid (**5**), 16,17-epoxy-15-[(2-methyl-1-oxo-2-butenyl)oxy]-4α,15β(Z)-kauran-18-oic acid (**6**), and 16,17-epoxy-15-[(2-methyl-1-oxo-2-butenyl)oxy]-4α,15β(E), kauran-18-oic acid (**7**).

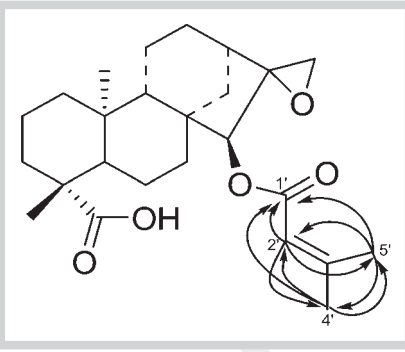

Fig. 2 Key HMBC correlations (H to C) for compound **8**.

The new compound **8** was isolated as a white amorphous powder; t_R 14.4 min, Alltima RP-18 column (10 µ, 4.6 × 250 mm i.d), H_2O/MeOH 3 : 7, 9 mL/min; R_f 0.54, silica gel 60 F_{254}, PET/EtOAc 80 : 20; UV (MeOH) λ_{max} (log ε) 220.5 (1.71) nm; IR (KBr) ν_{max} 1690, 1720, 1650 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75.5 MHz) (**Tables 2** and **3**); LREI-MS *m/z* (rel. int.) 416.3 $[M^+]$ (1); HREI-MS *m/z* 439.30451 $[M + Na]^+$ (calcd. for $C_{25}H_{36}O_5Na$ 439.30309); *anal.* C 72.06%, H 8.65%, calc. for $C_{25}H_{36}O_5$, C 72.06%, H 8.65%. All compounds were determined to be pure by NMR and LREI-MS. In all cases, the purity was assessed as > 97% by analytical HPLC.

The 48-hour [^3H]hypoxanthine-based antimalarial assay was conducted according to established procedures [19–22]. Cytotoxicity of the total extract, fractions, and pure compounds was determined with the KB (human oral epidermoid carcinoma) cell line as previously described [23–25].

Supporting information

HMBC, HMQC, and ^1H NMR spectra of compound **8**, as well as detailed protocols for the antimalarial and cytotoxicity assays, are available as Supporting Information.

Copies of original spectra of all known compounds are obtainable from the author of correspondence.

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