ISSN: 2161-1009
Biochemistry & Analytical Biochemistry

OPEN ACCESS Freely available online

Research Article

Herbicidal Quassinoids Isolated from *Ailanthus altissima* Leaves

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ABSTRACT

Bioassay-guided fractionation of the methanolic extract of *Ailanthus altissima* leaves led to the isolation of one new quassinoid, named 6-α-tigloyloxyailanthone (3), and three known quassinoids, ailanthone (1), 13, 18-dehydroglaucarubinone (2), and 6-α-tigloyloxychaparrinone (4) by a series of chromatographic methods. The structures of the isolates were established by one-dimensional (1D) and 2D-Nuclear Magnetic Resonance (NMR) analysis along with High- Resolution Time-Of-Flight Mass Spectrometry (HRTOFMS) and chemical methods. In addition, herbicidal activity against five grassy weeds and five broad-leaf weeds was evaluated.

Keywords: Quassinoids; Ailanthone; *Ailanthus altissima*; Simaroubaceae; Herbicidal activity

Abbreviations: COSY: Correlation Spectroscop Y; HMBC: Heteronuclear Multiple Bond Correlation; HPLC: High-Performance Liquid Chromatography; HRTOFMS: High-Resolution Time-Of-Flight Mass Spectrometry; NMR: Nuclear Magnetic Resonance; NOE: Nuclear Overhauser Effect; HSQC: Heteronuclear Single Quantum Correlation; ROESY: Rotating-Frame Nuclear Overhauser Effect Spectroscopy

INTRODUCTION

Quassinoids are a group of degraded triterpenes found in the family Simaroubaceae that undergo extensive oxidative biodegradation, leaving their carbon skeleton highly oxygenated [1-4]. Quassinoids are classified into five groups according to their basic structure, C-18, C-19, C-20, C-22, and C-25. The C-20 quassinoids have been extensively investigated due to their antileukemic activity discovered in the early 1970s [5]. Since 1960, hundreds of quassinoids have been isolated and identified from plants, but they seem harder to synthesize due to the presence of their highly oxygenated carbon framework. Many quassinoids display a wide range of biological activities *in vitro* or *in vivo*, including antitumor [6], antimalarial [7], antiviral [8], anti- inflammatory [9], antifeedant [10], insecticidal [11], antiulcer [12], and herbicidal activities [13]. In our ongoing investigations of structurally unique bioactive agents, such as herbicides derived from traditional plants, systematic phytochemical studies of *Ailanthus altissima* resulted in the isolation of one new quassinoid (3) and three known quassinoids (1, 2, and 4). In this paper, we describe the isolation, structural elucidation, and evaluation of the herbicidal activities of all isolates obtained.

MATERIALS AND METHODS

Experimental details

Plant material: Seeds of 10 weed species (*S. bicolor, E. crus-galli, A. smithii, D. sanguiinalis, P. dichotomiflorum, S. nigrum, A. indica, A. avicennae, X. strumarium, C. japonica*) were germinated in flats in a commercial greenhouse substrate and watered with tap water. The weeds were grown in a greenhouse at 30 ± 3/20 ± 3°C day/night temperature with a 14-h photoperiod.

Biological activity: The activity assay was performed 12 days after sowing for the foliar application of samples at each concentration with a laboratory spray gun. The herbicidal activity of the foliar application was evaluated by visual injury 14 days after treatment (0, no damage; 100, complete control).

Extraction and isolation: *A. altissima* leaves (5 kg) collected from Nonsan, Korea were dipped in MeOH at room temperature and filtered after three days. After concentrating the methanol, the extract was defatted with hexane, then partitioned between EtOAc and H_2O. A portion of the active EtOAc fraction (6 g)

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Received: 16-Nov-2023, Manuscript No. BABCR-23-23962; **Editor assigned:** 20-Nov-2023, Pre QC No. BABCR-23-23962 (PQ); **Reviewed:** 04-Dec-2023, QC No. BABCR-23-23962; **Revised:** 11-Dec-2023, Manuscript No. BABCR-23-23962 (R); **Published:** 18-Dec-2023, DOI: 10.35248/2161-1009.23.12.517

Citation: Kim YS, Moon SS, Choi JS (2023) Herbicidal Quassinoids Isolated from *Ailanthus altissima* Leaves. Biochem Anal Biochem. 12:517

was purified by gradient elution on a flash silica gel column using $CHCl_3$:MeOH (50:1, 20:1, 10:1, 5:1, and 100% MeOH). Fraction II [1.03 g, $CHCl_3$-MeOH (20:1)] and fraction III [1.32 g, $CHCl_3$: MeOH (10:1)], which were active in the bioassay, were further purified by ODS Sep-pak cartridge (Alltech, Deerfield, IL, USA) chromatography eluted with an increasing methanol concentration gradient (0%–100%) in water. The active fraction was finally purified by reversed-phase HPLC. Preparative HPLC (C18, 5 μm, 20 × 250 mm; COSMOSIL) was performed using 30%–50% aqueous MeOH, UV detection at 254 nm, a flow rate of 12 mL/min, and gradient elution for 50 min.

Structural analysis: Melting points were measured on a Fisher melting point apparatus and uncorrected. Optical rotations were measured on a Perkin Elmer 341-LC polarimeter. NMR spectra were recorded on a Bruker 900 and 700 spectrometer with standard pulse sequences, operated at 900 and 700 MHz for ^1H NMR and 226 and 176 MHz for ^{13}C NMR. Chemical shifts, measured in ppm, were referenced to solvent peaks (δ_H 2.50 and δ_C 39.5 for DMSO-d_6). HR-TOF-MS spectra were recorded in the positive ESI mode on a Waters Synapt G2 at the Korea Basic Science Institute.

Compound 1 (ailanthone):

^1H NMR (DMSO-d_6, 900 MHz) δ: 8.23 (^1H, s, 11-OH), 7.08 (^1H, d, J = 2.8 Hz, 1-OH), 5.99

(^1H, d, J = 0.9 Hz, H-3), 5.40 (^1H, d, J = 4.5 Hz, 12-OH), 5.05 (^1H, d, J = 0.9 Hz, Ha-21), 5.02

(^1H, d, J = 0.9 Hz, H_b-21), 4.55 (^1H, t, J = 2.8 Hz, H-7), 4.28 (^1H, s, H-1), 3.81 (^1H, d, J = 9.0

Hz, Ha-20), 3.27 (^1H, d, J = 9.0 Hz, H_b-20), 3.67 (^1H, d, J = 3.6 Hz, H-12), 2.88 (^1H, d, J = 10.8

Hz, H-5), 2.98 (1H, dd, J = 18.0, 14.4 Hz, Ha-15), 2.81 (^1H, s, H-9), 2.77 (^1H, dd, J = 13.5, 5.4,

H-14), 2.44 (^1H, dd, J = 18.0, 5.4 Hz, H_b-15), 2.03 (2H, m, H-6), 1.93 (3H, s, H-18), 1.06 (3H, s,

H-19) ppm; ^{13}C NMR (225 MHz, DMSO-d_6): 197.1 (C2), 169.1 (C16), 162.4 (C4), 146.6 (C13),

125.0 (C3), 117.6 (C21), 108.8 (C11), 82.4 (C1), 79.0 (C12), 77.5 (C7), 71.1 (C_{20}), 46.1 (C14),

44.5 (C8 and C10), 43.3 (C9), 41.2 (C5), 34.3 (C15), 25.1 (C6), 22.4 (C18), and 9.5 (C19) ppm;

HRTOFMS (positive ESI mode) m/z 399.1415 $[M + Na]^+$ (calcd for $C_{20}H_{24}O_7$+Na, 399.1420).

Compound 2 (13, 18-dehydroglaucarubinone):

^1H NMR (DMSO-d_6, 700 MHz) δ: 8.33 (^1H, s, 11-OH), 7.16 (^1H, d, J = 2.1 Hz, 1-OH), 5.99

(^1H, q, J = 0.7 Hz, H-3), 5.64 (^1H, br s, H-15), 5.35 (^1H, br s, 12-OH), 5.11 (^1H, s, 2'-OH), 5.09

(^1H, d, J = 1.4 Hz, H_a-21), 4.99 (^1H, br s, H_b-21), 4.70 (^1H, t, J = 2.8 Hz, H-7), 4.42 (^1H, d, J =

2.1 Hz, H-1), 3.81 (^1H, d, J = 8.4 Hz, H_a-20), 3.67 (^1H, d, J = 4.9 Hz, H-12), 3.32 (^1H, dd, J =

8.4 Hz, H_b-20), 3.08 (^1H, d, J = 11.9 Hz, H-5), 3.04 (^1H, d, J = 11.4 Hz, H-14), 2.97 (^1H, s, H-9),

2.06 (2H, m, H-6), 1.93 (3H, s, H-18), 1.70 (^1H, dq, J = 140.0, 7.0 Hz, H_a-3'), 1.53 (^1H, dq, J =

140.0, 7.0 Hz, H_b-3'), 1.30 (3H, s, H-5'), 1.06 (3H, s, H-19), 0.81 (3H, t, J = 7.0 Hz, H-4') ppm;

^{13}C NMR (176 MHz, DMSO-d_6): 197.0 (C2), 174.2 (C1'), 166.7 (C16), 162.4 (C4), 142.4 (C13),

124.8 (C3), 119.8 (C21), 108.6 (C11), 82.1 (C1), 78.7 (C12), 77.8 (C7), 73.9 (C2'), 70.7 (C_{20}),

68.2 (C15), 50.2 (C14), 46.4 (C8), 44.5 (C10), 44.0 (C9), 40.7 (C5), 32.8 (C3'), 25.8(C5'), 24.6 (C6), 22.2 (C18), 9.5 (C19), and 8.0 (C4') ppm; HRTOFMS (positive ESI mode) m/z 499.194

$[M + Na]^+$ (calcd for $C_{25}H_{32}O_9$+Na, 499.1944) and m/z 975.3983 $[2M + N]^+$ [calcd for $(C_{25}H_{32}O_9)_2$+Na, 975.3990].

Compound 3 (6-α-tigloyloxyailanthone):

^1H NMR (DMSO-d_6, 700 MHz) δ: 8.15 (^1H, s, 11-OH), 7.28 (^1H, d, J = 2.8 Hz, 1-OH), 6.91 (dq,

J = 7.7, 2.1 Hz, H3'), 6.02 (^1H, s, H-3), 5.55 (^1H, d, J = 4.2 Hz, 12-OH), 5.49 (^1H, dd, J = 11.9,

2.8 Hz, H-6), 5.02 (^1H, d, J = 1.4 Hz, H_a-21), 5.01 (^1H, s, H_b-21), 4.63 (^1H, d, J = 2.8 Hz, H-7),

4.39 (^1H, s, H-1), 3.91 (^1H, d, J = 8.4 Hz, H_a-20), 3.69 (^1H, t, J = 4.9 Hz, H-12), 3.46 (^1H, d, J =

11.9 Hz, H-5), 3.39 (^1H, dd, J = 8.4 Hz, H_b-20), 3.01 (^1H, dd, J = 18.4, 13.3 Hz, H_a-15), 2.85

(^1H, dd, J = 13.3, 5.6 Hz, H-14), 2.83 (^1H, s, H-9), 2.47 (^1H, dd, J = 18.4, 5.6 Hz, H_b-15), 1.94

(3H, s, H-18), 1.829 (3H, s, H5'), 1.823 (3H, d, J = 4.2 Hz, H4'), 1.21 (3H, s, H-19) ppm; ^{13}C

NMR (176 MHz, DMSO-d_6): 196.6 (C2), 168.2 (C16), 165.9 (C1'), 161.2 (C4), 146.1 (C13),

139.2 (C3'), 127.9 (C2'), 127.6 (C3), 117.5 (C21), 109.0 (C11), 82.4 (C1), 79.1 (C12), 77.5 (C7),

70.2 (C_{20}), 67.3 (C6), 47.2 (C10), 46.1 (C14), 45.2 (C8), 44.1 (C5), 42.0 (C9), 34.0 (C15), 24.7 (C18), 14.4 (C4'), 11.9 (C5'), and 10.9 (C19) ppm; HRTOFMS (positive ESI mode) m/z 497.1785 $[M + Na]^+$ (calcd for $C_{25}H_{30}O_9$+Na, 497.1788) and m/z 971.3670 $[2M + N]^+$ [calcd for $(C_{25}H_{30}O_9)$2+Na, 971.3678].

Compound 4 (6-a-tigloyloxychaparrinone):

^1H NMR (DMSO-d_6, 700 MHz) δ: 8.0 (^1H, s, 11-OH), 7.19 (^1H, d, J = 2.8 Hz, 1-OH), 6.91 (dq,

J = 7.7, 2.1 Hz, H3'), 6.0 (^1H, s, H-3), 5.49 (^1H, dd, J = 11.9, 2.8 Hz, H-6), 5.14 (^1H, d, J = 4.9

Hz, 12-OH), 4.56 (^1H, d, J = 2.8 Hz, H-7), 4.30 (^1H, d, J = 2.8 Hz, H-1), 3.93 (^1H, d, J = 8.4 Hz,

H_a-20), 3.64 (^1H, dd, J = 8.4 Hz, H_b-20), 3.39 (^1H, d, J = 11.9 Hz, H-5), 3.21 (^1H, t, J = 4.9 Hz,

H-12), 2.72 (^1H, dd, J = 18.2, 13.3 Hz, H_a-15), 2.61 (^1H, s, H-9), 2.40 (^1H, dd, J = 18.2, 5.6 Hz,

H_b-15), 2.09 (^1H, m, H-13), 2.05 (^1H, m, H-14), 1.94 (3H, s, H-18), 1.82 (3H, br s, H5'), 1.81

(3H, d, J = 7.7 Hz, H4'), 1.22 (3H, s, H-19), 0.85 (3H, d, J = 7.0 Hz, H-21) ppm; ^{13}C NMR (176

MHz, DMSO-d_6): 196.5 (C2), 168.9 (C16), 165.8 (C1'), 161.2 (C4), 139.0 (C3'), 127.8 (C2'),

127.5 (C3), 109.1 (C11), 82.5 (C1), 78.1 (C12), 77.6 (C7), 69.3 (C_{20}), 67.3 (C-6), 47.1 (C10),

45.7 (C8), 44.1 (C5), 41.8 (C9), 40.6 (C14), 30.2 (C13), 29.3 (C15), 24.5 (C18), 14.3 (C4'), 12.5 (C21), 11.8 (C5'), and 10.8 (C19) ppm; HRTOFMS (positive ESI mode) m/z 499.1944 $[M + Na]^+$ (calcd for $C_{25}H_{32}O_9$+Na, 499.1944) and m/z 975.3983 $[2M + N]^+$ [calcd for $(C_{25}H_{32}O_9)_2$+Na, 975.3990].

RESULTS AND DISCUSSION

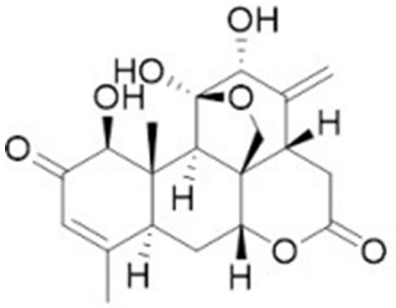
The MeOH extract of *Ailanthus altissima* leaves at 10 mg/mL showed 100% control of *Sorghum bicolor, Digitaria sanguinalis, Aeschynomene indica,* and *Abutilon avicennae*, and >90% control of *Echinochlia crus-galli, Panicum dichotomiflorum, Xanthium strumarium,* and *Calystegia japonica*. Herbicidal activity against *Agropyron smithii* was slightly weaker (Table 1). The main characteristic was its quick action upon foliar application. External symptoms began to appear within 24 h of exposure, and the weeds were completely controlled five days after treatment. MeOH extract showing herbicidal activity was purified by ethyl acetate fractionation and a series of chromatographic techniques, including silica gel column, C18 Sep-pak cartridge, Sephadex LH20, and preparative High-Performance Liquid Chromatography (HPLC). Four quassinoids, 1-4 (Figure 1), were isolated from the MeOH extract of *Ailanthus altissima* leaves and showed herbicidal activity of 98, 95, 85, and 75%, respectively, when applied to *D. sanguiinalis* at a concentration of 10 μg/mL (Table 2 and Figure 2).

Among these, 3 was a new compound, and the characterization and spectroscopic analysis of this compound were performed by data comparison with literature values [14]. The ^{1}H and ^{13}C NMR (nuclear magnetic resonance) data for these compounds are shown in the experimental section. The ^{1}H (Table 3) and ^{13}C NMR (Table 4) signals assignments were aided by HSQC (Heteronuclear Single Quantum Correlation), HMBC (Heteronuclear Multiple Bond Correlation), and ^{1}H-^{1}H COSY (Correlation Spectroscop Y) experiments. The other compounds were identified as ailanthone (1) [15], 13, 18-dehydroglaucarubinone (2) [16], and 6-α-tigloyloxychaparrinone (4) [17,18]. Compound 3 was obtained as a white solid. The molecular formula was established as $C_{25}H_{32}O_9$ based on a quasi-molecular ion at m/z 497.1785 $[M + Na]^+$ (calcd 497.1788) in its HRTOFMS. The ^{1}H NMR spectrum of 3 displayed signals for two olefinic protons [δ_H 6.91 (^{1}H, dq, J = 7.7, 2.1, H-3'), 6.02 (^{1}H, s, H-3)], an exo-methylene [δ_H 5.02 (^{1}H, d, J = 2.1, H_a-21), 5.01 (^{1}H, s, H_b-21)], four oxygenated methines [δ_H 5.49 (^{1}H, dd, J = 11.9, 2.8, H-6), 4.63 (^{1}H, d, J = 2.8, H-7), 4.39 (^{1}H, s, H-1), 3.69 (^{1}H, d, J = 3.5, H-12)], one oxygenated methylene [δ_H 3.91 and 3.39 (each ^{1}H, d, J = 8.4, H-20)], one methylene proton [δ_H 3.01 (^{1}H, dd, J = 18.4, 13.3, H_a- 15) and 2.40 (^{1}H, dd, J = 18.4, 5.6, H_b-15)] and four methyl groups [δ_H 1.94 (3H, s, H-18), 1.829 (3H, br s, H-5'), 1.823 (3H, d, J = 7.7, H-4'), and 1.21 (3H, s, H-19)]. The ^{13}C NMR spectrum exhibited 25 carbon signals, including three carbonyl signals, six olefinic carbon signals, one hemiketal carbon signal, four methyl carbon signals, two methylene carbon signals, seven methine carbon signals, and two quaternary carbon signals. Comparison of the NMR data of 3 with those of ailanthone (1) revealed that the two compounds possessed the same quassinoid moiety, except that the C6 position in ailanthone (1) was substituted with a tigloyloxy group in 3. The ^{1}H-^{1}H COSY between H-3' (δ_H 6.91) and H-4' (δ_H 1.823) and the HMBC correlation between H-3' (δ_H 6.91), H-5' (δ_H 1.829), and C1' (δ_C 165.8) and H-4' (δ_H 1.81) and C-2' (δ_C 127.8) confirmed the existence of a tigloyloxy group and that it was connected to the C6 position of 3, established by a long-range correlation between δ_H 5.49 (H-6 of quassinoid moiety) and carboxylic carbon (δ_C 165.9, C1' of tigloyloxy group) in the HMBC spectrum of 3. The correlations between H-9 and H-1/H-5 and between H-7 and H-14 in the ROESY spectrum suggested that the configuration of 3 was the same as that of ailanthone (1). And the ROESY correlation between H-3' and H-5' indicated that the geometry of the double-bond of the tigloyloxy group was Z-form. Thus, the structure of compound 3 was determined to be 6-α- tigloyloxyailanthone. The relative stereochemistry of 3 was confirmed from its 2D-ROESY NMR spectrum. The NOE correlations are shown by arrows in Figure 3 (2D-ROESY).

Table 1: Herbicidal activity of foliar application of methanol extracts from *Ailanthus altissima* to several weeds in a greenhouse condition.

Rates (mg/mL)	Herbicidal activity (%)									
	SORBI	ECHCG	AGRSM	DIGSA	PANDI	SOLNI	AESIN	ABUTH	XANSI	CAGHE
10	100	95	30	100	98	70	100	100	90	95
5	40	40	20	100	70	60	100	80	40	70
2.5	40	30	10	90	20	20	98	98	40	70
1	40	30	10	70	20	20	95	98	40	70

Note: SORBI: Sorghum bicolor, ECHCG: Echinochloa crus-galli, AGRSM: Agropyron smithii, DIGSA: *Digitaria sanguinalis*, PANDI: Panicum dichotomiflorum, SOLNI: Solanum nigrum, AESIN: Aeschynomene indica, ABUTH: Abutilon avicennae, XANSI: Xanthium strumarium, CAGHE: Calystegia japonica.



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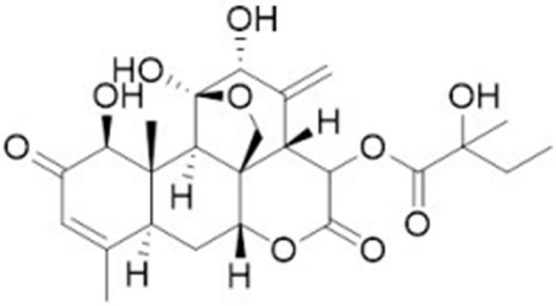

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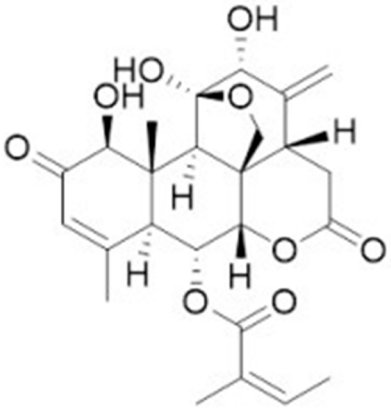

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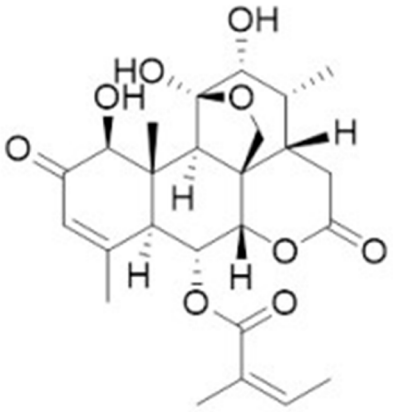

4

Figure 1: Structures of quassinoids compounds 1-4.

Table 2: Herbicidal activity of isolated compounds against *Digitaria sanguinalis*.

	Herbicidal activity at 10 μg/mL (%)
Compound 1	98
Compound 2	95
Compound 3	85
Compound 4	75

Figure 2: Herbicidal activity against *Digitaria sanguinalis* at same concentration of 10 μg/mL.

Table 3: 1H-NMR Data (DMSO-d6) for compounds 1-4.

Proton	Compound	121 M 78 F	121 M 78 F	121 M 78 F
	1	2	3	4
H-1	4.28(s)	4.42 (d, 2.1)	4.39 (s)	4.30 (d, 2.8)
H-3	5.99 (d, 0.9)	5.99 (q, 0.7)	6.02 (s)	6.0 (s)
H-5	2.88 (d, 10.8)	3.08 (d, 11.4)	3.46 (d, 11.9)	3.39 (d, 11.9)
H-6	2.03 (m)	2.06 (m)	5.49 (dd, 11.9,2.8)	5.49 (dd, 11.9, 2.8)
H-7	4.55 (t, 2.8)	4.70 (t, 2.8)	4.63 (d, 2.8)	4.56 (d, 2.8)
H-9	2.81 (s)	2.97(s)	2.83(s)	2.61(s)
H-12	3.67 (d, 3.6)	3.67 (d, 4.9)	3.69 (d, 3.5)	3.21 (t, 4.9)
H-13	-	-	-	20.9 (m)
H-14	2.77 (dd, 13.5, 5.4)	3.04 (d, 11.4)	2.85 (dd, 13.3, 5.6)	2.05 (m)
Ha-15	2.98 (dd, 18.4, 13.5)	5.64 (br s)	3.01 (dd, 18.4, 13.3)	2.72 (dd, 18.2, 13.3)
Hb-15	2.44 (dd, 18.4, 5.4)		2.47 (dd, 18.4, 5.6)	2.40 (dd, 18.2, 5.6)
H-18	1.93 (s)	1.93 (s)	1.94 (s)	1.94 (s)
H-19	1.06 (s)	1.06 (s)	1.21 (s)	1.22 (s)
Ha-20	3.81 (d, 9.0)	3.81 (d, 8.4)	3.91 (d, 8.4)	3.93 (d, 8.4)
Hb-20	3.27 (d, 9.0)	3.32 (d, 8.4)	3.39 (d, 8.4)	3.64 (d, 8.4)
Ha-21	5.05 (d, 0.9)	5.09 (d, 1.4)	5.02 (d, 1.4)	-
Hb-21	5.02 (d, 0.9)	4.99 (br s)	5.01 (br s)	-
Ha-3'	-	1.70 (dq, 14.0,7.0)	6.91 (dq, 7.7,2.1)	6.91 (dq, 7.7, 2.1)
Hb-3'	-	1.53 (dq, 14.0,7.0)	-	-
H-4'	-	0.81 (t, 7.0)	1.823 (d, 4.2)	1.81 (d, 7.7)
H-5'	-	1.30 (s)	1.829 (s)	1.82 (br s)
1-OH	7.08 (s)	7.16 (d, 2.1)	7.28 (s)	7.19 (d, 2.8)
11-OH	8.23 (s)	8.33 (s)	8.15 (s)	8.0 (s)
12-OH	5.40 (d, 4.5)	5.35 (br s)	5.55 (d, 4.2)	5.14 (d, 4.9)

Table 4: 13C-NMR Data (DMSO-d6) for compounds 1-4.

Carbon	Compound			
	1	2	3	4
C-1	82.4	82.1	82.4	82.5
C-2	197.1	197	196.6	196.5
C-3	125	124.8	127.6	127.5
C-4	162.4	162.4	161.1	161.2
C-5	41.2	40.7	44.1	44.1
C-6	25.1	24.6	67.3	67.3
C-7	77.5	77.8	77.6	77.6

C-8	44.5	46.4	45.2	45.7
C-9	43.3	44	42	41.8
C-10	44.5	44.5	47.2	47.1
C-11	108.8	108.6	109	109.1
C-12	79	78.7	79.1	78.1
C-13	146.6	142.4	146.1	30.2
C-14	46.1	50.2	46.1	40.6
C-15	34.3	68.2	34	29.3
C-16	169.1	166.7	168.2	168.9
C-17	22.4	22.2	24.7	24.5
C-18	9.5	9.5	10.9	10.8
C-19	71.1	70.7	70.2	69.3
C-20	117.6	119.8	117.5	12.5
C-1'	-	174.2	165.9	165.8
C-2'	-	73.9	127.9	127.8
C-3'	-	32.8	139.2	139
C-4'	-	8	14.4	14.3
C-5'	-	25.8	11.9	11.8

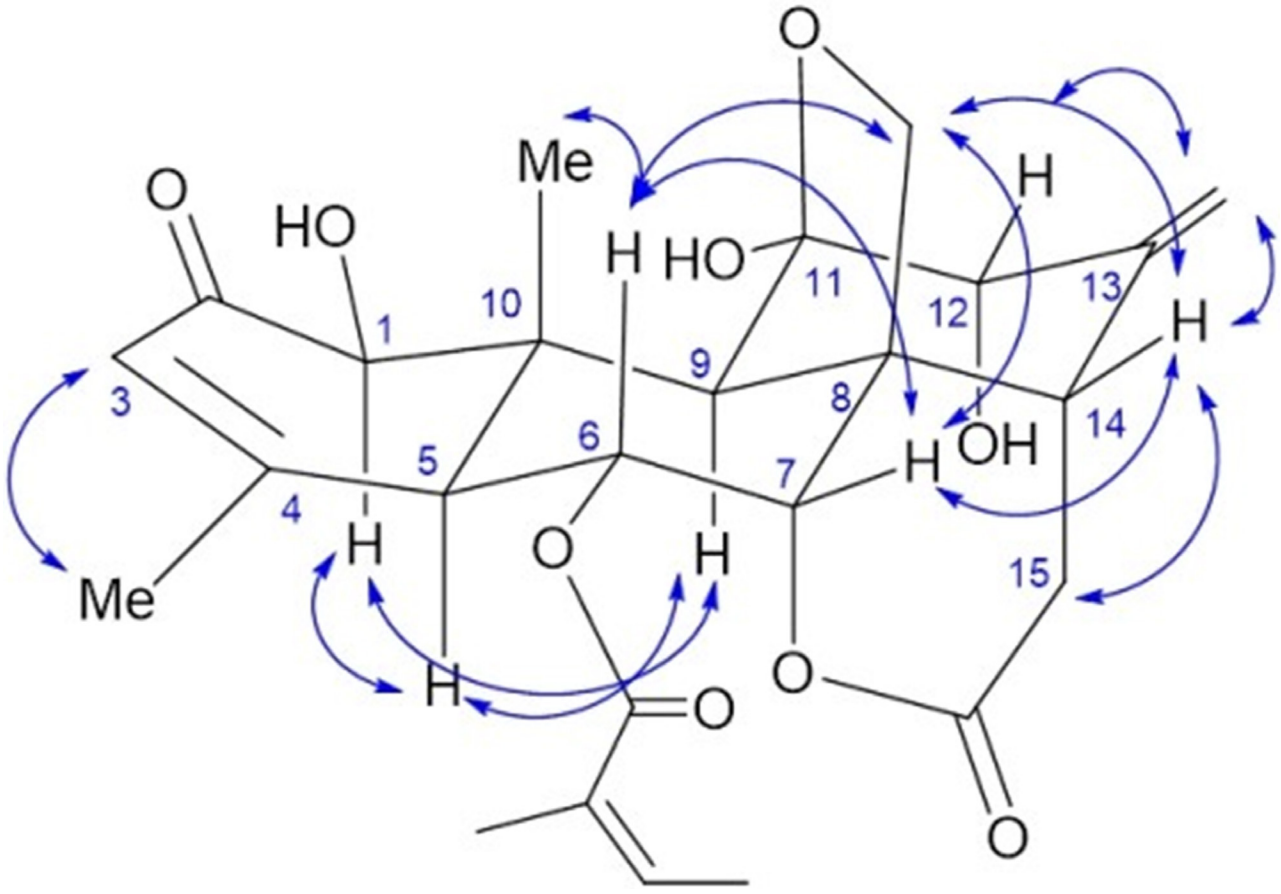

Figure 3: 2D ROESY (NOE) correlation of 3.

CONCLUSION

Herbicidal activity of methanol extract of *A. altissima* leaves with foliar application on *D. sanguiinalis* at 2, 5, and 10 mg mL-1 was 90, 100 and 100%, respectively, the main herbicidal symptoms were chlorosis or burn-down and followed by necrosis and eventual death. On 10 weed species, the methanol extract showed strong herbicidal activity and it showed excellent herbicidal activity particularly on *A. indica* and *A. avicennae* even at the lowest concentration of 1 mg mL-1. Three known quassinoid compounds (1, 2, 4) and one unknown compound (3) isolated from methanol extract showed herbicidal activity of 98, 95, 75, and 85%, respectively, against D. sanguinalis at 10 μg mL-1. Compound 3, a white solid, was identified as 6-α- tigloyloxyailanthone with a molecular formula of $C_{25}H_{32}O_9$ by the analyses of HR-TOF-MS and ^1H and ^{13}C NMR and 2D NMR spectral data.

This study showed that *A. altissima* extracts have potential as bioherbicide and one unknown compounds may be used as lead compounds for development of new herbicides.

SUPPORTING INFORMATION

Structures and spectra of compound 1, 2, 3 and 4 (Figures S1-S16).

AUTHOR CONTRIBUTIONS

The authors confirm contribution to the paper as follows: study conception and design: Y. S. Kim, S. S. Moon; data collection: J. S. Choi, S. S. Moon; analysis and interpretation of results: Y. S. Kim, S. S. Moon, J. S. Choi; draft manuscript preparation: Y. S. Kim, J. S. Choi. All authors reviewed the results and approved the final version of the manuscript.

ACKNOWLEDGMENTS

This work was carried out with the support of the Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ01612820 23), Rural Development Administration, Republic of Korea.

COMPETING INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

1. Cormier MJ, Flasch H, Franck B, Hori K, Jaenicke L, Keller-Schierlein W, et al. Quassinoid bitter principles. Fortschr Chem Org Naturs.1973:101-150.
2. Vieira IJ, Braz-Filho R. Quassinoids: structural diversity, biological activity and synthetic studies. Stud Nat Prod. 2006;33:433-492.
3. Guo Z, Vangapandu S, Sindelar RW, Walker LA, Sindelar RD. Biologically active quassinoids and their chemistry: Potential leads for drug design. Curr Med chem. 2005;12(2):173-190.
4. Alves IA, Miranda HM, Soares LA, Randau KP. Simaroubaceae family: Botany, chemical composition and biological activities. Rev Bras Farmacogn 2014;24:481-501.
5. Elson S, Howe I, Jarman M, McDougal PG, Polonsky J, Schmuff NR, et al. Quassinoid bitter principles II. Fortschr Chem Org Naturst.1985:221-264.
6. Fukamiya N, Okano M, Miyamoto M, Tagahara K, Lee KH. Antitumor agents, 127. Bruceoside C, A new cytotoxic quassinoid glucoside, and related compounds from *Brucea javanica*. J Nat Prod. 1992;55(4):468-475.
7. Ang HH, Chan KL, Mak JW. *In vitro* antimalarial activity of quassinoids from *Eurycoma longifolia* against Malaysian chloroquine-resistant *Plasmodium falciparum* isolates. Planta Medica. 1995;61(02):177-178.
8. Apers S, Cimanga K, Berghe DV, Van Meenen E, Longanga AO, Foriers A, et al. Antiviral activity of simalikalactone D, a quassinoid from *Quassia africana*. Planta Med. 2002;68(01):20-24.
9. Silva RL, Lopes AH, França RO, Vieira SM, Silva EC, Amorim RC, et al. The quassinoid isobrucein B reduces inflammatory hyperalgesia and cytokine production by post-transcriptional modulation. J Nat Prod. 2015;78(2):241-249.
10. Leskinen V, Polonsky J, Bhatnagar S. Antifeedant activity of quassinoids. J Chem Ecol. 1984;10(10):1497-1507.
11. Daido M, Fukamiya N, Okano M, Tagahara K, Hatakoshi M, Yamazaki H, et al. Antifeedant and insecticidal activity of quassinoids against diamondback moth (*Plutella xylostella*). Biosci Biotechnol Biochem. 1993;57(2):244-246.
12. Tada H, Yasuda F, Otani K, Doteuchi M, Ishihara Y, Shiro M, et al. New antiulcer quassinoids from *Eurycoma longifolia*. Eur J Med Chem 1991;26:345-349.
13. Heisey RM, Kish Heisey T. Herbicidal effects under field conditions of *Ailanthus altissima* bark extract, which contains ailanthone. Plant Soil. 2003;256:85-99.
14. Kubota K, Fukamiya N, Tokuda H, Nishino H, Tagahara K, Lee KH et al. Quassinoids as inhibitors of Epstein-Barr virus early antigen activation. Cancer Letters 1997;113()1-2:165-168.
15. Kubota K, Fukamiya N, Hamada T, Okano M, Tagahara K, Lee KH, et al. Two new quassinoids, ailantinols A and B, and related compounds from *Ailanthus altissima*. J Nat Prod 1996;59(7):683-686.
16. Polonsky J, Varon Z, Jacquemin H, Pettit GR. The isolation and structure of 13, 18-dehydroglaucarubinone, a new antineoplastic quassinoid from Simarouba amara. Experientia. 1978;34:1122-1123.
17. Carter CA, Tinto WF, Reynolds WF, McLean S. Quassinoids from Quassia multiflora: Structural assignments by 2D Nmr spectroscopy. J Nat Prod.1993;56(1):130-133.
18. Okunade AL, Bikoff RE, Casper SJ, Oksman A, Goldberg DE, Lewis WH, et al. Antiplasmodial activity of extracts and quassinoids isolated from seedlings of *Ailanthus altissima* (Simaroubaceae). Phytotherapy Research 2003;17(6):675-677.