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Iridoid glycosides from the root of *Acanthus sennii*

[Glicósidos iridoides de la raíz de *Acanthus sennii*]

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Abstract

Context: *Acanthus sennii* is a plant traditionally used for the treatment of antifungal, cytotoxic, anti-inflammatory, antipyretic, antioxidant, insecticidal, hepatoprotective, immunomodulatory, anti-platelet aggregation and anti-viral potential.

Aims: To investigate the phytochemical constituents of roots of *Acanthus sennii*.

Methods: Phytochemical screening tests were conducted to identify the class of compounds present in the root extract. Silica gel column chromatographic technique was applied to separate the constituents of the extracts. Various spectroscopic techniques (IR, ^{1}H NMR, ^{13}C NMR, DEPT-135, COSY, gHSQC, and gHMBC) were applied to determine the structures of isolated compounds.

Results: Phytochemical screening of the dichloromethane/methanol (1:1) and methanol (100%) root extract of the plant revealed the presence of phenolic compounds, steroids, flavonoids, and terpenes. Chromatographic separation of dichloromethane/methanol (1:1) root extract of *Acanthus sennii* yielded two iridoid glycosides (**1**, **2**).

Conclusions: The roots of *Acanthus senni* contain various class of constituents such as flavonoids, phenols, terpenoids, tannins, and iridoid glycosides identified through phytochemical screening test and purification process, which might be responsible for the traditional use of the plant. To the best of our knowledge, these compounds are isolated for the first time from this genus.

Keywords: *Acanthus sennii;* phytochemical screening; iridoid glycosides.

Resumen

Contexto: *Acanthus sennii* es una planta usada tradicionalmente para el tratamiento antifúngico, citotóxico, anti-inflamatorio, antipirético, antioxidante, insecticida, hepatoprotector, inmunomodulador, antiagregante plaquetario y potencial antiviral.

Objetivos: Investigar los constituyentes fitoquímicos de la raiz de *Acanthus sennii*.

Métodos: Se llevaron a cabo pruebas de detección de fitoquímicos para identificar la clase de compuestos presentes en el extracto de raíz. Técnicas de cromatografía en columna de sílica gel fueron aplicadas para separar los constituyentes de los extractos. Varias técnicas espectroscópicas (IR, ^{1}H NMR, ^{13}C NMR, DEPT-135, COSY, gHSQC, and gHMBC) fueron aplicadas para determinar las estructuras de los compuestos aislados.

Resultados: El cribado fitoquímico de los extractos de raíz de la planta en diclorometano/metanol (1:1) y metanol (100%) revelaron la presencia de compuestos fenólicos, esteroides, flavonoides y terpenos. La separación cromatográfica del extracto de diclorometano/metanol (1:1) de raíz de *Acanthus senni* rindió dos glicósidos iridoides (**1, 2**).

Conclusiones: Las raíces de *Acanthus senni* contienen varias clases de constituyentes como, flavonoides, fenoles, taninos y glicósidos iridoides, identificados a través de pruebas fitoquímicas y procesos de purificación, los cuales pueden ser los responsables del uso tradicional de la planta. Hasta donde alcanza nuestro conocimiento, estos compuestos se aislaron por primera vez a partir de este género.

Palabras Clave: *Acanthus sennii*; cribado fitoquímico; glicósidos iridoides.

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INTRODUCTION

Acanthus is a genus of flowering plants belongs to the family *Acanthaceae*. The *Acanthus* family is fairly large with some 2500-3000 species in about 250 genera. The family is distributed in tropical and subtropical habitats mainly around Indo-Malaysia, Africa, Brazil and Central America. The *Acanthaceae* family possess antifungal, cytotoxic, anti-inflammatory, anti-pyretic, antioxidant, antiviral, insecticidal, hepatoprotective, immunomodulatory, and anti- platelet aggregation (Tripetch et al., 2001; Tomas et al., 2009; Awan and Aslam, 2014; Ming-Yu et al., 2014).

Acanthus sennii (Fig. 1) is endemic to Ethiopia (Melesse et al., 2015; Teshome and Kelbessa, 2013) with different local names; i.e. *Kusheshile* (Amharic) (Ermias et al., 2011; Muthuswamy and Abay, 2009), *Chocha* (Kembatigna), (Melesse et al., 2015), *Sokoro* (Oromifa) (Rainer et al., 2011).

Figure 1. Picture of *A. sennii*
(Photo taken by Etagen A., Wolaita Sodo, November 2015).

Acanthus sennii is traditionally used for treating scorpion sting with root decoction given orally and to treat bleeding and stabbing pain leaf paste is applied (Giday et al., 2007; Awas and Demissew, 2009; Muthuswamy and Abay, 2009). The leaves are used as a medicine with butter and applied to wounds (Rainer et al., 2011). In our ongoing project to study the medicinal plants of southern Ethiopia, we hereby present a comprehensive phytochemical analysis of the roots of *A. sennii*.

MATERIAL AND METHODS

Instrumentation

UV-Vis spectrum was measured with GENESY's spectrometer (200-600 nm) in methanol at room temperature. Infrared (KBr pellet) spectrum was recorded on Perk-Elmer BX infrared spectrometer in the range 400-4000 cm^{-1}. Nuclear Magnetic Resonance (NMR) analysis was recorded on a Bruker Avance 400 MHz spectrometer with tetramethylsilane (TMS) as internal standard and deuterated dimethyl sulfoxide (DMSO-d_6) as solvent. Structural assignments were done on the basis of ^{1}H NMR, ^{13}C NMR, DEPT-135 chemical shift value, Gradient Heteronuclear Multiple Quantum Coherence (gHMQC), Heteronuclear Single Quantum Coherence Spectroscopy (gHSQC), Gradient Heteronuclear Multiple Bond Coherence (gHMBC), and Correlation Spectroscopy (COSY) spectra. Thin Layer Chromatography (TLC) was done using silica gel 60 F254. Column chromatography was performed on silica gel 60 (60-100 mesh).

Plant material collection and preparation

The root of *A. sennii* material was collected in November 2015 in Southern Nations, and Nationalities Peoples Region, Wolaita zone, Wolaita Sodo area, which is 329 km from Addis Ababa, the capital of Ethiopia and 150 km from Hawassa University. The plant species was confirmed by botanist Mr. Markos Kuma from Wolaita Sodo University and the specimen was deposited at the herbarium of Wolaita Sodo University, Ethiopia (AS-001-2016). The collected root part of *A. sennii* was dried and grinded in to powder using mortar and was made ready for further analysis.

Preparation of crude dichloromethane/methanol extraction

The grounded (500 g) root of was *Acanthus sennii* soaked into 3 L of dichloromethane/methanol (1:1) solvents at room temperature in the Erlenmeyer flask. After shaking well, the flask containing the solution was put on an orbital shaker and left for 12 h at a speed of 120 revolutions per minute. After 12 h the solution was filtered using 15 cm size Whatman filter paper. The filtered solution was concentrated using rotary evaporator at a temperature of about 40-45°C, air dried and weighted to yield 35 g of a brownish crude extract.

Isolation and purification of compounds

About 35 g of the brownish methanol extract was subjected to silica gel column chromatographic separation (150 g) silica gel) and eluted with increasing gradient ethyl acetate in *n*-hexane followed by methanol (100%). A total of 34 fractions (each 30 mL) were collected. The constituent profile of each fraction was monitored by TLC (70:30; *n*-hexane/ethyl acetate) and visualized under UV-Vis light (λ 254 and 366 nm). Fractions 27-31 showed two spots each on TLC result in (70:30) *n*-hexane/ethyl acetate solvent system in both wave lengths (254 and 365 nm). Then, these fractions were combined, dried and washed sequentially with *n*-hexane for several times and the TLC of each precipitate was checked. One clear spot with R_f value of 0.42 was observed (compound **2**, 45 mg). Fractions 32-34 showed single spot with a minor impurity with R_f value 0.64 in *n*-hexane/ethyl acetate (70:30) solvent system. Thus, these three fractions were combined and washed continuously with *n*-hexane, monitored by TLC, to give a yellowish powder (compound **1**, 10 mg).

Preliminary phytochemical screening

Phytochemical screening tests were carried out on the crude extract of dichloromethane/methanol (1:1) and 100% methanol using standard procedures to identify the chemical constituents present (Ganesh and Vennila, 2011; Tiwari et al., 2011).

Test for alkaloids

To 1 mL of 1% HCl was added to 3 mL of the extract in a test tube. The mixture was heated for 20 min, cooled and filtered. Then, 1 mL of the filtrate was tested with 0.5 mL Wagner's, Hager's and Mayer's reagents. Formation of reddish brown precipitate for Dragendorff's and Wagner's reagents, yellow precipitate for Hager's and cream precipitate for Mayer's indicated the presence of alkaloids (Tiwari et al., 2011).

Test for flavonoids

Flavonoids were determined by Mg-HCl reduction test. A piece of magnesium ribbon (powder) and 3 drops of concentrated hydrochloric acid were added to 3 mL of the test extract. A red coloration indicated the presence of flavonoids. Five milliliters of dilute ammonia solution was added to 5 mL of the aqueous filtrate of extract followed by the addition of 1 mL concentrated H_2SO_4. A yellow coloration indicated the presence of flavonoids. The yellow colure disappeared on standing position (Ganesh and Vennila, 2011).

Test for phenols

Ferric chloride test: Extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish black colure indicates the presence of phenols (Ganesh and Vennila, 2011).

Test for glycosides

To 2 mL of extract 2 drops of Molisch's reagent was added and shaken well. Two milliliters of concentrated H_2SO_4 was added on the sides of the test tube. A reddish violet ring appeared at the junction of two layers immediately indicated the presence of carbohydrates (Ganesh and Vennila, 2011).

Test for terpenoids

About 5 mL of the extract was mixed with 2 mL of chloroform and 3 mL of concentrated H_2SO_4 was added. A reddish brown coloration at the interface confirmed the presence of terpenoids (Ganesh and Vennila, 2011).

Test for tannins

About 0.2 g of the dried powdered samples was boiled in 10 mL of distilled water in a test tube and then filtered. The addition of 0.1% $FeCl_3$ solution resulted in a characteristic blue, blue-black, green or blue-green color, which confirmed the presence of tannins (Ganesh and Vennila, 2011).

Test for saponins

About 0.2 g of powdered sample extract was boiled in 2 mL of distilled water on a water bath and filtered. A fraction of aqueous filtrate measuring 1 mL was mixed with 2 mL of distilled water and shaken vigorously to form a stable persistent froth. The frothing was mixed with about three drops of olive oil and shaken vigorously. Formation of an emulsion confirms the presence of saponins (Okamoto et al., 2006).

RESULTS AND DISCUSSION

Preliminary phytochemical screening

Preliminary screening tests of the crude extract of dichloromethane/methanol (1:1) revealed the presence of flavonoids, glycosides, terpenes, phenols, tannins and absence of alkaloids and saponins (Table 1).

Table 1. Phytochemical screening of the roots of *Acanthus sennii*

Metabolite group	Reagent	Present/Absent
Alkaloids	Dragedoff's reagents	-
Flavonoids	Dilute ammonia solution	+
Phenols	$FeCl_3$	+
Glycosides	Conc HCl/ Molisch	+
Terpenoids	Chloroform and conc H_2SO_4	+
Tannins	$FeCl_3$	+
Saponins	Warring in water bath	-

(+) presence, (-) absence

Spectroscopic analysis

Compound **1** was obtained as a yellow powder (10 mg) from the dichloromethane/methanol (1:1) extract with R_f value of 0.64 under 70/30 *n*-hexane/ethyl acetate solvent system. The UV spectrum of indicated absorbance of peaks λ_{max} at 220, which indicated the presence of an iridoid enol ether system and transitions of lone pair of electrons n-σ* (C-O moiety). The IR spectrum revealed vibrations at 3400cm^{-1} (hydroxyl group), and at 2936 cm^{-1} (due to C-H stretching).

The ^{1}H NMR spectrum (400 MHz, Table 2) revealed the presence four methylenes at δ 3.65 (*dd*, *J*=6.5, 1.8, H-3) and δ 3.77 (*d*, *J*=1.5 H-10) and at δ 1.68 (dd, *J*=6.5, 3.0, H-4), δ 1.78 (t, H-6), respectively. Of these two of them are oxymethylenes (H-3 and H-10). Four methines were observed at δ 4.95 (t, H-1), δ 1.65 (m, H-5), δ 3.66(t, H-7), 2.21 (brd, H-9). The presence of a sugar moiety was evident from the anomeric peak at δ 4.6 (d, H-1″) and glycoside monosaccharide peaks at δ 3.18 (dd, *J*= 8.3, H-2″), δ 3.38 (t, H-3″), δ 3.28 (t, H-4″), δ 3.36 (m, H-5″), and δ3.59 (dd, *J*= 8, 6.0, H-6″). The ^{13}C NMR spectrum revealed a total of 15 carbons of which six of them were for a sugar moiety and the remaining nine peaks belong to iridoid skeleton. Two oxygenated methylenes at δ 61.6 and 63.4 (C-3 and C-10, respectively), two methyelenes at δ 39.1 (C-6) and 29.3 (C-4), two oxygenated methines at δ 77.4 (C-7) and δ 92.1 (C-1), two methines at δ 24.2 (C-5) and δ 45.6 (C-9) and one quaternary carbon at δ 82.9 (C-8) (Table 2) were all in good agreement with the ^{1}H NMR spectra. The HSQC spectrum suggested direct connectivity between C-1 and H-1, C-3 and H-3, C-4 and H-4, C-5 and H-5, C-9 and H -9 and C-1″ and H-1″ (Table 2). The HMBC spectrum showed important correlations between H-3 and C-1, H-5 and C-9, H-9 and C-1, C-8, C-10. These correlations clearly suggest an iridoid skeleton and the presence of the sugar moiety at C-1 position was further substantiated by correlations between H-1″ at δ 5.03 with δ104.4 (C-1). Moreover, the position of oxymethylene (CH_2OH) at C-8 was proved from the HMBC correlation between at δ3.10 (H-9) with carbons at δ 63.4 (C-10) (see Table 3 below). Thus, based on the above spectroscopic data and comparison with literature (Biswanath et al., 2007), the structure of compound **1** (Fig. 1) was proposed to be a derivative of stegoside II iridoid glycoside.

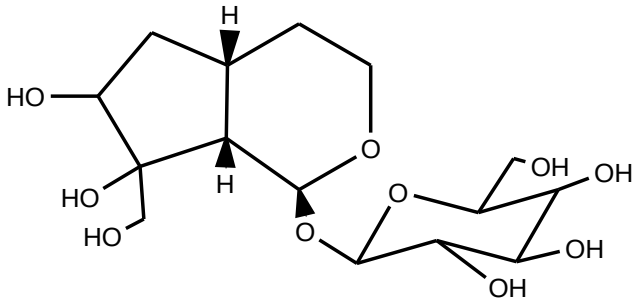

Figure 1. Important HMBC correlation of compound **1**.

Compound **2** was obtained as a brownish powder (45 mg) with R_f value of 0.42 under 30% ethyl acetate/*n*-hexane solvent system. The UV-Vis spectrum showed a similar pattern to that of compound-**1** with λ_{max} at 220 and 280 nm, which indicated the presence of an iridoid enol ether system and transitions of lone pair of electrons n-σ* and n-π*, respectively. The IR spectrum indicated the broad vibration at 3400 cm^{-1} (due to a hydroxyl group), around 2936 cm^{-1} vibrations (due to C-H stretching), vibrations at 1640 cm^{-1} (due to C=C).

The ^{1}H NMR spectrum (400 MHz, Table 3) revealed the presence one methyl at δ1.31 (1H, s, H-10); one methylene at δ1.78 (t, H-6); six methines at δ5.34 (1H, s, H-1), δ6.40 (1H, d, *J*=5.1,H-3), δ4.60 (1H, overlapped H-4), δ2.18 (m, H-5), δ 3.31 (1H, overlapped H-7) and 2.30 (brd, 10.2, H-9), respectively. The presence of a sugar moiety was evident from the anomeric peak at δ5.03 (d, H-1′′) and glycoside monosaccharide peaks at δ3.20 (dd, 9.0, 8.3 H-2′′), δ 3.49 (t, H-3′′), δ3.54 (t, H-4′′), δ3.73 (m, H-5′′), δ3.79` (dd, *J*=1.2, 8, 6.0, H-6′′). The ^{13}C NMR spectrum revealed a total of 15 carbons of which six of them were for a sugar moiety. One methyl at δ18.3 (C-10); one methylene at δ49.1(C-6); six methines; of which three of them were oxygenated at δ98.7 (C-1), δ143.9 and δ82.4 (C-7) and other three were at δ99.2 (C-4), δ38.5 (C-5) and δ56.2 (C-9); and one quaternary carbon at δ82.9 (C-8). Thus, based on the above spectroscopic data and comparison with literature (Biswanath et al., 2007) and that of spectra of compound **1**, the structure of compound **2** (Fig. 3) was proposed to be a derivative of stegoside II iridoid glycoside.

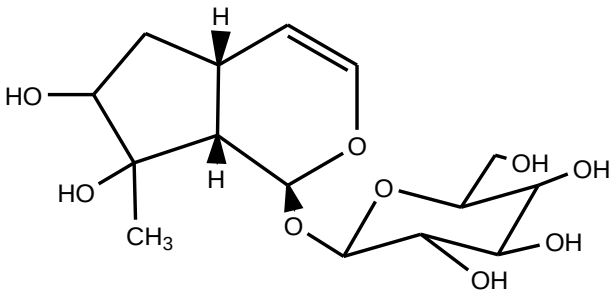

Figure 3: Structure of compound **2**.

Table 2. ^{1}H and ^{13}C Nuclear Magnetic Resonance spectroscopic data of compound **1**.

C/H	δ_C	δ_H (ppm) J in Hz	Δ (DEPT-35)	HSQC	HMBC	(Biswanath et al., 2007).	
						δ_C	δ_H
Aglycone							
1	104.4	4.50 (d, *J*=9,0)	CH (104.4)	$H_1 \rightarrow C_1$		92.7	5.45 (s)
3	62.6	3.65 (dd)	CH_2 (62.6)	$H_3 \rightarrow C_3$	$H_3 \rightarrow C_1$	139.4	6.16 (1 H)
4	24.2	2.21 (dd)	CH_2 (24.2)	$H_4 \rightarrow C_4$		104.9	4.85 (1 H)
5	29.3	1.5 (m)	CH (29.3)	$H_5 \rightarrow C_5$	$H_5 \rightarrow C_9$	40.3	2.67 (m, H-5)
6	39.1	1.78 (dt)	CH_2 (39.1)			77.2	3.90 (1 H)
7	77.4	3.66 (t)	CH (77.4)			49.1	3.66 (t)
8	82.9		C			78.5	-
9	45.6	3.10 (brd)	CH (45.6)	$H_9 \rightarrow C_9$	$H_9 \rightarrow C_{1,8,10}$	50.8	2.30 (brd)
10	63.4	3.67 (1 H)	CH_2 (63.4)			24.2	1.31 (s)
Glucose							
1′′	92.1	5.03 (*d*, *J*=8,0)	CH (92.1)	$H_{1''} \rightarrow C_{1''}$	$H_{1''} \rightarrow C_{1,5''}$	98. 7	4.62
2′′	77.5	3.76 (dd)	CH (77.5)	$H_{2''} \rightarrow C_{2}''$		73.6	3.18 (dd)
3′′	74.6	3.73 (t)	CH (74.6)			76.8	3.38 (t)
4′′	77.1	3.40 (t)	CH (77.1)			70.7	3.28 (t′)
5′′	73.2	3.46 (m)	CH (73.2)			77.0	3.36 (m)
6′′	60.8	3,79 (dd)	CH_2 (60.8)			61.4	3,59 (dd)

δ_C: carbon chemical shift value; δ_H: proton chemical shift value; J: coupling constant; Δ (DEPT-35): DEPT-135 chemical shift value; HSQC: Heteronuclear Single Quantum Coherence Spectroscopy; HMBC: Heteronuclear Multiple Bond Coherence.

Deuterated dimethyl sulfoxide (DMSO-d_6) was used as solvent. Nuclear Magnetic Resonance (NMR) analysis was recorded on a Bruker Avance 400 MHz spectrometer.

Table 3. ^{1}H and ^{13}C Nuclear Magnetic Resonance spectroscopic data of compound **2**.

Position	Compound 2		Biswanath et al., 2007.	
	δC	δH	δC	δH
Aglycone				
1	98.7	5.34 (s)	92.7	5.45 (1 H, s)
3	143.9	6.40 (d)	139.4	6.16 (1 H, d)
4	99 .2	4.60	104.9	4.85
5	38.5	2.18 (m)	40.3	2.67 (m)
6	49.1	1.78	77.2	3.90 (1 H)
7	82.4	3.31 (t,)	49.1	3.66 (t)
8	82.9	-	78.5	-
9	56.2	2.30 (brd)	50.8	2.30 (brd)
10	18.3	1.31 (s)	24.2	1.31 (1 H,s)
Glucose				
1′	98. 3	5.03	98. 7	4.62 (H-1′)
2′	72.4	3.20 (dd, J=9.0, 8.0)	73.6	3.18 (dd, J=9.0, 8.3)
3′	76.6	3.49 (t)	76.8	3.38 (t)
4′	70.2	3.54 (t)	70.7	3.28 (t)
5′	77.4	3.73 (m)	77.0	3.36 (m)
6′	62.4	3.79 (dd)	61.4	3,59 (dd)

δC: carbon chemical shift value; δH: proton chemical shift value. Deuterated dimethyl sulfoxide (DMSO-d_6) was used as solvent. Nuclear Magnetic Resonance (NMR) analysis was recorded on a Bruker Avance 400 MHz spectrometer.

CONCLUSIONS

This work is the first attempt to phytochemically analyze the roots of *Acanthus sennii* from Ethiopian flora. Phytochemical screening of the roots extract of *Acanthus sennii* revealed that the presence of glycosides, terpenoids, flavonoids, phenolics and tannins. Chromatographic separation of the dichloromethane/methanol (1:1) yielded stegioside derivative iridoid glycosides. To the best of our knowledge, this is the first report on the presence of such kind of iridoid derivatives from the genus *Acanthus* indigenous to Ethiopian flora. Iridoid glycosides commonly occur in the order *Scrophulariales* to which the *Acanthaceae* family belongs. The finding of these pharmacologically important secondary metabolites from root extracts brings the attention of experts to look more on the medicinal importance of the plant.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Author contributions:

Contribution	Assefa E	Alemayhu I	Endale M	Mammo F
Concepts or Ideas	X		X	
Design	X		X	
Definition of intellectual content	X	X	X	
Literature search	X	X	X	X
Experimental studies	X	X	X	X
Data acquisition	X	X	X	X
Data analysis	X	X	X	X
Statistical analysis	X	X	X	X
Manuscript preparation	X	X	X	
Manuscript editing	X	X	X	X
Manuscript review	X	X	X	X

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