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Deciphering the Chemical Structures with Trypanocidal Properties and Identifying Distinctive Compounds in *Scutellaria* and *Artemisia* Plants Native to Mongolia

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A thesis presented to meet the requisites for the Doctor of Philosophy degree

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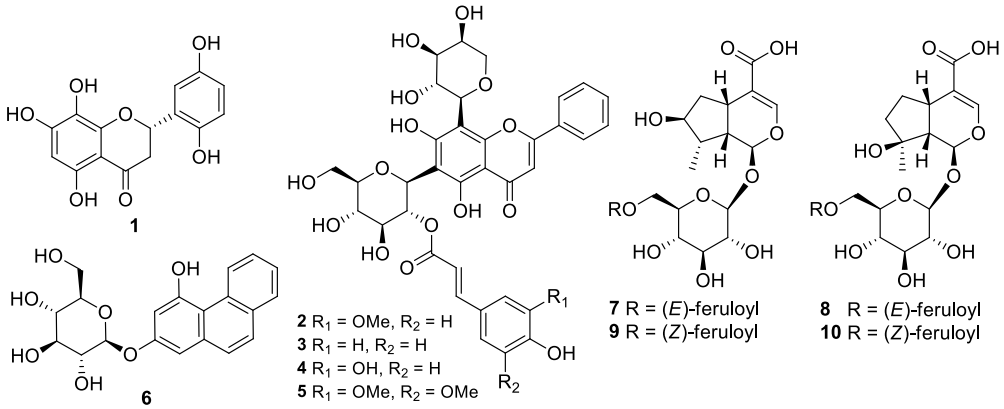
1. **Stipan Nurbyek**, Buyanmandakh Buyankhishig, Keisuke Suganuma, Yoshinobu Ishikawa, Mika Kutsuma, Marie Abe, Kenroh Sasaki, Bekh-Ochir Davaapurev, Javzan Batkhuu, Toshihiro Murata. Phytochemical investigation of *Scutellaria scordiifolia* and its trypanocidal activity. Phytochemistry 209 (113615), 2023
2. **Stipan Nurbyek**, Toshihiro Murata, Keisuke Suganuma, Yoshinobu Ishikawa, Buyanmandakh Buyankhishig, Takashi Kikuchi, Tseesuren Byambajav, Bekh-Ochir Davaapurev, Kenroh Sasaki, Javzan Batkhuu. Isolation and evaluation of trypanocidal activity of sesquiterpenoids, flavonoids, and lignans in *Artemisia sieversiana* collected in Mongolia. Journal of Natural Medicines 74 (750-757), 2020
3. **Stipan Nurbyek**, Toshihiro Murata Buyanmandakh Buyankhishig, Bekh-Ochir Davaapurev, Javzan Batkhuu. The seasonal variation for chemical constituents of *Artemisia sieversiana*. Mongolian Journal of Agricultural Sciences 28(03), 2019

I. Abstracts

This thesis includes general introduction of the study with three chapters. Each chapter's abstracts are presented below.

Chapter I: Phytochemical investigation of *Scutellaria scordiifolia* and its trypanical activity

This investigation unveiled 10 previously uncharacterized compounds, encompassing a flavanone (compound **1**), four chrysin derivatives with *C*-glycoside structures (compounds **2** − **5**), a phenanthrene glucoside (compound **6**), 4 iridoid glucosides (compounds **7** − **10**), in addition to 31 previously documented substances. The sugar configurations in the *C*-glycosides were determined by comparing their electric circular dichroism spectra with calculated data. Certain compounds within the categories of flavanones (compounds **1** and **17**), flavonols (compounds **11** − **13**), flavone (compound **14**), and specific flavone glucuronides (compounds **15** and **16**) exhibited significant trypanocidal effects against *Trypanosoma congolense*. The activity data and quantitative analysis of flavonoids using high-performance liquid chromatography (HPLC) from the aerial parts of *S. scordiifolia* indicate their potential effectiveness in treating diseases caused by the aforementioned trypanosomes.

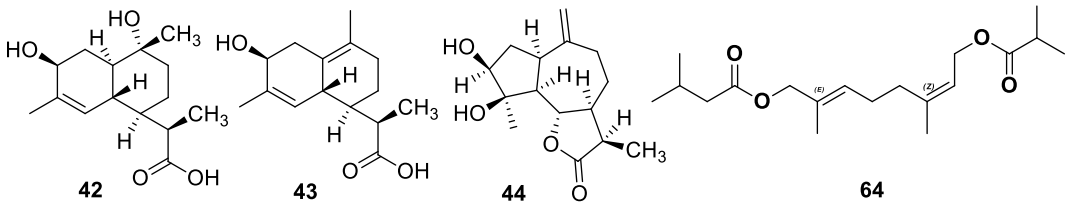


The new compounds purified from *Scutellaria scordiifolia*

Chapter II: Phytochemical investigation on *Artemisia sieversiana*

In this investigation, we isolated three new sesquiterpenoids (labeled as **42** − **44**) and one monoterpenoid (**64**) along with known substances from *A. sieversiana*, examining their characteristics. The complete structure of compound **42** was determined

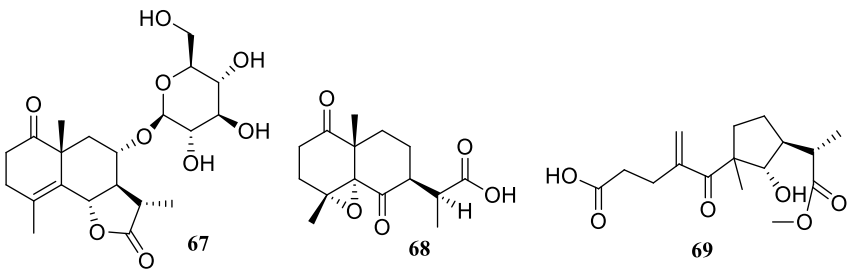
using single-crystal X-ray diffraction crystallography, revealing a configuration distinct from previously reported compounds with similar structures. Additionally, two other novel sesquiterpenoids (**43** and **44**) with analogous structures and determined their configurations. Furthermore, we evaluated the anti-trypanosomal effects of the isolated compounds (**42** − **59**) against *Trypanosoma congolense*, the pathogen responsible for fatal trypanosomosis in animals. Flavonoids and lignans were identified as the active components, displaying IC_{50} values ranging from 2.9 to 90.2 µM.



Isolated new compounds from *A.sieversiana*

Chapter III: Phytochemical constituents of *Artemisia adamsii*

Within this study, three new sesquiterpenoids were isolated together with 23 known compounds which are flavonoids, coumarins and quinic acid derivatives. Additionally, all the isolated compounds were mentioned for the first time from *A. adamsii*. Until recently, comprehensive scientific information about the phytochemicals in this plant has been lacking. As a result, the reputed traditional uses of the plant have not been adequately supported by scientific evidence. Our findings, which reveal the phytochemicals of *A. adamsii*, may contribute to further research aimed at understanding ecological interactions with animals and other plant species, as well as exploring the therapeutic applications of this plant.



Molecular sturucture of new sesquiterpenoids from *Artemisia adamsii*

II. GENERAL INTRODUCTION

i. The importance of the study of plants and infectious diseases of livestock animals in Mongolia

The plants in Mongolia has a crucial role as traditional medicinal resources and fodder for livestock animals. Nomadic culture has a strong connectivity with both livestock animals and the nature. A significant proportion, approximately 80% of Mongolia's agricultural sector is centered around animal husbandry, making it the primary source of livelihood for about one-third of the population (Kadirbyek D., 2023). This emphasizes the pivotal role of livestock animals and plants for the country.

Mongolia boasts rich biodiversity and a longstanding tradition of utilizing natural products for medicinal purposes (Murata T., 2021). Within this context, numerous yet scientifically unexplored interactions exist between nomadic culture and indigenous plants, microorganisms, and environmental elements. Additionally, Mongolia's distinctive geography and climate create a diverse array of habitats and ecosystems, fostering a broad spectrum of plant and animal species. Which is expected as the great potential for uncovering novel natural products with medicinal properties.

Infectious diseases among livestock animals in Mongolia pose significant challenges to the nation's agricultural sector and the livelihoods of its nomadic inhabitants. The harsh climatic conditions, coupled with traditional nomadic herding practices, create an environment conducive to the spread of various infectious diseases. Some key infectious diseases affecting livestock in Mongolia include: Foot and mouth disease (FMD), malaria, babesiosis, and trypanosomosis (Altangerel., 2012; McFadden., 2015; Munkhjargal., 2013; Suganuma., 2016).

Notably, diseases like dourine, resulting from *Trypanosoma equiperdum* infection, exert a substantial impact on Mongolian horses, leading to considerable economic losses for local farmers (Mizushima D.,2020). Our research group has been evaluating the growth-inhibitory activity of plant-derived components against several species of trypanosomes, and we have been conducting a study using *T. congolense*, which causes animal Nagana disease in Africa (Munsimbwe, 2022). In this study, the effects of the purified compounds on *T. congolense* of the same genus were evaluated as a first screening, with a view to future application to *T. equiperdum*, which is a problem in Mongolia.

The epidemiological mapping of trypanosomes in Mongolia (Mizushima D.,2020)

ii. Objective of the study

The primary objective of our research is to delineate the phytochemical constituents of widely distributed some specific plants on Mongolian rangelands. The plants discussed in this study are widely distributed and recognized by local nomadic people as medicinal and livestock feeding plants. In our case, our efforts are directed towards discovering the phytochemical novelty of the plants and search the treatments for local livestock animal diseases from indigenous plant sources.

Chapter I. Phytochemical investigation of *Scutellaria scordiifolia* and its trypanocidal activity

1.1 Introduction

Scutellaria scordiifolia Fisch. ex Schrank is a medicinal herb belonging to the Lamiaceae family. This plant is naturally found in regions of China, Russia, and Mongolia, primarily in the wilderness of forest-steppe areas, mountain slopes, riverbanks, thickets, and forest edges. It serves as a source of nutrition for domestic animals like cattle, horses, camels, which is observed in Mongolia (Ligaa, 1996, Olennikov and Chirikova, 2013; Li et al., 2020).

Figure 1. The flowering *Scutellaria scordiifolia*

Mongolian traditional medicine has a long history of using *S. scordiifolia* to treat various ailments, including fever, hepatomegaly, cirrhosis, snakebite detoxification, and malignant tumors (Ligaa, 1996). Additionally, it is believed to be effective in managing internal organ inflammations such as pneumonia and myocarditis (Shen et al., 2021). The plant has also been used in the treatment of malaria (Karimov and Botirov, 2017). Recent research has highlighted its antifungal properties (Giordani et al., 2020). Notably, *S. scordiifolia* contains flavones such as apigenin, baicalein, chrysin, oroxylin, luteolin, scutellarein, scutellarin, and wogonin, as well as flavonoid glycosides like baicalin, chrysin-7-*O*-glucuronic acid, and wogonosides (Olennikov and Chirikova, 2013; Ligaa, 1996).

Understanding the chemical composition of *S. scordiifolia* is vital for assessing its ecological role and investigating its potential in treating infectious diseases caused by pathogens. This study reports the extraction and identification of flavonoids,

phenanthrene, and iridoids from *S. scordiifolia*, along with their evaluation for trypanocidal activity.

1.2 **Outcomes and discussions**

1.2.1 **Identification of isolated compounds**

From the extracts of the above-ground portions of *S. scordiifolia*, a total of ten previously uncharacterized substances, comprising a flavanone numbered **1**, four chrysin compounds with *C*-glycoside structures marked as **2** − **5**, a phenanthrene glucoside denoted as **6**, and four iridoid compounds featuring glycosides with the numbers **7** − **10**, were separated (Fig. 2).

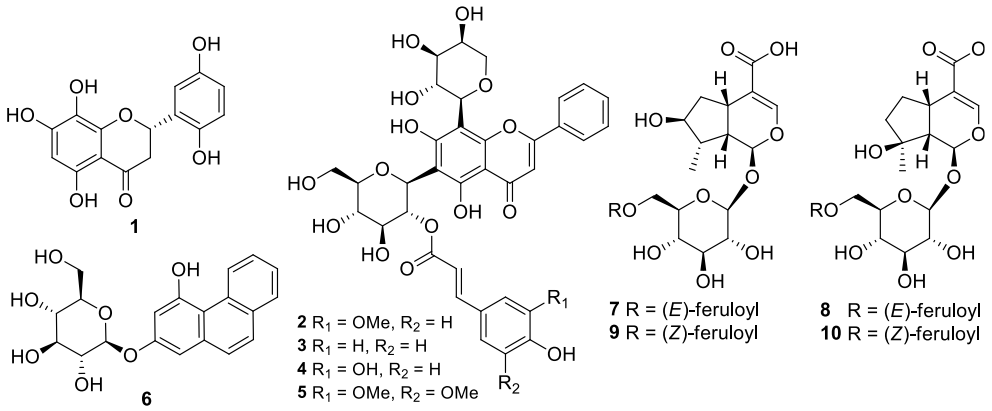


Fig. 2. Isolated new compounds (Nurbyek et al., 2023)

Additionally, 31 already documented compounds were also identified in these extracts (Fig. 3 and 4). Those compounds were identified by comparing with existing references of scutellarein (5,6,7,4′-tetrahydroxyflavone) (**11**) (Xia et al., 2007), luteolin (5,7,3′,4′-tetrahydroxyflavone) (**12**) (Park et al., 2007), 5,6,7,3′,4′-pentahydroxyflavone (**13**) (Gao and Kawabata, 2004), 7-*O*-β-D-glucuronopyranosyloxy-5,8-dihydroxyflavone (**14**) (Yung-Qi et al., 1988), 7-*O*-β-D-glucuronopyranosyloxy-5,3′,4′ -trihydroxyflavone (**15**) (Vanhoenackera et al., 2002), 7-*O*-β-D-glucuronopyranosyloxy-5,6,3′,4′-tetrahydroxyflavone (**16**) (Lu et al., 2010), (2*S*)-5,6,7,4′ -tetrahydroxyf lavanone (**17**) (Miyake et al., 2003), 8-*C*-α-L-arabinopyranosyl 6-*C*-β-D-glucopyranosyl-5,7-dihydroxyflavone (**18**) (Takagi et al., 1981; Xie et al., 2003), 6-*C*-α-L-arabinopyranosyl-8-*C*-β-D-glucopyranosyl-5,7-dihydroxyflavone (**19**) (Takagi et al., 1981; Xie et al., 2003), 6-*C*-α-L-arabinopyranosyl-8-*C*-β-D-glucopyranosyl-5,7,4′-trihydroxyflavone (**20**) (Xie et al., 2003), 6-*C*-β-D-glucopyranosyl-5,7-dihydroxyflavone (**21**) (Takagi et al., 1981;

Xie et al., 2003; Chen et al., 2003), 8-*C*-β-D-glucopyranosyl-5,7-dihydroxyflavone (**22**) (Brazier-Hicks et al., 2009), 5,7,2′,5′-tetrahydroxyflavone (**23**) (Miyaichi et al., 2006), 7-*O*-β-D-glucuronopyranosyloxy-5-hydroxyflavone (**24**) (Yung-Qi et al., 1988), wogonoside (7-*O*-β-D-glucuronopyranosyloxy-5-hydroxy-8- methoxyflavone) (**25**) (Wu et al., 2005), 7-*O*-β-D-glucuron opyranosyloxy-5,8,4′-trihydroxyflavone (**26**) (Lu et al., 2010), 7-*O*-β-D-glucuronopyranosyloxy-5,2′-dihydroxyflavone (**27**) (Yung-Qi et al., 1988; Karimov et al., 2017), 7-*O*-β-D-glucuronopyranosyloxy-5- hydroxy-8,2′-dimethoxyflavone (**28**) (Miyaichi et al., 2006), 7-*O*-β-D-glucuronopyranosyloxy-5,2′,5′-trihydroxyflavone (**29**) (Miyaichi et al., 2006), 2′-*O*-β-D-glucopyranosyloxy-5,7-dihydroxyflavone (**30**) (Miyaichi et al., 2006), 2-*O*-β-D-glucopyranosyloxy-5,7-dihydroxy-8-methoxyflavone (**31**) (Miyaichi et al., 2006), (2*S*)-2′ -*O*-β-D-glucopyranosyloxy-5,7,4,5′-tetrahydroxyflavanone (**32**) (Fu et al., 2009), 6′-*O*-(*E*)-*p*-coumaroyl-8-*epi*-loganic acid (**33**) (Gousiadou et al., 2007), martynoside (34) (Çalis¸ et al., 1993), cistanoside E (**35**) (Kobayashi et al., 1985), 6-*O*-(*E*)-*p*-feruloyl-(α/β)-glucopyranoside (36) (Bokern et al., 1991), 6-*O*-(*E*)-*p*-caffeoyl-(α/β)-glucopyranoside (**37**) (Hussein et al., 2003), 3-*O*-β-D-glucopyranosyloxy-5-phenylvaleric acid (**38**) (Fujita et al., 1996), kankanoside E (**39**) (Miyaichi et al., 2006), kankanoside O (**40**) (Morikawa et al., 2010), and tryptophan (**41**).

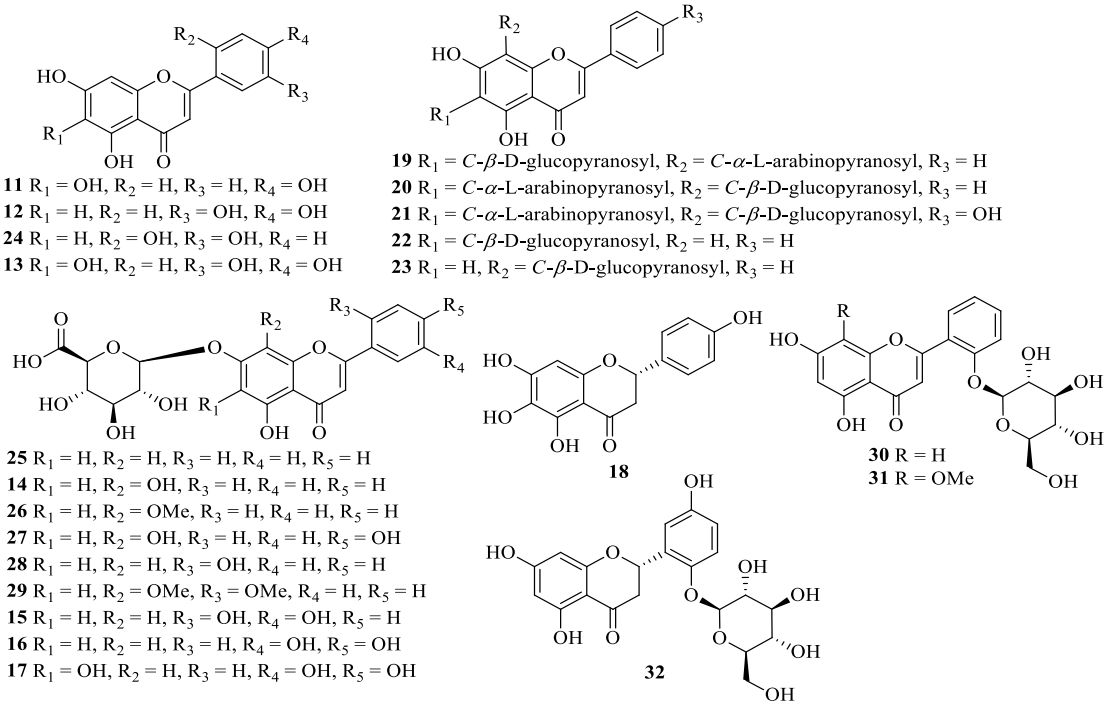


Fig. 3. Molecular structures of flavonoids and flavanones from *Scutellaria scordiifolia*

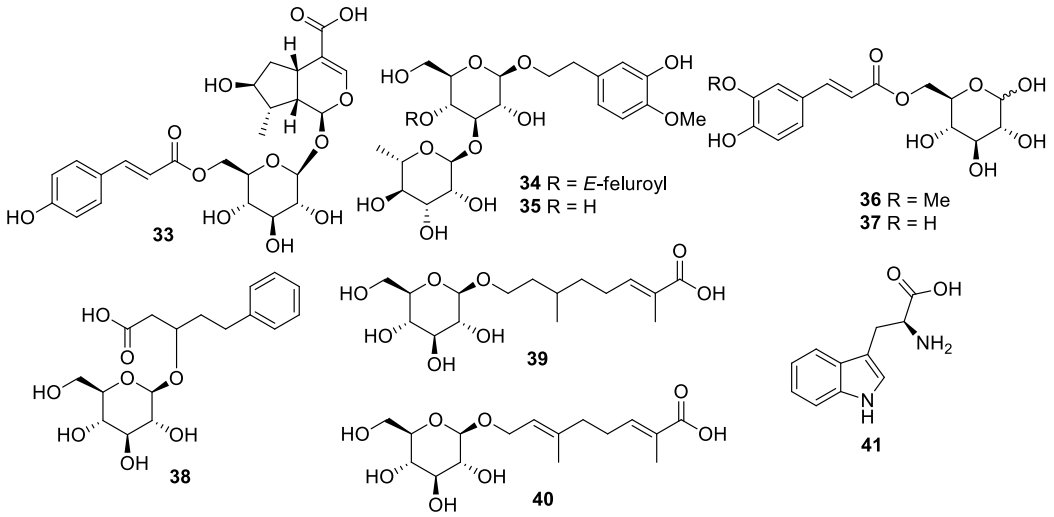


Fig. 4. Molecular structures of the other compounds

Compound **1**: Pale yellow amorphous solid; $[\alpha]^{22}_{D}$ − 24 (*c* 0.19, MeOH); UV (MeOH) λmax (log *ε*) 296 (3.26) nm; ECD (*c* 0.00020, MeOH) ((*θ*)) 207 (− 4600), 221 (+4300), 244 (− 1200), 258 (− 300), 290 (− 10300), 313 (+1900), 331 (− 200), 365 (+300) nm; molecular formula $C_{15}H_{12}O_7$ was confirmed through high-resolution fast atom bombardment mass spectrometry (HRFABMS) data. Specifically, the data at *m/z* = 327.0490 $(M+Na)^+$ matched the calculated value for $C_{15}H_{12}O_7Na^+$ at 327.0480. The assigned data of the ^1H and ^{13}C nuclear magnetic resonance (NMR) of **1** are presented in Table 1.

Table 1. ^1H and ^{13}C NMR spectroscopic data for **1** − **5**

	1[a]		**2**[b]		**3**[b]		**4**[b]		**5**[b]	
C#	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
2	74.1	5.58, dd (12.5, 3.0)	163.1		163.1		163.4[c]		163.1[c]	
3	41.6	3.05, dd (17.0, 12.5) 2.72, dd (17.0, 3.0)	104.5	6.83, s	104.5	6.83, s	104.5	6.81, s	104.5	6.81, s
4	196.5		181.9		181.8		181.8[c]		181.9[c]	
5	155.8		159.2		159.2		159.5		159.3[c]	
6	95.4	5.94, s	106.9		107.0		106.9		106.2	
7	156.4		161.8		162.0		161.1[c]		162.0	
8	125.6		103.9		103.9		103.9		103.9[c]	
9	149.6		154.1		154.2		154.2[c]		154.5[c]	
10	101.6		103.1		103.0[c]		103.1[c]		103.4[c]	
1'	125.8		130.5		130.5		130.5		130.5	

2'	146.2		126.5	8.12, d (9.0)	126.5	8.13, d (9.0)	126.5	8.12, d (9.0)	126.5	8.12, d (9.0)
3'	116.1	6.68, d (9.0)	128.6	7.55, m	128.7	7.56, m	128.6	7.55, m	128.6	7.55, m
4'	115.5	6.59, dd (9.0, 3.0)	131.4	7.55, m	131.4	7.56, m	131.4	7.55, m	131.4	7.55, m
5'	149.9		128.6	7.55, m	128.7	7.56, m	128.6	7.55, m	128.6	7.55, m
6'	113.4	6.93, d (3.0)	126.5	8.12, d (9.0)	126.5	8.13, d (9.0)	126.5	8.12, d (9.0)	126.5	8.12, d (9.0)
1"			70.8	5.01, d (10.0)	70.8	5.03, d (10.0)	70.8	5.02, d (10.0)	70.4	5.01, d (10.0)
2"			72.0	5.53, dd (10.0, 7.0)	72.1	5.55, dd (10.0, 7.0)	72.1	5.53, dd (10.0, 7.0)	72.1	3.55, dd (10.0, 7.0)
3"			76.1	3.55[c]	76.1	3.55[c]	76.1	3.55[c]	76.1	3.55[c]
4"			70.4	3.36[c]	70.4	3.38[c]	70.4	3.38[c]	70.3	3.37[c]
5"			81.3	3.39[c]	81.3	3.38[c]	81.2	3.38[c]	81.3	3.39[c]
6"			60.8	3.73[c]	60.8	3.73[c]	60.8	3.73[c]	60.8	3.70[c]
				3.59[c]		3.58[c]		3.58[c]		3.56[c]
1'''			75.0	4.81, d (10.0)	75.0	4.82, d (9.5)	75.0	4.80, d (10.0)	75.0	4.81, d (10.0)
2'''			69.1	4.00, dd (10.0, 9.0)	69.1	4.02, t (9.5)	69.1	4.02, dd (10.0, 9.0)	69.1	4.01, dd (10.0, 9.0)
3'''			74.0	3.53[c]	74.1	3.53[c]	74.1	3.53[c]	74.0	3.55[c]
4'''			68.3	3.87[c]	68.4	3.88[c]	68.4	3.88[c]	68.4	3.85[c]
5'''			70.2	3.93, dd (12.0, 2.0)	70.2	3.93, dd (12.0, 2.0)	70.2	3.93, dd (12.0, 2.0)	70.3	3.92[c]
				3.69[c]		3.70, br d (12.0)		3.70, br d (12.0)		3.70[c]
1''''			125.4		124.9		125.4		124.3	
2''''			111.4	7.13, d (1.5)	129.5	7.39, d (8.5)	114.4	6.91, d (1.5)	106.2	6.84, s
3''''			147.7		115.4	6.76, d (8.5)	145.2		147.9	
4''''			148.9		158.5		147.8		138.2	
5''''			115.3	6.74, d (8.0)	115.4	6.76, d (8.5)	115.5	6.71, d (8.0)	147.9	
6''''			122.2	6.97, dd (8.0, 1.5)	129.5	7.39, d (8.5)	120.6	6.85, dd (8.0, 1.5)	106.2	6.84, s
7''''			143.9	7.32, d (16.0)	143.6	7.34, d (16.0)	144.0	7.25, d (16.0)	144.1	7.32, d (16.0)
8''''			114.6	6.16, d (16.0)	114.3	6.12, d (16.0)	114.1	5.99, d (16.0)	115.1	6.21, d (16.0)
9''''			164.9		164.9		164.8		164.8	
OMe			55.6	3.79, s					56.0	3.77, s

[a]In DMSO-d6 solution at 24 °C, [b]In DMSO-d_6 solution at 80 °C [c]Unclear signal due to the presence of rotamers and low amount, and the value is expected by 2D NMR spectra

In the aromatic field (^{1}H NMR): Compound **1** indicating the presence of a benzene ring from the following shifts δ_H 6.68 (1H, *d*, *J* = 9.0 Hz, H-3′), 6.59 (1H, *dd*, *J* = 9.0, 3.0 Hz, H-4′), and 6.93 (1H, *d*, *J* = 3.0 Hz, H-6′). Coupling shift at δ_H 5.58 (1H, *dd*, *J* = 12.5, 3.0 Hz, H-2), 3.05 (1H, *dd*, *J* = 17.0, 12.5 Hz, H-3α), and 2.72 (1H, *dd*, *J* = 17.0, 3.0 Hz, H-3*β*) in the aliphatic field suggested oxygenated methine-methylene moieties. A singlet proton at δ_H 5.94 (1H, s, H-6) and five hydroxy broad singlet resonances (δ_H 11.73, 10.59, 9.07, 8.85, and 8.15) indicated a pentahydroxy flavanone skeleton. In the ^{13}C NMR spectrum, showed signals supporting a pentahydroxy flavanone structure, including carbonyl carbon at δ_C 196.5 (C-4) and aromatic (δ_C 155.8, 95.4, 156.4, 125.6, 149.6, 101.6, 125.8, 146.2, 116.1, 115.5, 149.9, and 113.4 (C-5–10 and C-1′–6′)) and aliphatic (δ_C 74.1 (C-2), 41.6 (C-3)) carbon resonances. Those signals confirmed with HMBC spectrum of **1**, the key correlations including couplings of the solitary aromatic proton (H-6) with C-5, C-8, and C-10, and hydroxy proton (5-OH) with C-5, C-6, and C-10 (Fig. 5). These correlations established the presence of a 5,7,8-trihydroxy A-ring in this flavanone (Miyaichi et al., 1987; Sordon et al., 2016). About the B-ring of the **1**, trisubstituted benzene ring was expected, the proton resonances assigned to the B-ring indicated a 2',5'-dihydroxy phenyl moiety. HMBCs and ROE correlation supported this inference (Fig. 5). The negative Cotton effect at 290 nm in the ECD spectrum suggested a 2*S*-absolute configuration (Sabrin et al., 2021), leading to the determination of (*S*)-2-(2',5'-dihydroxyphenyl)-5,7,8-trihydroxychroman-4-one (Fig. 6).

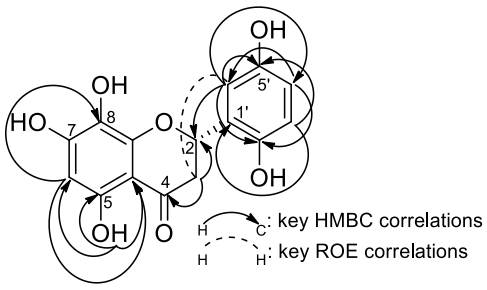


Fig. 5. Key HMBC correlation of **1**

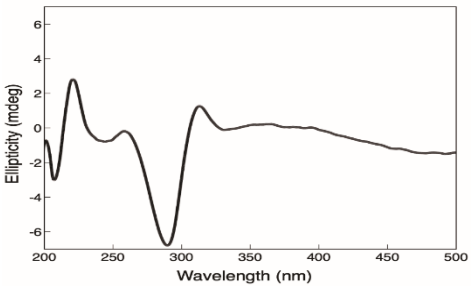


Fig. 6. ECD spectrum of **1**

In the ^{1}H-NMR spectra of **2** – **5** at 23°C, the aromatic proton resonances were observed as broad signals. This is due to the presence of rotamers at room temperature (Frank et al., 2012), and this spectroscopic feature appears to be common to flavone *C*-glycosides (Xie et al., 2003). Because variable-temperature NMR recordings can

overcome the broadness and obscurity of ^{1}H and ^{13}C NMR signals (Lewis et al., 2000), the NMR spectra of **2** – **5** were recorded at 80°C (Table 1).

Compound **2**. Pale yellow amorphous solid; $[\alpha]^{23}_{D}$ − 76 (*c* 1.0, MeOH); UV (MeOH) λmax (log *ε*) 278 (3.62), 321 (3.47) nm; ECD (*c* 0.00020, MeOH) ((*θ*)) 225 (− 16400), 281 (+13,400), 342 (− 18000), 404 (+2000) nm; The (+)-HRFABMS spectra established that the molecular formula of **2** is $C_{36}H_{36}O_{16}$ (*m/z* = 747.1891 $(M+Na)^{+}$, calcd for $C_{36}H_{36}O_{16}Na^{+}$: 747.1900), which is larger than that of 8-*C*-α-L-arabinopyranosyl-6-*C*-β-D-glucopyranosyl-5,7-dihydroxyflavone by one $C_{10}H_8O_3$. In the ^{13}C NMR spectrum of **2**, one carbonyl and 14 aromatic (δ_C 163.1, 104.5, 181.9, 159.2, 106.9, 161.8, 103.9, 154.1, and 103.1 of C-2–10 and 130.5, 126.5, 128.6, 131.4, 128.6, and 126.5 of C-1′–6′) carbon resonances corresponding to the chrysin moiety and 11 oxygenated (δ_C 70.8, 72.0, 76.1, 70.4, 81.3, and 60.8 of C-1″to 6″and 75.0, 69.1, 74.0, 68.3, and 70.2 of C-1‴to 5‴) carbon resonances corresponding to *C*-glucopyranosyl and *C*-arabinopyranosyl moieties were observed. In addition, one *O*-methyl (δ_C 55.6), eight aromatics (δ_C 125.4, 111.4, 147.7, 148.9, 115.3, 122.2, 143.9, and 114.6 of C-1⁗ –8⁗), and one carbonyl (δ_C 164.9, C-9⁗) carbon suggested the presence of a phenylpropanoid moiety. In the ^{1}H NMR spectrum of **2**, aromatic proton resonances at δ_H 8.12 (2H, d, J = 7.0 Hz, H-2′ and H-6′) and 7.55 (3H, m, H-3, H-4′, and H-5′) were assigned to the phenyl moiety as the B-ring of chrysin. In the aromatic region, a set of *o* and *m* coupling proton resonances at δ_H 7.13 (1H, d, J = 1.5 Hz, H-2⁗), 6.74 (1H, d, *J* = 8.0 Hz, H-5⁗), and 6.97 (1H, dd, *J* = 8.0, 1.5 Hz, H-6⁗) and a set of olefinic proton resonances at δ_H 7.32 (1H, *d*, *J* = 16.0 Hz, H-7⁗) and 6.16 (1H, d, *J* = 16.0 Hz, H-8⁗) were observed. These resonances indicated the presence of a phenylpropanoid moiety, and the large value of the coupling constant *J*7⁗ -8⁗ = 16.0 suggested their *E*-configuration.

In the HMBC spectrum of **2**, an *O*-methyl proton resonance was long-range coupling with C-3⁗, while H-2⁗ and H-6⁗ were long-range coupling with C-7⁗ (Fig. 7). These correlations indicated the presence of a feruloyl moiety as the phenylpropanoid moiety of **2**. The proton (δ_H 5.53, 1H, dd, *J* = 10.0, 7.0 Hz, H-2″) and carbon (δ_C 72.0, C-2″) resonances of the glucopyranosyl moiety were shielded compared to those of 8-C-α-L-arabinopyranosyl-6-C-β-D-glucopyranosyl-5,7-dihydroxyflavone (Takagi et al., 1981; Xie et al., 2003), indicating that the feruloyl moiety bonded to the oxygen of C-2″ via an

ester bond. The HMBCs from the anomeric proton resonance of glucopyranosyl at δ_H 5.01 (1H, d, *J* = 10.0 Hz, H-1″) to C-5, C-6, and C-7 and from the anomeric proton resonance of arabinopyranosyl at δ_H 4.81 (1H, d, *J* = 10.0 Hz, H-1‴) to C-7, C-8, and C-9 supported the existence of a 6-glucopyranosyl-8-arabinopyranosyl moiety in **2**.

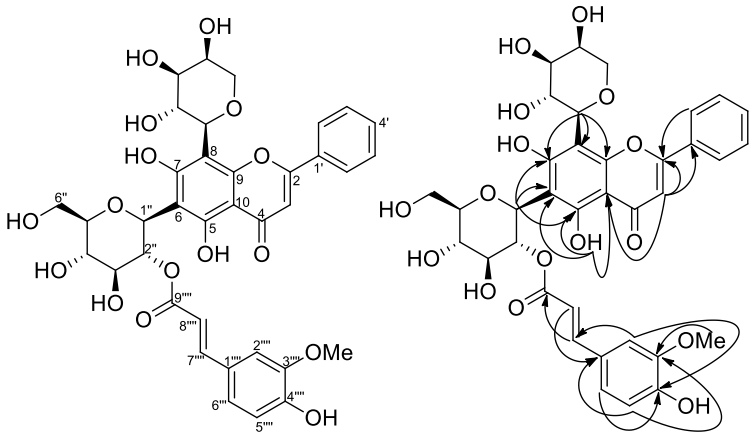


Figure 7. The molecular structure and key HMBC correlations of **2**

When the sugars are assumed to be D-glucose and L-arabinose, the coupling constants of the two sugar moieties (*J* = 10.0 Hz) suggested the existence of a *C*-glycosyl bond and the β- and α-configurations of D-glucose and L-arabinose (Xie et al., 2003; Shie et al., 2010; Dou et al., 2002). From these data, **2** was identified to be 8-*C*-α-L-arabinopyranosyl-6-*C*-(2″-*O*-(*E*)-feruloyl-β-D-glucopyranosy-l)-5,7-dihydroxyflavone.

Compound **3**: Pale yellow amorphous solid; $[\alpha]^{21}_{D}$ − 95 (*c* 0.48, MeOH); UV (MeOH) λmax (log *ε*) 278 (3.70), 313 (3.56) nm; ECD (c 0.00020, MeOH) ((*θ*)) 225 (− 23700), 281 (+12,400), 331 (− 23500), 408 (− 1200) nm; with a molecular formula of $C_{35}H_{34}O_{15}$, was determined from its (+)-HRFABMS spectrum (*m/z* = 717.1787 $(M+Na)^+$) to be smaller than compound **2** by one CH_2O molecule (Takagi et al., 1981; Xie et al., 2003). The ^{1}H NMR spectrum of **3** revealed a set of *o*-coupling aromatic proton resonances at δ_H 7.39 (2H, d, *J* = 8.5 Hz, H-2‴″ and H-6‴″) and 6.76 (2H, d, *J* = 8.5 Hz, H-3‴″ and H-5‴″). Additionally, olefinic proton resonances at δ_H 6.12 (1H, d, J = 16.0 Hz, H-8‴″) and aromatic protons (H-3‴″ and H-5‴″) showed long-range coupling with C-1‴″ (δ_C 124.9) in the HMBC spectrum. These data indicated the presence of a *p*-coumaroyl moiety in place of the feruloyl moiety of **2**. Therefore, compound **3** was determined to be 8-α-L-arabinopyranosyl-6-(2″-*O*-(*E*)-p-coumaroyl-*C*-β-D-glucopyranosyl)-5,7-

dihydroxyflavone.

Similarly, the molecular formula of compound **4** was determined to be $C_{35}H_{34}O_{16}$ (*m/z* = 733.1765 $(M+Na)^+$), smaller than that of **2** by one CH_2 molecule. $[\alpha]^{21}_D$ − 110 (*c* 0.41, MeOH); UV (MeOH) λmax (log *ε*) 277 (3.51), 323 (3.29) nm; ECD (*c* 0.00020, MeOH) ((*θ*)) 225 (− 16300), 283 (+9700), 347 (− 15200), 404 (+200) nm. Deshielded chemical shift values of C-2‴′, C-3‴′, and C-4‴′ (δ_C 114.4, 145.2, and 147.8, respectively) indicated the presence of a caffeoyl moiety in place of the feruloyl moiety of **2**. Therefore, compound **4** was identified as 8-*C*-α-L-arabinopyranosyl-6-*C*-(2″-*O*-(*E*)-caffeoyl-β-D-glucopyranosyl)-5,7-dihydroxyflavone.

Compound **5**, Pale yellow amorphous solid; $[\alpha]^{21}_D$ − 65 (*c* 0.31, MeOH); UV (MeOH) λmax (log *ε*) 276 (3.47), 320 (3.26) nm; ECD (*c* 0.00020, MeOH) ((*θ*)) 225 (− 13000), 296 (+5200), 350 (− 11200), 405 (− 2200) nm, the molecular formula of $C_{37}H_{38}O_{17}$ (*m/z* = 777.1974 $(M+Na)^+$), was larger than that of **2** by one CH_2O molecule. The ^{1}H NMR spectrum of **5** exhibited *O*-methyl (δ_H 3.77, 6H, s) and singlet aromatic (δH 6.84, 2H, s, H-2‴′ and H-6‴′) proton resonances, suggesting the presence of a sinapoyl moiety. Therefore, compound **5** was identified as 8-*C*-α-L-arabinopyranosyl-6-*C*-(2″-*O*-(*E*)-sinapoyl-*β*-D-glucopyranosyl)-5,7-dihydroxyflavone.

The determination of the absolute configuration of the glycosyl group is often challenging due to the resistance of the *C*-glycosyl C–C bond to acid hydrolysis (Harborn, 1965). To address this, computed ECD spectra of several trial compounds, including 8-*C*-α-D-arabinopyranosyl-6-C-β-D-glucopyranosyl-5,7-dihydroxyflavone, 8-*C*-α-D-arabinopyranosyl-6-*C*-β-L-glucopyranosyl-5,7-dihydroxyflavone, 8-*C*-α-L-arabinopyranosyl-6-*C*-β-D-glucopyranosyl-5,7-dihydroxyflavone, and 8-*C*-α-L-arabinopyranosyl-6-*C*-β-L-glucopyranosyl-5,7-dihydroxyflavone, were calculated. The computed ECD spectrum of 8-*C*-α-L-arabinopyranosyl-6-*C*-β-D-glucopyranosyl-5,7-dihydroxyflavone exhibited the closest similarity to the experimental data of the identified compound **1**.

In the ECD spectra (Fig. 8) of **2** – **5**, a significant negative Cotton effect was observed in the 330–350 nm region, contrasting with the absence of Cotton effect in that region in the spectrum of 8-*C*-α-L-arabinopyranosyl-6-*C*-*β*-D-glucopyranosyl-5,7-dihydroxyflavone. This difference in Cotton effects could aid in identifying the configurations of C-1″ and C-2″. Further analysis involved comparing the experimental and calculated ECD spectra to confirm the presence of D-glucosyl moieties in **2** – **5**. The study verified the utility of ECD spectra in determining the absolute configurations of acylated C-glycosyl moieties.

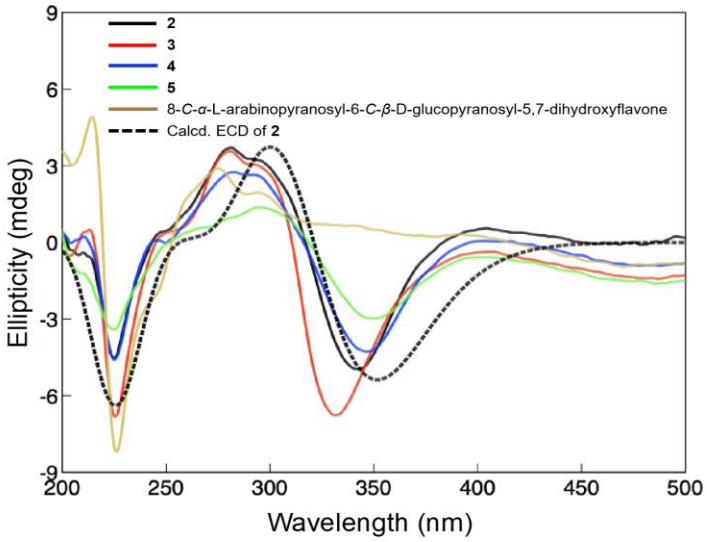


Fig. 8. The ECD spectrum of compound **2-5**

Degradation of **2** – **5** using K_2CO_3 produced 8-*C*-α-L-arabinopyranosyl-6-*C*-*β*-D-glucopyranosyl-5,7-dihydroxyflavone, establishing that **2** – **5** shared identical configurations with the reported compound. This reaffirmed the effectiveness of the ECD spectrum in determining the absolute configuration of the acylated *C*-glycosyl moiety.

Compound **6**. Pale yellow amorphous solid; $[\alpha]^{19}_{D}$ − 46.3 (*c* 0.35, MeOH). The molecular formula of compound **6** was determined to be $C_{20}H_{20}O_7$ from an ion peak at *m/z* = 373.1287 $(M+H)^+$ in the (+)-HRFABMS spectrum. In the ^{1}H NMR spectrum of **6**, a *p*-coupling system comprising two protons (δ_H 6.93, 1H, d, *J* = 2.5 Hz, H-1 and δ_H 7.07, 1H, d, *J* = 2.5 Hz, H-3), an *o*- and *m*-coupling system comprising four protons (δ_H 9.65, 1H, d, J = 9.0 Hz, H-5; δ_H 7.55, 1H, m, H-6; δ_H 7.45, 1H, dt, *J* = 9.0, 8.0 Hz, H-7; and δ_H 7.78, 1H, d, J = 8.0 Hz, H-8), and an *o*-coupling system comprising two protons (δ_H 7.63, 1H, d, J = 9.0 Hz, H-9 and δ_H 7.52, 1H, d, *J* = 9.0 Hz, H-10) were observed in its aromatic

field. The proton resonance at δ_H 9.65 (H-5) was shielded and shifted due to its hydrogen bond with the oxygen atom of the 4-hydroxy group (Sutherland et al., 1991). In the aliphatic field, the resonances of protons on oxygenated carbons were observed, suggesting the presence of a glucosyl moiety. The ^{13}C NMR resonances corresponding to the aforementioned protons were determined from the heteronuclear multiple-quantum correlation (HMQC) spectrum of **6**. The resonances were similar to those of 2,4,6-trihydroxyphenanthrene, except for the presence of an oxygenated aromatic carbon (Shie et al., 2010). The six oxygenated carbon resonances (δ_C 102.7, 75.1, 78.4, 71.3, 78.8, and 62.6, C-1′–6′) suggested that the sugar moiety of **6** was a glucopyranosyl moiety.

Table 2. ^{1}H and ^{13}C NMR spectroscopic data for compounds **6** in MeOH-d_4

Position	δ_C	δ_H (J in Hz)	Position	δ_C	δ_H (J in Hz)
1	107.5	6.93, d (2.5)	8a	133.1	
2	158.9		9	129.2	7.63, d (9.0)
3	104.1	7.07, d (2.5)	10	127.5	7.52, d (9.0)
4	157.2		10a	137.2	
4a	116.0		1'	102.7	5.28, d (8.0)
4b	131.7		2'	75.1	3.83, m
5	129.3	9.65, d (9.0)	3'	78.4	3.56, m
6	127.5	7.55, m	4'	71.3	3.52, m
7	125.9	7.45, dt (9.0, 8.0)	5'	78.8	3.83, m
8	129.2	7.78, d (8.0)	6'	62.6	3.96, dd (12.0, 2.0) 3.76, dd (12.0, 5.5)

In the HMBC spectrum of **6** (Fig. 9), H-3 was coupled with C-1, C-2, C-4, and C-4a (δ_C 107.5, 158.9, 157.2, and 116.0, respectively), C-4a with H-1 and H-10, H-10 with C-1 and C-8a (δ_C 133.1), and C-8a with H-5 and H-7. These correlations support the presence of a 2,4-dioxygenatedphenanthrene moiety. The anomeric proton of glucopyranosyl at δ_H 5.28 (1H, d, J = 8.0 Hz, H-1′) was correlated with C-2 in the HMBC spectrum (Fig. 9), suggesting that the glucosyl moiety was bonded to C-2. The HPLC sugar analysis and the coupling constant of the anomeric proton resonance (J = 8.0 Hz) indicated that **6** contained a β-D-glucosyl moiety. The H-1′ proton was long-range coupled with C-2 (δ_C 158.9). From these data, compound **6** was determined to be 2-*O*-β-D-glucopyranosyloxy-4-hydroxyphenanthrene.

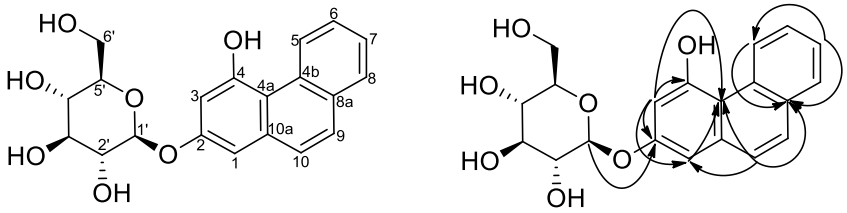


Fig. 9. Molecular structure and key HMBC correlation of **6**

Compounds **7**–**10** were determined to have the identical molecular formula of $C_{26}H_{32}O_{13}$. This determination was made by (+)-HRFABMS (High-Resolution Fast Atom Bombardment Mass Spectrometry), and the observed *m/z* values for the (M+Na) + ions were as follows:

- Compound **7**: *m/z* = 575.1749
- Compound **8**: *m/z* = 575.1734
- Compound **9**: *m/z* = 575.1751
- Compound **10**: *m/z* = 575.1722

These observed *m/z* values closely matched the calculated value for $C_{26}H_{32}O_{13}Na^+$ (calculated *m/z* = 575.1740), confirming the molecular formula for all four compounds. The use of (+)-HRFABMS is instrumental in providing accurate mass measurements, aiding in the determination of the molecular composition of the compounds.

The ^{1}H and ^{13}C NMR spectra of compounds **7–10** exhibited common features characteristic of iridoid glycosides. The ^{1}H and ^{13}C NMR spectra of compound **7** were specifically compared to those of 6'-*O*-(*E*)-p-coumaroyl-8-*epi*-loganic acid (Gousiadou et al., 2007). The resonances assigned to the phenylpropanoid moiety in compound **7** differed from those in 6'-*O*-(*E*)-*p*-coumaroyl-8-*epi*-loganic acid. Notably, the *O*-methyl proton (δ_H 3.89, 3H, s) and its corresponding carbon (δ_C 56.5) resonances were present in compound **7**. Furthermore, the HMBC spectrum of **7** showed a correlation between the *O*-methyl proton and C-3″ (δ_C 149.4), while the ROESY spectrum displayed a correlation between the *O*-methyl proton and H-2″ (δ_H 7.19, 1H, br s). These findings suggest the presence of an *O*-methyl group attached to the phenylpropanoid moiety in compound **7**, differentiating it from 6'-*O*-(*E*)-p-coumaroyl-8-*epi*-loganic acid.

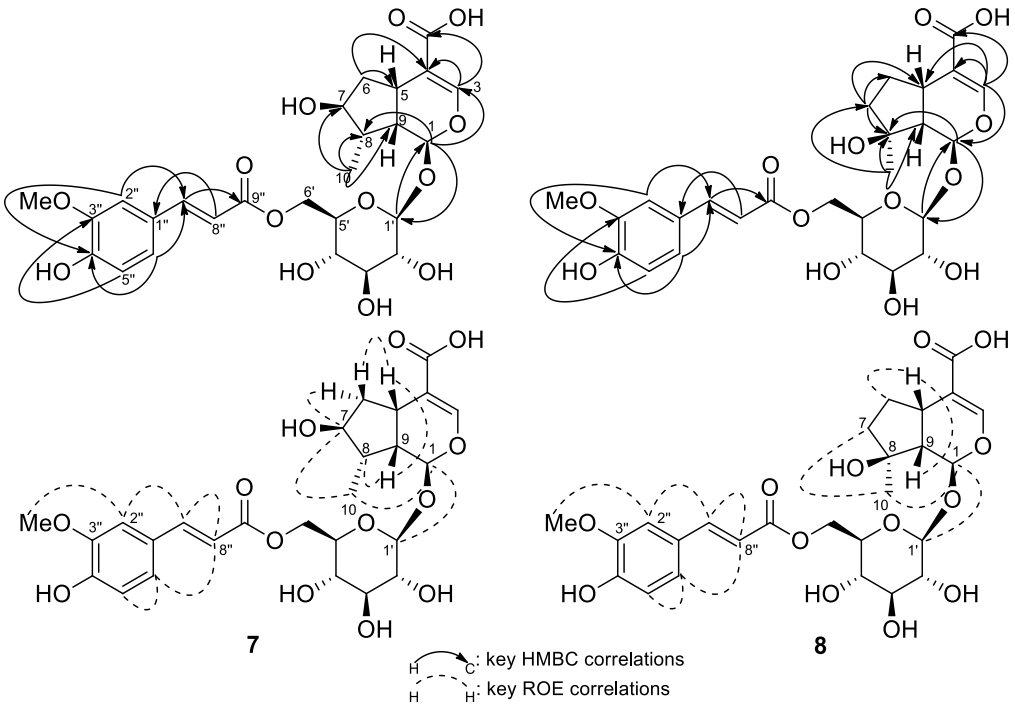


Fig. 10. Key HMBC and ROESY correlations of **7** and **8**

Table 3. ^{1}H and ^{13}C NMR spectroscopic data for compounds **7** − **10** (in MeOH-d_4)

	7		**8**		**9**		**10**
#	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_H (*J* in Hz)
1	96.2	5.30, d (5.0)	95.7	5.23, d (6.0)	96.2	5.27, d (5.0)	5.18, d (6.0)
2							
3	152.6	7.40, s	152.0	7.40, s	152.5	7.38, s	7.34, br s
4	113.8		113.4		113.3		
5	31.9	3.01, m	33.2	3.16, m	31.6	3.01, m	3.15, m
6	41.4	2.01, m	30.9	2.25, m	41.3	2.02, m	2.24, m
		1.78, m		1.37, m		1.78, m	1.35, m
7	79.0	3.80, br t (5.0)	40.0	1.67, br d (7.0)	79.2	3.73, m	1.58, m
8	45.5	2.09, m	81.0		45.3	2.05, overlap	
9	43.2	2.48, m	52.2	2.11, dd (9.0, 8.0)	43.0	2.49, m	2.10, dd (9.0, 6.0)
10	14.4	1.02, d (7.0)	25.0	1.30, s	14.4	1.00, d (7.0)	1.28, s
11	170.6		170.8		170.8		
1′	99.8	4.69, d (8.5)	99.9	4.72, d (8.0)	99.9	4.65, d (8.0)	4.68, d (8.0)
2′	74.8	3.23, m	74.8	3.23, dd (9.0, 8.0)	74.7	3.20, dd (9.0, 8.0)	3.21, d (9.0, 8.0)
3′	77.9	3.38$^{(over)}$	77.9	3.38$^{(over)}$	77.9	3.37$^{(over)}$	3.3-3.6$^{(over)}$
4′	71.8	3.38$^{(over)}$	71.7	3.38$^{(over)}$	71.8	3.35$^{(over)}$	3.3-3.6$^{(over)}$
5′	75.8	3.55, m	75.7	3.56, m	75.6	3.52, m	3.50, m

6′	64.3	4.51, br d (12.0)	64.2	4.52, dd (12.0, 2.5)	64.4	4.47, br d (12.0)	4.47, dd (12.0, .2.0)
1″	127.7		127.6		128.1		
2″	111.7	7.19, br s	111.6	7.18, d (2.0)	115.2	7.77, br d	7.84, d (1.5)
3″	149.7		149.4		148.4		
4″	150.7		150.7		150		
5″	115.3	113	115.2	6.80, d (8.5)	115.8	6.77, d (7.5)	6.76, d (8.5)
6″	124.3	7.07 br d (8.5)	124.3	7.05, dd (8.5, 2.0)	126.9	7.15, br d (7.5)	7.15, dd (8.5, 1.5)
7″	147.2	7.64, d (16.0)	147.1	7.62, d (16.0)	145.7	6.88, d (13.0)	6.87, d (13.0)
8″	116.5	6.39, d (16.0)	116.5	6.38, d (16.0)	116.2	5.81, d (13.0)	5.79, d (13.0)
9″	169.0		169.0		168.1		
OMe	56.5	3.89, s	56.5	3.89, s	56.5	3.87, s	3.87, s

The structural elucidation of compounds **7**, **8**, **9**, and **10** involved detailed analysis of NMR spectra, ROESY correlations, and HPLC sugar analysis.

Compound **7** (6′-*O*-(*E*)-feruloyl-8-*epi*-loganic acid): Colorless amorphous solid; $[\alpha]^{23}_{D}$ − 51 (*c* 0.47, MeOH). The (*E*)-feruloyl group was identified to be bonded to the glucosyl moiety at C-6′ in 8-*epi*-loganic acid. The ROESY spectrum (Fig. 10) established the *α*-orientation of H-1, 8-methyl, H-7, and H-6α, indicating their relative configuration. HPLC sugar analysis confirmed the presence of a D-glucose moiety with a *β*-configuration (Tanaka et al., 2007). Compound **7** was determined to be 6′-*O*-(*E*)-feruloyl-8-*epi*-loganic acid.

Compound **8** (6′-*O*-(*E*)-feruloyl-mussaenosidic acid): Colorless amorphous solid; $[\alpha]^{21}_{D}$ − 40 (*c* 0.50, MeOH). The NMR spectra of **8** were similar to those of 6′-*O*-(*E*)-*p*-coumaroyl-mussaenosidic acid (Taskova et al., 1998), with the key difference being the presence of a feruloyl group instead of a *p*-coumaroyl moiety. The ROESY spectrum (Fig. 10) indicated the α-orientation of H-1 and 8-methyl and β-orientation of H-5 and H-9. HPLC sugar analysis confirmed the presence of a *β*-D-glucopyranosyl moiety (Tanaka et al., 2007). Compound **8** was determined to be 6′-*O*-(*E*)-feruloyl-mussaenosidic acid.

Compounds **9** (6′-*O*-(*Z*)-feruloyl-8-*epi*-loganic acid) colorless amorphous solid; $[\alpha]^{24}_{D}$ − 48 (c 0.13, MeOH) and **10** (6′-*O*-(*Z*)-feruloyl-mussaenosidic acid) colorless amorphous solid; $(\alpha)^{21}_{D}$ − 45 (*c* 0.40, MeOH). These compounds were considered artifacts resulting from the exchange of *E*- and *Z*-configurations under ultraviolet irradiation. The coupling

constant of the olefinic proton resonances indicated that they are in the (*Z*)-configuration. Compound **9** was determined to be 6′-*O*-(*Z*)-feruloyl-8-*epi*-loganic acid. Compound **10** was determined to be 6′-*O*-(*Z*)-feruloyl-mussaenosidic acid.

1.2.2. **Trypanocidal activity of isolated compounds from *S.scordiifolia***

The trypanocidal activity of isolated compounds (**1**–**41**) and characteristic flavones from Scutellaria plants, including baicalein, baicalin, chrysin, and wogonin, against *T. congolense* was evaluated. The results are summarized in Table 4.

Table 4. IC_{50} value of active compounds against *Trypanosoma congolense*

Sample	IC_{50} (μM)
	mean ± SD
1	8.07± 5.33
11	19.0± 3.06
12	21.0± 0.47
13	5.77± 1.22
14	36.4± 9.73
15	27.8± 4.83
16	11.9± 1.67
17	14.0± 8.18
baicalein	44.6± 42.2
baicalin	49.9± 19.5
chrysin	19.4± 6.32
wogonin	7.03± 0.74
Pentamidine	0.23 ± 0.02
Diminazene	0.23 ± 0.05

The treatment was replaced three times
The isolated other compounds in this study were inactive at 25 μg/ml.

Compounds without sugar moieties, such as (*S*)-2-(2′,5′-dihydroxyphenyl)-5,7,8-trihydroxychroman-4-one (**1**, IC_{50} 8.07 μM), baicalein (IC_{50} 44.6 μM), chrysin (IC_{50} 19.4 μM), scutellarein (**11**, IC_{50} 19.0 μM), luteolin (**12**, IC_{50} 21.0 μM), 5,6,7,3′,4′-pentahydroxyflavone (**13**, IC_{50} 5.77 μM), 5,6,7,3′,4′-pentahydroxyflavone (**14**, IC_{50} 36.4 μM), and (2*S*)-5,6,7,4′-tetrahydroxyflavanone (**17**, IC_{50} 14.0 μM), exhibited potent trypanocidal activity.

Chrysin and baicalein are known for various biological and pharmaceutical activities, and their chemical structures have characteristic B-rings lacking oxygen (Pingili et al., 2019). Chrysin lacks a hydroxy group at C-6, distinguishing it from

baicalein, which contains a pyrogallol moiety in its A-ring. These compounds are common constituents of Scutellaria plants (Olennikov and Chirikova, 2013; Shen et al., 2021), and *S. baicalensis*, a species similar to *S. scordiifolia*, is used for medicinal purposes.

The study also analyzed the major flavonoids in the leaves, stems, and roots of *S. scordiifolia* using HPLC. The leaves contained scutellarein (0.231%), chrysin (0.599%), chrysin-7-*O*-glucuronide (1.095%), and various other flavonoids. Livestock animals, except goats, are likely to efficiently ingest these flavones by consuming the aerial parts of *S. scordiifolia*.

1.3 **Conclusions**

An unidentified flavanone, along with four chrysin *C*-glycosides, a phenanthrene glucoside, and four iridoid glucosides, were detected in an extract derived from the aerial components of *S. scordiifolia*. Additionally, 31 recognized compounds were present in this extract. The absolute configurations of C-2 in the flavanone and D-glucose in the *C*-glycosides were ascertained through ECD spectra. Several flavonoids isolated from this plant displayed trypanocidal activities, highlighting the aerial parts of *S. scordiifolia* as a valuable source of these bioactive flavonoids. Analyzing the flavonoid content, as well as the chemo phenetic and functional characteristics of flavonoid-*C*-glycosides, iridoids, and other compounds in Scutellaria plants, holds significance due to their ecological interactions with grazing and burrowing animals, coupled with the medicinal applications associated with these plants.

1.4 **Experimental**

The instruments used in the study, as well as the plant materials and experimental procedures related to the trypanocidal activity, are explained in detail on page 50-52.

1.4.1. **Extraction, isolation and purification process**

The dried aerial parts of *S. scordiifolia* (105.6 g) were extracted using 1.5 L of acetone/H_2O (4:1) at 20°C and 1.5L at 60°C in two consecutive steps. The resulting extract was dissolved in 1L of H_2O, and less polar compounds were removed using 1 L of Et_2O. The aqueous extract (75.8 g) underwent Diaion HP-20 chromatography, yielding

fractions eluted with different solvent systems showed below in table 5.

Table 5. Isolation of crude extract

Fractions	Amount (g)	Elution
1A	40.0	H_2O
1B	8.0	MeOH/H_2O (1:4)
1C	5.1	MeOH/H_2O (2:3)
1D	4.3	MeOH/H_2O (3:2)
1E	3.6	MeOH/H_2O (4:1)
1F	1.2	MeOH
1G	0.728	Acetone

Fractions 1B–F underwent ODS-SM-50C-M column chromatography using the respective solvent systems mentioned below:

Fractions from 1B: Fractions 2A–K obtained using 1:9 and 2:3 MeOH/H_2O, fractions from 1C: fractions 3A–O obtained using 3:7 and 3:2 MeOH/H_2O, fractions from 1D: fractions 4A–Q obtained using 3:7 and 3:2 MeOH/H_2O, fractions from 1E: fractions 5A–L obtained using 1:1 and 4:1 MeOH/H_2O, Fractions from 1F: fractions 6A–M obtained using 3:2 and 4:1 MeOH/H_2O. This detailed extraction and chromatographic process resulted in the isolation of various fractions enriched with different compounds from the aerial parts of *S. scordiifolia*.

To isolate various compounds, different fractions were subjected to high-performance liquid chromatography (HPLC) separation using specific columns and mobile phase compositions. Details are provided below:

Fraction 2F (330.4 mg): Column: C_{30}-UG-5 (1:4 acetonitrile/H_2O with 0.2% trifluoroacetic acid (TFA)), compounds obtained: **15** (13.6 mg), **16** (2.6 mg), and **29** (1.9 mg). Fraction 2K (250.6 mg): Column: TSKgel ODS-120T (1:4 acetonitrile/H_2O with 0.2% TFA), compounds obtained: **26** (17.3 mg) and **27** (17.9 mg). Fraction 3F (455.4 mg): Columns: TSKgel ODS-120T (1:9 acetonitrile/H_2O) and C_{30}-UG-5 (3:17 acetonitrile/H_2O), compounds obtained: **33** (2.0 mg), **37** (2.6 mg), and **41** (10.3 mg). Fraction 3G (328.8 mg): Columns: TSKgel ODS-120T (3:17 acetonitrile/H_2O) and C_{30}-

UG-5 (3:17 acetonitrile/H_2O) compound obtained: **35** (23.7 mg). Fraction 3J (242.5 mg): Columns: TSKgel ODS-120T (1:4 acetonitrile/H_2O) and C30-UG-5 (1:4 acetonitrile/H_2O), compound obtained: **20** (3.0 mg). Fraction 3K (247.0 mg): Column: TSKgel ODS-120T (1:4 acetonitrile/H_2O with 0.2% TFA) compound obtained: **32** (112.2 mg). Fraction 3L (73.1 mg): Column: TSKgel ODS-120T (1:3 acetonitrile/H_2O with 0.2% TFA) compound obtained: **19** (19.5 mg). Fraction 3M (296.1 mg): Column: TSKgel ODS-120T (7:13 acetonitrile/H_2O with 0.2% TFA) compounds obtained: **38** (37.0 mg) and **40** (4.2 mg). Fraction 3N: compound obtained: **24** (122.4 mg). Fraction 4E (103.7 mg): Columns: TSKgel ODS-120T (3:17 acetonitrile/H_2O), Capcell Pak C8 (1:4 acetonitrile/H_2O), Mightysil RP-18 GP (1:9 acetonitrile/H_2O), compounds obtained: **20** (4.0 mg) and **35** (3.6 mg). Fraction 4F (119.1 mg): Columns: TSKgel ODS-120T (3:17 acetonitrile/H_2O with 0.2% TFA) and Mightysil RP-18 GP (1:9 acetonitrile/H_2O with 0.2% TFA), compound obtained: **36** (2.4 mg). Fraction 4H (430.9 mg): Column: TSKgel ODS-120T (1:4 acetonitrile/H_2O with 0.2% TFA), compound obtained: **32** (136.9 mg). Fraction 4I (358.5 mg): Columns: TSKgel ODS-120T (1:4 acetonitrile/H_2O with 0.2% TFA) and C_{30}-UG-5 (3:7 acetonitrile/H_2O with 0.2% TFA), compound obtained: **1** (4.0 mg). Fraction 4J (260.0 mg): Columns: TSKgel ODS-120T (1:4 acetonitrile/H_2O with 0.2% TFA), C_{30}-UG-5 (1:3 acetonitrile/H_2O with 0.2% TFA), and Mightysil RP-18 GP (1:4 acetonitrile/H_2O with 0.2% TFA), compound obtained: **22** (4.5 mg). Fraction 4K (360.7 mg): Columns: TSKgel ODS-120T (1:4 acetonitrile/H_2O with 0.2% TFA) and C_{30}-UG-5 (1:4 and 1:3 acetonitrile/H_2O with 0.2% TFA), compound obtained: **40** (2.2 mg). Fractions 4L and 4M (368.9 mg): Columns: TSKgel ODS-120T (3:7 acetonitrile/H_2O with 0.2% TFA) and C_{30}-UG-5 (1:4 acetonitrile/H_2O with 0.2% TFA), compounds obtained: **7** (4.7 mg), **8** (1.8 mg), **9** (1.3 mg), **10** (0.4 mg), **13** (14.7 mg), and **22** (5.3 mg). Fraction 4N (388.0 mg): Columns: TSKgel ODS-120T (3:7 acetonitrile/H_2O with 0.2% TFA), Capcell Pak C_8 (3:7 acetonitrile/H_2O with 0.2% TFA), and Mightysil RP-18 GP (1:3 acetonitrile/H_2O with 0.2% TFA), compound obtained: **39** (4.9 mg). Fraction 5B (165.2 mg): Column: TSKgel ODS-120T (1:4 acetonitrile/H_2O with 0.2% TFA), compound obtained: **32** (19.6 mg). Fraction 5C (145.8 mg): Columns: TSKgel ODS-120T (1:3 acetonitrile/H_2O with 0.2% TFA), C_{30}-UG-5 (1:3 acetonitrile/H_2O with 0.2% TFA), and Mightysil RP-18 GP (1:3 acetonitrile/H_2O with 0.2% TFA), Compounds Obtained: **18** (12.6 mg), **19** (6.3 mg), **7** (2.5 mg), **33** (2.2 mg). Fraction 5D (171.4 mg): Columns:

TSKgel ODS-120T (1:3 acetonitrile/H_2O with 0.2% TFA) and C_{30}-UG-5 (3:7 acetonitrile/H_2O with 0.2% TFA), compounds obtained: **17** (5.0 mg) and **38** (30.7 mg). Fraction 5E (98.9 mg): Columns: TSKgel ODS-120T (1:3 acetonitrile/H_2O with 0.2% TFA), C_{30}-UG-5 (3:7 acetonitrile/H_2O with 0.2% TFA), and Mightysil RP-18 GP (1:3 acetonitrile/H_2O with 0.2% TFA), compounds obtained: **4** (4.1 mg) and **34** (15.0 mg). Fractions 5F and 5G (281.5 mg): Columns: TSKgel ODS-120T (1:3 acetonitrile/H_2O with 0.2% TFA), C_{30}-UG-5 (7:13 acetonitrile/H_2O with 0.2% TFA), compounds obtained: **2** (6.9 mg), **6** (4.1 mg), **25** (3.4 mg), **21** (3.3 mg), **23** (2.4 mg), **30** (5.1 mg), **31** (9.9 mg), **5** (3.1 mg), **11** (39.7 mg), **14** (8.8 mg), **3** (4.8 mg), **28** (1.4 mg). Fractions 5H, 5I, and 5J (229.8 mg): Column: TSKgel ODS-120T (3:7 acetonitrile/H_2O with 0.2% TFA), compound obtained: **12** (83.8 mg).

1.4.2. **The process of sugar identification**

The isolated compounds (**6**, **7**, and **8**), each weighing 1.0 mg, underwent hydrolysis using a mixture of HCl (3.5 N, 200 μL) and MeOH (200 μL) at 60°C for 30 minutes. Following hydrolysis, the resulting mixtures were passed through HP-20 resin columns (6 mm × 80 mm; eluted with approximately 3 mL of H_2O) to obtain the hydrolyzed sugar fractions. After vacuum drying, each sugar fraction was subjected to a reaction with L-cysteine methyl ester (5 mg) in pyridine (0.5 mL) for 60 minutes at 60°C. Subsequently, *o*-tolyl isocyanate (5 μL) was added to the mixture (Tanaka et al., 2007).

The reaction mixtures were then analyzed using HPLC with the following parameters:

- Column: Accalim 120 C_{18}, 5 μm; 4.6 mm × 250 mm
- Mobile phase: acetonitrile/H_2O with 0.2% TFA (1:3)
- Flow rate: 1.0 mL/min
- Detection: UV at 250 nm

The glucosidic derivatives of compounds **6**, **7**, and **8** were appeared at t_R = 18.8, 18.9, and 18.9 minutes, respectively. To verify the absolute configuration of the sugar component, they were compared with standards of D- and L-glucose derivatives (t_R = 18.8 and 17.5 minutes, respectively). This analysis was crucial for determining the specific arrangement of the sugar moieties in the isolated compounds.

1.4.3. **Calculation of ECD**

This specific part of the research was conducted in collaboration with Yoshinobu Ishikawa (Faculty of Pharmaceutical Sciences, Shonan University of Medical Sciences). The stable conformation analyses and ECD calculations for compounds **2** and 8-*C*-α-L-arabinopyranosyl-6-*C*-β-D-glucopyranosyl-5,7-dihydroxyflavone were conducted as shown in previous report (Murata et al., 2019).

Conformational Analysis:

- A conformational analysis was performed using a shell script reported by (Ishikawa, 2013).
- 300 energy-minimized three-dimensional structures of the stereoisomers of **2** and 8-*C*-α-L-arabinopyranosyl-6-*C*-β-D-glucopyranosyl-5,7-dihydroxyflavone were generated from the two-dimensional chemical structures.
- Open Babel and Balloon (O'Boyle et al., 2011; Vainio and Johnson, 2007) were utilized for the conversion of two-dimensional structures to three-dimensional structures.
- The single-point energy of each conformer was calculated using the PM7 Hamiltonian in MOPAC2016 (Stewart Computational Chemistry). (Stewart., 2016)

Geometry Optimization

- The lowest-energy conformers obtained from the conformational analysis were geometrically optimized.
- A B3LYP/6-31+G(d,p) level of theory was employed for optimization in the gas phase.
- Gaussian 09 (Gaussian, Inc.) was used for the optimization calculation (Frisch et al., 2016).

ECD Calculation:

- Electronic Circular Dichroism (ECD) calculations were conducted at the B3LYP/6-31G(d,p) level of time-dependent density functional theory (TDDFT) in MeOH.
- The conductor-like polarizable continuum model (CPCM) was applied using

Gaussian 09.

- ECD spectra were obtained from 45 calculated excitation energies and rotational strengths.
- The spectra were represented as the sum of Gaussian functions centered at the wavelength of each transition with a parameter σ (width of the band at half height) set to 0.40 eV.

These computational methods were employed to explore the stable conformations and predict the ECD spectra for compounds **2** and 8-*C*-α-L-arabinopyranosyl-6-*C*-β-D-glucopyranosyl-5,7-dihydroxyflavone.

1.4.4. **Degradation of compounds 2–5 with K_2CO_3**

Compounds **2** (1.0 mg), **3** (0.5 mg), **4** (1.0 mg), and **5** (1.0 mg) were separately hydrolyzed with saturated K_2CO_3 in MeOH-d_4 (0.6 mL) at 20ºC for 12 h. The reaction mixtures were analyzed by HPLC (Acclaim 120 C_{18},5 μm 120 Å column, 4.6 mm × 250 mm, Thermo scientific; mobile phase, (1:4) acetonitrile/H_2O with 0.2% TFA, 1.0 mL/min; detector, UV at 256 nm). The alkaline-hydrolyzed flavone *C*-glycoside of **2**–**5** with their acyl groups removed was determined to be 8-C-α-L-arabinopyranosyl-6-C-β-D-glucopyranosyl-5,7-dihydroxyflavone (t_R = 12.2 min) by comparison with the isolated 8-*C*-α-L-arabinopyranosyl-6-*C*-β-D-glucopyranosyl-5,7-dihydroxyflavone and 6-*C*-α-L-arabinopyranosyl-8-*C*-β-Dglucopyranosyl-5,7-dihydroxyflavone (t_R = 12.2 and 9.40 min, respectively).

1.4.5. **Quantitative HPLC analysis of some main flavonoids in the leaves, stems, and roots of *S.Scutellaria***

Quantitative HPLC analysis of baicalin, baicalein, chrysin, chrysin-7-*O*-glucuronide, luteolin, scutellarin, wogonin, and wogonoside in the leaves, stems, and roots of *S. scordiifolia* was conducted using standard and purified samples. The following details describe the experimental setup and conditions:

Standard Samples: Authentic and purified samples of the compounds were used.

- Baicalin (Fujifilm Wako Pure Chemical Corporation, Osaka, Japan)
- Baicalein (Tokyo Chemical Industry, Tokyo, Japan)

- Chrysin (Fujifilm Wako Pure Chemical Corporation)
- Chrysin-7-*O*-glucuronide (purified by HPLC)
- Luteolin (LKT Laboratories, Inc., Saint Paul, Minnesota, USA)
- Scutellarein (purified by HPLC)
- Wogonin (Tokyo Chemical Industry)
- Wogonoside (Tokyo Chemical Industry)

HPLC Conditions:

- Reversed-phase HPLC system:
- Pump: PU4180, JASCO Co., Tokyo, Japan
- Column: Acclaim 120 C_{18}, 5μm, 120Å, 4.6mm × 250mm, Thermo Scientific, USA
- Flow rate: 1.0 mL/min
- Detector: Photodiode array detector, JASCO Co.

Analysis Procedure:

1. HPLC analysis was performed three times for each standard sample at concentrations of 1.0 mg/mL, 0.5 mg/mL, 0.1 mg/mL, and 0.05 mg/mL.

2. Mean values were calculated and presented.

Calibration Curves: Calibration curves for the compounds used in the quantitative analyses were established with the following parameters:

Baicalin:
- Solvent system: acetonitrile/H_2O containing 0.2% TFA (1:3 v/v)
- UV at 276 nm
- Calibration curve: y = 6453503x, R^2 = 0.999

Baicalein:
- Solvent system: acetonitrile/H_2O containing 0.2% TFA (2:3 v/v)
- UV at 276 nm
- Calibration curve: y = 8686258x, R^2 = 0.999

Chrysin:
- Solvent system: acetonitrile/H_2O containing 0.2% TFA (2:3 v/v)
- UV at 264 nm
- Calibration curve: y = 9943470x, R^2 = 0.99

Chrysin-7-*O*-glucuronide:

- Solvent system: acetonitrile/H_2O containing 0.2% TFA (1:3 v/v)
- UV at 264 nm
- Calibration curve: y = 5893217x, R^2 = 0.997

Luteolin:

- Solvent system: acetonitrile/H_2O containing 0.2% TFA (1:3 v/v)
- UV at 252 nm
- Calibration curve: y = 5038702x, R^2 = 0.99

Scutellarin:

- Solvent system: acetonitrile/H_2O containing 0.2% TFA (1:3 v/v)
- UV at 336 nm
- Calibration curve: y = 8000259x, R^2 = 0.997

Wogonin:

- Solvent system: acetonitrile/H_2O containing 0.2% TFA (2:3 v/v)
- UV at 272 nm
- Calibration curve: y = 10790837x, R^2 = 0.999

Wogonoside:

- Solvent system: acetonitrile/H_2O containing 0.2% TFA (1:3 v/v)
- UV at 272 nm
- Calibration curve: y = 6527178x, R^2 = 0.999

Sample Preparation:

DMSO/H_2O (1:1) solution (200 μL) was added separately to each sample tube (1.5 mL) containing 10 mg of the powders of the leaves, stems, and roots of *S. scordiifolia*. The samples were sonicated for 3 hours at 35°C.

Chapter II. Phytochemical investigation on *Artemisia sieversiana*

2.1 Introduction

Artemisia sieversiana Ehrh. ex Willd holds significant cultural importance as a traditional medicinal herb in various regions of Asia, including Mongolia, Tibet and China. In Mongolia, it is commonly used to address fevers, throat inflammation, and pneumonia using its flowers (Ligaa., 2005). During spring, this plant plays a crucial role in the dietary patterns of nomadic Mongolian livestock, particularly camels, goats, and sheep. However, in the summer, animals tend to avoid consuming *A. sieversiana*, resuming their intake during the autumn (Damiran,2005). Nomadic people have noticed that when their livestock ingest *A. sieversiana* in the fall, the resulting milk develops an undesirable taste and odor, while the meat becomes tougher. The diet in Mongolia generally based on animal products such as meat, milk and dairy products (Odsuren T., 2014). Therefore, obtaining accurate information about the chemical composition of commonly consumed forage plants for livestock is very vital.

Fig. 11. Grazing livestock animals and unearthed root of *Artemisia sieversiana*

A. sieversiana, known for its medicinal applications, has been studied for its chemical constituents and their biological properties (Liu S-J., 2017). In our current study, we have identified three novel sesquiterpenoids from the aerial parts of *A. sieversiana*, and their absolute configurations, differing from known isomers, were determined using single-crystal X-ray diffraction. Additionally, a new hydroxynerol-type compound was isolated from the roots. Regarding to phytochemicals, main compounds from the root and aerial parts of *A. sieversiana* are quite similar (sesartemin, yangambin, epiyangambin, diayangambin, tricin, and chlorogenic acid derivatives). Notably, sesquiterpenoids were not found in the root extract in our investigation. However, lignans with trypanocidal

activity, such as epiyangambin, were present in both parts of *A. sieversiana*. To assess the impact of this plant on animals, we have examined the inhibitory properties of the isolated compounds, including sesquiterpenoids, flavonoids, and lignans, against *Trypanosoma congolense*.

2.2 **Result and Discussion**

2.2.1 **Identification of isolated compounds**

Three new sesquiterpenoids (**42** − **44**) (Fig. 12) and known compounds were isolated from the extracts of dried *A. sieversiana* areolas via HPLC. The known compounds were identified comparing with existing references, costuslactone B (**45**) (Tan RX., 1999), artabsinolide A (**46**) (Beauhaire J., 1982), absinthin (**47**) (Bohlmann F., 1985), anabsinthin (**48**) (Turak A., 2014), absinthin A (**49**) (Turak A., 2014), chrysoeriol (**50**) (Kim JH., 2014), tricin (**51**) (Kong C., 2004), chrysosplenetin (**52**) (Sy L-K., 1998), e (**53**) (Gođevac D., 2015, Beck MA., 1999), epiyangambin (**54**) (MacRar WD., 1985), de-*O*-methylepimagnolin A (**55**) (Miyazawa M., 1994), yangambin (**56**) (Bai H., 2005), diayangambin (**57**) (Solís PN., 2005), sesamin (**58**) (Li C-Y., 2005), epi-aschantin (**59**) (Ahmed AA., 2002), spinacetin 3-*O*-β-D-glucopyranoside (**60**) (Gođevac D., 2015), kaempferol 3-*O*-β-D-glucopyranoside (**61**) (Kazuma K., 2003), 1,5-dicafeoylquininic acid (**62**) (Murata Y., 1995), and 1,5-dicafeoylquininic acid methyl ester (**63**) (Murata Y., 1995). The structural determination of new terpenoids and activities of the isolated compounds is discussed below.

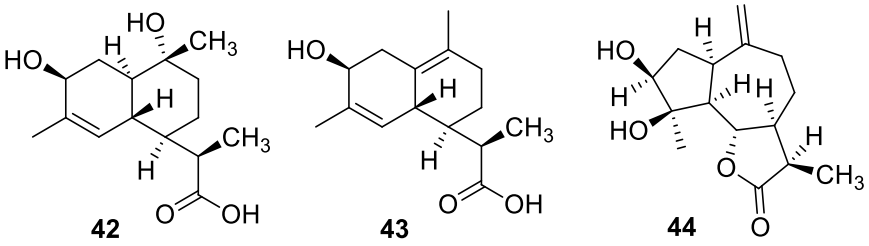


Fig. 12. Isolated new compounds from aerial parts of *A.sieversiana*

Compound **42**, obtained as colorless crystals from MeOH with a melting point of 233–234°C, displayed characteristics including ^{1}H NMR (refer to Table 6), ^{13}C NMR (refer to Table 6), and $[\alpha]^{21}_{D}$ −101 (*c* 0.3, MeOH). In the positive-ion mode of HREIMS, it exhibited a molecular ion peak at *m/z* 268.1667 $(M)^{+}$ (calculated for $C_{15}H_{24}O_4$,

268.1675). The molecular formula $C_{15}H_{20}O_5$ was assigned based on HR electron ionization (EI) MS analysis, with the calculated *m/z* for $C_{15}H_{20}O_5$ being 268.1675 and the measured m/z of the $(M)^+$ ion as 268.1667. The ^{13}C NMR spectrum with 15 resonances suggested the compound's sesquiterpenoid nature. Distinct features included a carbonyl carbon at C-12 (δ_C 179.0), olefinic carbons at C-3 (δ_C 139.0) and C-4 (δ_C 126.7), and oxygenated carbons at C-2 (δ 71.9) and C-9 (δ_C 72.0). The ^1H NMR spectrum revealed three methyl protons at δ_H 1.73 (3H, s, H-14), 1.13 (3H, d, *J*=7.0 Hz, H-13), and 1.08 (3H, s, H-15). An olefinic proton was observed at δ_H 5.62 (1H, d, *J*=2.0 Hz, H-4). The HMBC (Fig. 13) spectrum showed long-range coupling, such as H-13 with C-6, C-11, and C-12, and H-14 with C-2, C-3, and C-4, indicating the presence of three isobutyl moieties at C-3, C-6, and C-9.

^1H-^1H COSY correlations supported the presence of an aliphatic coupling system, indicating the main skeleton of **42** contained a chain, and its predicted molecular structure was identified as 2,9-dihydroxymuurol-3(4)-en-12-oic acid (Liu S-J., 2014, Feng Z-M., 2009). However, despite using the same solvent for NMR analysis (DMSO-d_6) (refer to Table 6), the results did not match those reported by Liu et al. and Feng et al., suggesting a difference in stereochemistry. The relative configurations were determined from NOE correlations (refer to Fig. 14). The H-5/H-1β (δ_H 1.22, 1H, t, *J*=10.5 Hz) and H-5/H-15 correlations suggested that H-5 and CH_3-9 had *β*-configurations.

Table 6. NMR spectroscopic data for compound **42**

Position	**42**[a]			**42**[b]		
	δ_C	δ_H (*J* in Hz)	HMBC	δ_C	δ_H (*J* in Hz)	HMBC
1	33.9	2.32, m 1.22, t (10.5)	2, 3, 5, 9, 10	33.0	2.21, dd (11.5, 5.0), 1.02	
2	71.9	4.08, m		69.3	3.87, m	
3	139.0			138.2		
4	126.7	5.62, d (2.0)	2, 3, 5, 6, 10, 14	124.6	5.47 brs	2, 6, 10, 14
5	41.9	2.00, m		39.9	1.84, m	
6	46.5	1.34[c]		44.6	1.21	
7	26.3	1.73[c] 1.34		25.1	1.55-1.65 1.19	
8	42.7	1.78[c] 1.46[c]		41.6	1.55-1.65	
9	72.0			69.5		
10	50.4	1.38, m		48.9	1.18	9
11	40.5	2.86, m	5, 6, 7, 12, 13	38.8	2.73, q (7.0)	12, 13

12	179.0			176.2		
13	15.0	1.13, d (7.0)	6, 11, 12	14.3	1.02, d (7.0)	6, 11, 12
14	19.7	1.73, s	2, 3, 4	19.5	1.63, s	2, 3, 4
15	20.5	1.08, s	8, 9, 10	20.4	0.92, s	8, 9, 10

[a]In methanol-d_4 solution. [b]In DMSO-d_6 solution. [c]Unclear signal pattern due to overlapping

Additionally, the H-2/H-10 correlation suggested they had α configurations. While correlations between H-5 and H-10 were observed in the spectra of the known compounds (Liu S-J., 2014, Feng Z-M., 2009), H-5/H-10 and H-10/H-15 correlations were not present in the NOE spectra of **42**. This absence supported the conclusion that **42** had a 5*R*,10*R* configuration. To further confirm the structure, including its absolute configuration, single-crystal X-ray diffraction (SC-XRD) was performed after generating a cuboidal crystal in MeOH. The SC-XRD results established the 2*S*,5*R*,6*R*,9*R*,10*R*,11*R* configurations.

Despite the SC-XRD analysis suggesting a 5*R*,6*R* configuration, a NOE correlation between H-5 and H-6 was observed, likely due to the vicinal nature of H-5 and H-6 protons. Combining these findings, **42** was characterized as (*R*)-2-((1*R*,4*R*,4a*R*,6*S*,8a*R*)-4,6-dihydroxy-4,7-dimethyl-1,2,3,4,4a,5,6,8a-octahydronaphthalen-1-yl) propanoic acid and given the name tsarvanin A.

Compound **43**, presenting as a colorless amorphous solid, displayed characteristics including ^{1}H NMR (refer to Table 7), ^{13}C NMR (refer to Table 7), and $[\alpha]^{20}_D$ −11 (*c* 0.3, MeOH). In the negative-ion mode of HRFABMS, it exhibited a molecular ion peak at *m/z* 249.1495 $(M–H)^-$ (calculated for $C_{15}H_{21}O_3$, 249.1491). Notably, **43** possessed two fewer hydrogen atoms and one less oxygen atom compared to **42**. The ^{13}C NMR spectrum indicated fifteen carbon resonances, confirming its sesquiterpenoid nature. Distinct features included a carbonyl carbon at C-12 (δ_C 180.5), two sets of olefinic carbons at C-3 (δ_C 137.1) and C-4 (δ_C 126.5), and at C-9 (δ_C 127.0) and C-10 (δ_C 126.5), along with an oxygenated carbon at C-2 (δ_C 71.1). The HMBC spectrum (Fig. 13) revealed a methyl singlet at δ_H 1.66 (3H, s) with long-range couplings to C-8 (δ_C 31.9), C-9, and C-10, indicating a methyl group on an olefinic carbon instead of the oxygenated carbon found in **43**.

Remaining HMBC correlations (Fig. 13) mirrored those of **43**, including those

between H-4 (δ_H 5.57, 1H, brs) and C-2, C-5 (δ_C 40.1), C-6 (δ_C 44.0), C-10, and C-14 (δ_C 18.9); H-13 (δ_H 1.21, 3H, d, J=7.0 Hz) and C-6, C-11 (δ_C 39.7), and C-12; and H-14 (δ_H 1.77, 3H) and C-2, C-3, and C-4. Based on these findings, the proposed molecular structure of **43** was identified as 2-(6-hydroxy-4,7-dimethyl-1,2,3,5,6,8a-hexahydronaphthalen-1-yl) propanoic acid, representing a dehydrated derivative of **42**.

Although only key ROESY correlations were observed between H-5 and H-6 and between H-11 and H-13 (refer to Fig. 14), a 2*S*,5*R*,6*R*,11*R* configuration was suggested, assuming a shared biosynthetic pathway with **42**. Similar to **42**, the vicinal protons H-5 and H-6 in 2 explained the ROESY correlation between them. Consequently, the structure of 43 was identified as (*R*)-2-((1*R*,6*S*,8a*R*)-6-hydroxy-4,7-dimethyl-1,2,3,5,6,8a-hexahydronaphthalen-1-yl) propanoic acid (refer to Fig. 13) and named tsarvanin B.

Compound **44** was characterized with the molecular formula $C_{15}H_{22}O_4$, determined through its HR fast atom bombardment (FAB) MS spectrum. The $(M + Na)^+$ ion exhibited a measured *m/z* of 289.1418, closely aligning with the calculated *m/z* for $C_{15}H_{22}O_4Na$ at 289.1416. The ^1H and ^{13}C NMR spectra of **44** (refer to Table 7) closely resembled those of 8,9-dihydroxy-3,9-dimethyl-6-methylenedecahydroazuleno(4,5-b) furan-2(3*H*)-one (Sigstad EE., 1991). Notably, key HMBC correlations (Fig. 13) provided significant support for the proposed molecular structure, including correlations between H-13 and C-7, C-11, and C-12, as well as between the olefinic methylene protons (H-14a and H-14b) and C-1, C-9, and C-10. Additionally, correlations between methyl H-15 and C-3, C-4, and C-5 further validated this structure. However, discrepancies in NMR chemical shifts and key NOE correlations compared to reported compounds indicated a different configuration for **44** (see Fig. 14). Notably, NOE correlations between H-1 and H-5, and between H-5 and H-7, suggested a 1*R*,5*S*,7*S*-configuration (Sigstad EE., 1991). Correlations between H-7 and H-11, and between H-6 and H-13, supported a 5*S*,6*S*,7*S*,11*R* configuration akin to reported compounds. Yet, specific NOE correlations, such as between H-3 and H-5, and between H-5 and H-15, pointed to a 3*S*,5*S* and 4*R*,5*S* configuration, respectively. The NOE correlation between H-5 and H-15 suggested a 4*R*,5*S* configuration, aligning **44** with the latter compound. Additionally, the NOE correlation between H-3 and H-15 indicated a 3*S*,4*R* configuration. In conclusion, based on these findings, compound **44** was identified as (1*R*,3*S*,4*R*,5*S*,6*S*,7*S*,11*R*)-3,4-

dihydroxyguai-10(14)-en-6,12-olide, or (3*R*,3a*S*,6a*R*,8*S*,9*R*,9a*S*,9b*S*)-8,9-dihydroxy-3,9-dimethyl-6-methylenedecahydroazuleno(4,5-b) furan-2(3*H*)-one, and named tsarvanin C.

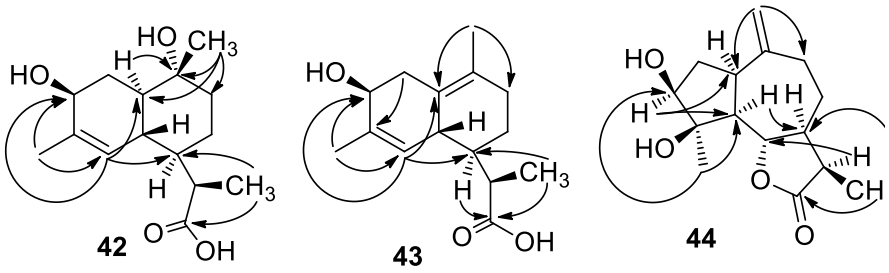


Fig. 13. Key HMBC correlations of new compounds

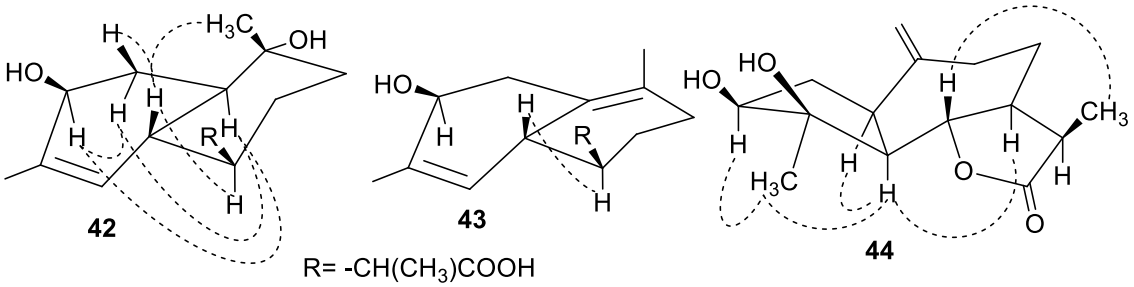


Fig. 14. The key NOE correlations of **42** - **44**

Table 7. ^{1}H and ^{13}C NMR spectroscopic and HMBC data for compounds **43** − **44**

	43[a]			**44**[b]		
Position	δ_C	δ_H (J in Hz)	HMBC	δ_C	δ_H (J in Hz)	HMBC
1	37.2	3.07, dd (12.5, 5.5)	2, 3, 5, 10	45.4	3.10, m	2, 5, 10
		1.88[c]	2, 10			
2	71.1	4.08, m	3	36.8	2.36, m	
					1.66, m	5, 3
3	137.1			82.0	3.76, d (4.0)	1, 5
4	126.5	5.57, brs	2, 5, 6, 10, 14	83.8		
5	40.1	2.81, brs		53.8	2.28, t (11.0)	4, 6, 7, 10
6	44.0	1.27[c]	11, 12	84.4	4.40, t (11.0)	
7	23.4	1.80[c] 1.27[c]		48.2	2.43, m	
8	31.9	1.97[c]		30.7	1.91, m	10
					1.38, m	
9	127.0			39.9	2.62, m	10
					1.87, dd (13.0, 4.0)	
10	126.5	1.38, m		150.7	1.18	
11	39.7	2.90, brdd (7.0)	12, 13	40.6	2.65, m	6
12	180.0			182.8		
13	15.2	1.21, d (7.0)	6, 11, 12	11.7	1.13, d (8.0)	7, 11, 12
14	18.9	1.77, s	2, 3, 4	112.0	4.93, s	1, 9, 10
					4.91, s	1, 9, 10

15	18.5	1.66, s	8, 9, 10	24.0	1.43, s	3, 4, 5

[a]In chloroform-*d* solution. [b]In methanol-d_4 solution. [c]Unclear signal pattern due to overlapping

Total of 9 compounds were isolated from the extract of *A. sieversiana* roots including a hydroxynerol derivative new compound (Fig. 15) (**64)**. The other known compounds 8-isovaleryoxy-nerylisovalerate (**65**) (Bohlman F., 1985), lignans (**54**, **55**, **56**, **57**, **59**), sesartemin (**66**) and tricin (**51**). The main compounds which were isolated from the root and aerial part of *A. sieversiana* approximately identical (sesartemin, yangambin, epiyangambin, diayangambin, tricin and chlorogenic acid derivatives) to each other.

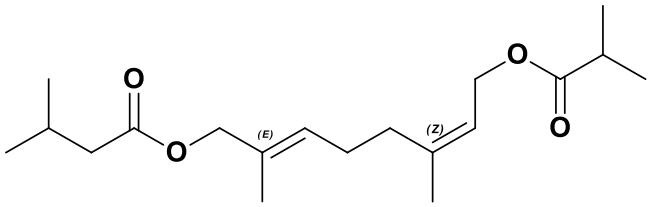


Fig. 15. Molecular structure of new compound **64**

Compound **64**: Colorless amorphous solid; ^{1}H and ^{13}C NMR (Table 8); HRFABMS (positive-ion mode) *m/z* 347.2204 $(M+H)^+$ (calcd for $C_{19}H_{32}O_4Na$, 347.2204. The ^{1}H-NMR data of compound **64** indicated existence of 2,6-dimethylocta-2,6-dien-1-yl with following shifts δ_H 4.45 (2H, s, H-1), 5.45 (1H, brs, H-3), 2.14 (4H, m, H-4, 5), 5.37 (1H, brt, 7.0), 4.55 (2H, d, 7.0, H-8) in aliphatic region with 3-methylbutanoate δ_H 2.21 (2H, brd, 7.0, H-2), 2.11 (1H, m, H-3), 0.96 (3H, d, 6.5) and 8-(isobutyryloxy) at δ_H 2.54 (1H, sep, 6.5) and 1.16 (3H, d, 6.5).

Table 8. ^{1}H and ^{13}C NMR spectroscopic data for compound **64**

C#	δ_C	δ_H (*J* in Hz)	C#	δ_C	δ_H (*J* in Hz)	C#	δ_C	δ_H (*J* in Hz)
1	69.8	4.45 (brs)	1'	173.0		1"	177.2	
2	130.9		2'	43.5	2.21 (brd, 7.0)	2"	34.0	2.54, (sep, 6.5)
3	128.5	5.45 (brs)	3'	25.4	2.11 (m)	3"	19.1	1.16 (d, 6.5)
4	31.6	2.14 (m)	4'	22.7	0.96 (d, 6.5)			
5	26.4	2.14 (m)						
6	119.8							
7	141.7	5.37 (brt, 7.0)						
8	60.8	4.55 (d, 7.0)						
9	14.0	1.65 (s)						
10	23.5	1.77 (s)						

In the HMBC spectrum (Fig. 16) cross-peaks was observed on methyl protons δ_H 1.65 (3H, s, H-9) and 1.77 (3H, s, H-10) to δ_C 130.9 (C-2) and 119.8 (C-6) respectively. δ_H 2.21 (2H, brd, 7.0, H-2') showed the correlation with δ_C 173.0 (C-1'), 22.7 (C-4'), 25.4 (C-3') which is existence of 3-methylbutanoate group and correlation between δ_H 4.45 (2H, brs, H-1) to δ_C 173.0 (C-1') showed 3-methylbutanoate connected with 2,6-dimethylocta-2,6-dien-1-yl. And from δ_H 4.55 (2H, d, 7.0, H-8) cross peak was observed with δ_C 177.2 (C-1'') which proves connection of isobutyryloxy group to 2,6-dimethylocta-2,6-dien-1-yl. The relative configurations were determined from the NOE correlations (Fig. 16). We observed with the *E* configuration on the H-2 because of significant enhancement of the 4.45 (2H, brs, H-1) upon irradiation of 5.45 (1H, brs, H-3) and the NOE correlations 5.45(H-3)/4.45(H-1), 4.45(H-1)/5.45(H-3) and 1.65(CH_3-9), 1.65(CH_3-9)/4.45(H-1) suggested that H-2 had *E* configuration, 5.37(H-7)/4.55(H-8) and 1.77(CH_3-10), 4.55(H-7)/4.45(H-1) and 5.37(H-7), 1.77(CH_3-10)/5.37(H-7) correlations exhibited H-6 had *Z* configuration.

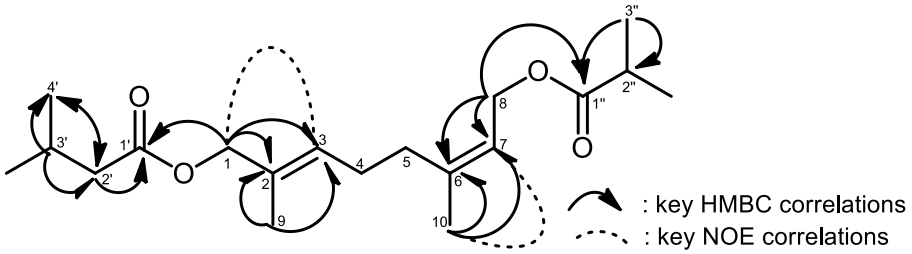
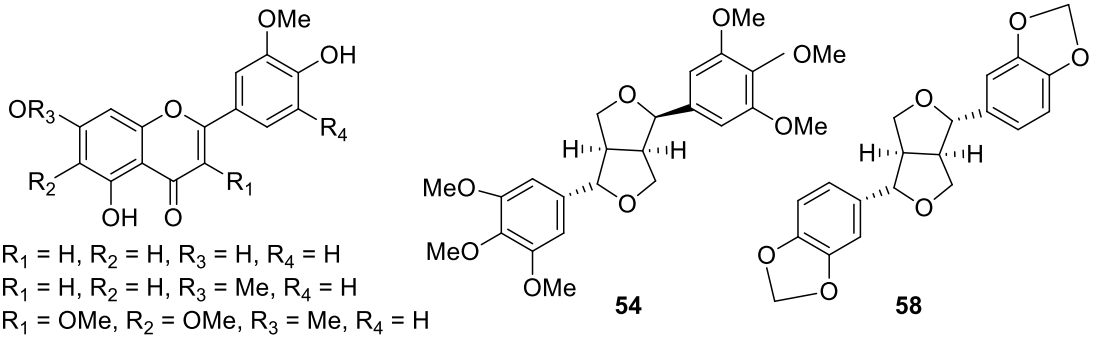


Fig. 16. The key HMBC and NOE correlations of compound **64**

2.2.1 **Trypanocidal activity of the isolated compounds from *A. sieversiana***

Isolated compounds **42–59** were tested for their inhibitory activities against *T. congolense*. Some flavonoids (**50**–**52**) and lignans (**54** and **57**) had trypanocidal activity, and their IC_{50} values ranged from IC_{50} 2.9 to 90.2 μM (Fig. 17, Table 9). Although the sesquiterpenoids isolated in this study did not have shown trypanocidal activity against *T. congolense*, the structurally similar sesquiterpenoids guaianolide (Cogo J., 2012) and nobilin (Mieri MD., 2015) were reported to have trypanocidal activity against *T. cruzi* and *T. brucei*, respectively. Simple flavonoids, including isorhamnetin, luteolin, and quercetin have been investigated and are known to have trypanocidal potential (Cockram PE., 2018). In this work, the simple flavonoids had trypanocidal activity against *T.*

congolense, while the flavonoid glycosides did not contain active components. The lignans also had moderate trypanocidal activity, and lignans were identified as active compounds against *T. congolense* (Odonbayar B., 2019). Although the aldehyde group was a key structure in that report (Odonbayar B., 2019), **54** and **58** did not contained aldehyde groups. Although compounds **54–59** had similar chemical structures, we did not identify specific structural features that affected their activities against *T. congolense*. However, lignans, which were phenylpropanoid dimers, displayed potential trypanocidal activity. This study adds to the body of knowledge about the relationships between the structures and trypanocidal activities of terpenoids, flavonoids, and lignans.



50 R_1 = H, R_2 = H, R_3 = H, R_4 = H
51 R_1 = H, R_2 = H, R_3 = Me, R_4 = H
52 R_1 = OMe, R_2 = OMe, R_3 = Me, R_4 = H

Fig. 17. Molecular structure of trypanocidal active compounds from *A. sieversiana*

Table 9. Evaluation of chemicals extracted from *Artemisia sieversiana* for their inhibitory action against *T. congolence*.

Compounds	IC_{50} (μM)
50	16.5
51	90.2
52	2.9
54	17.2
58	10
Pentamidine	0.169
Diminazene	0.109

The other isolated compounds found inactive at 50μg/mL

2.3 **Conclusion**

We have isolated 31 compounds from the aerial and underground (roots) parts of *Artemisia sieversiana*, including three new sesquiterpenoids and one nerol hydroxy-type new compound. The molecular structure elucidation of compound **42** was established

through single-crystal X-ray diffraction crystallography. Additionally, the trypanocidal activity of the isolated compounds was also tested, resulting in the discovery of some lignans and flavonoids with anti-trypanocidal activity which are including in the both parts (aerial parts and roots). The phytochemical profiles of the aerial parts and roots were almost identical. Further quantitative analyses of the secondary metabolites and investigations into biological activities related to livestock animal diseases and ecological significance are needed for a comprehensive understanding.

2.4 **Experimental**

The instruments used in the study, as well as the plant materials and experimental procedures related to the trypanocidal activity, are explained in detail on page 50-52.

2.4.1 **Extraction, isolation and purification process**

Aerial parts of *Artemisia sieversiana* were collected in 3 different seasons. Aerial from *A. sieversiana* collected in October (155 g) extracted three times using 4:1 acetone/H_2O and obtained 11 g of crude extract.

Table 10. Isolation of crude extract

Fractions	Amount (g)	Elution
1A	-	H_2O
1B	1.1	MeOH–H_2O (1:4)
1C	0.60	MeOH–H_2O (2:3)
1D	1.6	MeOH–H_2O (3:2)
1E	0.78	MeOH–H_2O (4:1)
1F	5.5	MeOH

Separation of Fraction 1B: Column: ODS-SM-50C-M (1:9–2:3 MeOH/H2O), C_{30}-UG-5 (1:9 acetonitrile/H_2O). Obtained compounds: **55** (4.0 mg), **62** (1.8 mg). Fraction1D: Column: ODS-SM-50C-M (3:2 MeOH/H_2O), C_{18}-AR-II, (3:17, 1:4, and 1:3 acetonitrile /H_2O with 0.2% TFA). Obtained compounds: **42** (17.9 mg), **46** (4.7 mg), **53** (8.5 mg), **63** (115.1 mg), and **64** (21.8 mg). Fraction 1E: Column: ODS-SM-50C-M (3:2 MeOH/H_2O), subfractions were purified via HPLC with Develosil C30-UG-5 (1:3 acetonitrile/H_2O with 0.2% TFA) and Cosmosil C_8 column (1:4 acetonitrile/H_2O with 0.2% TFA) and RP18-GP column (1:3 acetonitrile/H_2O with 0.2% TFA). Obtained compounds: **44** (5.9 mg), **53** (11.2 mg), **60** (1.9 mg), and **61** (1.2 mg).

A. sieversiana dried whole parts (200 g) were collected in May and extracted in three steps using a 4:1 acetone/water ratio, yielding an 18.6 g crude extract. After dissolving the crude extract in water and adding it to dichloromethane (DCM), the extract separated into fractions that were soluble in DCM (8.6 g) and water (9.3 g). Nine fractions with different solvent compositions were obtained by further processing the DCM fraction using HP-20 column chromatography. Further purification procedures were then carried out on a few selected fractions using high-performance liquid chromatography (HPLC) and ODS-SM-50C-M column chromatography with various stationary phases and solvent systems (Table 11).

Table 11. Isolation of crude extract

Fractions	Amount (mg)	Elution
3A	55.2	MeOH/H_2O (1:1)
3B	432.0	MeOH/H_2O (1:1)
3C	153.4	MeOH/H_2O (3:2)
3D	4.0	MeOH/H_2O (3:2)
3E	892.0	MeOH/H_2O (4:1)
3F	1300	MeOH/H_2O (4:1)
3G	536.1	MeOH
3H	1300	MeOH
3I	13.1	Acetone

Purification of Specific Fractions:

Fraction 3B: Column: ODS-SM-50C-M (3:2 MeOH/H_2O), ARII (2:3 acetonitrile/H_2O with 0.2% TFA). Compounds obtained: Compound **47** (3.9 mg). Fraction 3C: Column: ODS-SM-50C-M (1:1 acetonitrile/H_2O), (C_8, 2:3 acetonitrile/H_2O with 0.2% TFA). Compounds obtained **55** (12.7 mg). Mixture of Fr-3E and Fr-3F: Column: ODS-SM-50C-M (3:2 MeOH/H_2O), (ODS-120 T, C_{30}-UG-5, AR-II with various acetonitrile/H_2O ratios and 0.2% TFA). Obtained compounds **55** (2.7 mg), **56** (20.3 mg), **57** (122.8 mg), **58** (10.3 mg), and **59** (26.7 mg).

Three extractions were performed using 4:1 acetone/H_2O on 500 g of dried areolas from *A. sieversiana* that were collected in August. 42 g of crude extract were recovered following the solvent's evaporation at decreased pressure. The extract was dissolved in water, mixed with dichloromethane (DCM), and separated into two fractions: one that was water soluble (21 g) and the other that was DCM soluble (11 g). On a silica gel column, the DCM fraction was separated using an elution gradient of 100:0–0:100 hexane/acetone. Fr-2A − 2 K was obtained by normal phase column chromatography.

The purification processes: The mixture of Fr-2G and 2H was washed while going through HP-20 column using 80% MeOH for elution and the obtained extract was subjected to HPLCs (ODS-120 T, 3:7 acetonitrile/H_2O prepared with 0.2% TFA; C_{30}-UG-5, 7:13 acetonitrile/H_2O prepared with 0.2% TFA; C_8, 2:3 acetonitrile/H_2O prepared with 0.2% TFA; RP18-GP, 2:3 acetonitrile/H_2O prepared with 0.2% TFA) to obtain compounds **43** (2.7 mg), **45** (4.8 mg), **48** (109.4 mg), and **49** (2.0 mg).

Fr-2I was washed while going through HP-20 column using 80% MeOH for elution (35 g), and the obtained extract was subjected to HPLCs (ODS-120 T, 2:3 acetonitrile/H_2O prepared with 0.2% TFA; C_{30}-UG-5, 2:3 acetonitrile/H_2O prepared with 0.2% TFA; C_8, 2:3 acetonitrile/H_2O prepared with 0.2% TFA) to obtain compounds **48** (15.4 mg) and **50** (2.0 mg).

Fr-2J was washed while going through HP-20 column using 80% MeOH for elution (35 g), and the obtained extract was subjected to HPLCs (ODS-120 T, 2:3 acetonitrile/ H_2O prepared with 0.2% TFA; C_{30}-UG-5, 1:1 acetonitrile/H_2O prepared with 0.2% TFA; C_8, 1:1 acetonitrile/H_2O prepared with 0.2% TFA) to obtain compounds **48** (4.2 mg), **51** (0.8 mg), **52** (6.3 mg), **54** (23.1 mg), and **55** (6.5 mg).

Dried roots of *A.sieversiana* (550g) were extracted 3 times using 4:1 acetone/distilled water to yield 70 g of crude extract. The extract was subjected to open column chromatography on HP-20 (500 g) porous polymer gel using water and methanol for elution. Six fractions (ASR-1A to ASR-1G) were obtained as shown in table 12.

Table 12. Separation of crude extract of *A.sieversiana* roots.

Fractions (ASR)	Amount (g)	Elution
1A	26.3	H_2O
1B	2.44	MeOH–H_2O (1:4)
1C	1.48	MeOH–H_2O (2:3)
1D	1.97	MeOH–H_2O (3:2)
1E	2.1	MeOH–H_2O (4:1)
1F	11.5	MeOH
1G	7.8	Acetone

ASR-1D injected to ODS-SM-50C-M column using 2:2, 3:2, 4:1 MeOH/H_2O for elution and obtained subfractions were purified by HPLCs (ODS 120T 4.5:0.5 (acetonitrile/H_2O with 0.2 % TFA), ARII (2:3 acetonitrile/H_2O with 0.2% TFA), C_{30}-UG-5 (1:1 acetonitrile/H_2O with 0.2% TFA; C_8, 1:1 acetonitrile/H_2O with 0.2% TFA)) to obtain compounds **56** (1.2mg) and **57** (3.7mg).

ASR-1E: Subjected to ODS-SM-50C-M (2:3, 3:2 MeOH/H_2O) and subfractions were purified by HPLCs with (ODS 80Ts (4.5:0.5 acetonitrile/H_2O with 0.2 % TFA), ARII (2:3 acetonitrile/H_2O with 0.2% TFA), C_{30}-UG-5, 1:1 acetonitrile/H_2O with 0.2% TFA; C_8, (1:1 acetonitrile/H_2O with 0.2% TFA)). And Compounds obtained: **51** (0.8mg), **54** (92.9mg), **55** (4.6 mg), **56** (1.0g), **57** (99.0mg), **59** (110.1mg), and **66** (12.7 mg).

ASR-1F injected to ODS-SM-50C-M column using 3:2, 4:1, 5:0 MeOH/H_2O for elution and subfractions were purified by HPLCs (ODS 80Ts (2:3 acetonitrile/H_2O with 0.2 % TFA), C_{30}-UG-5, 2:3, 1:1 acetonitrile/H_2O with 0.2% TFA; C_8, 1:1 acetonitrile/H_2O with 0.2% TFA) to obtain compound **64** and **65.**

2.4.2 **SC-XRD analysis of compound 42**

This study was conducted by Yoshinobu Ishikawa (University of Shizuoka) within collaboration. Colorless compound **42** was crystallized from MeOH. SCXRD was performed using an XtaLAB Synergy-S diffractometer (Rigaku) equipped with a Cu Kα radiation source (λ = 1.54184 Å) at 93.15 K. Data collection, cell refinement, and data reduction were performed using the CrysAlisPro program. Olex2 (Dolomonov OV.,

2009) and the ShelXT (Sheldrick GM., 2015) structure solution program with intrinsic phasing were used to determine the structure of **42**. Structural refinement was performed using the ShelXL (Sheldrick GM., 2015) refinement package with least squares minimization. The crystal structure of compound ($C_{15}H_{24}O_4$, MW 268.34 g/mol) was assigned to the orthorhombic crystal system (0.170 ×0.142×0.117 mm3) in space group P212121, where Z=4, a=7.91956(7) Å, b = 11.29500(9) Å, c = 16.35715(12) Å, and α=β=γ=90°. The unit cell volume was 1,463.17(2) Å3 at (T) 93.15 K. The calculated density (ρ) was 1.218 g/cm3, and the Cu Kα absorption coefficient (μ) was 0.705 mm–1. In the 2θ range from 9.516˚ to 154.414°, 22,485 reflections were recorded, and 3026 were unique (Rint=0.0347). The final R1 was 0.0276 (≥ 2σ(I)), and wR2 was 0.0711 (all reflections). The value of the Flack parameter was –0.04(6), which indicated that the absolute structure was correctly determined. Additional crystallographic data for this compound can be found in Cambridge Crystallographic Data Center (CCDC) 1981977 and contained in the supplementary for this paper. The data can be obtained free of charge at https://www.ccdc.cam.ac.uk/conts/retri eving.html or from the CCDC (12 Union Road, Cambridge CB21EZ, UK; fax: + 44 1223 336033; e-mail: deposit@ ccdc.cam.ac.uk).

Chapter III. Phytochemicals from *Artemisia adamsii*

3.1 **Introduction**

Artemisia adamsii is predominantly distributed in Khentii, Khangai, Mongol Daguur, Mongol-Altai, Middle Khalkh, Eastern Mongolia, valleys, Gobi-Altai, and mostly grows in rocky, gravelly, dry steppes with soft soils, springs, salt marshes, and coastal ditches (Ligaa U., 2015; Shatar S., 2011). Regarding its edibility, animals typically avoid consuming this plant when it's green. However, during dry seasons (fall and winter), camels, horses, and cows consume it moderately. Despite its high protein and fat content, the plant is unpalatable for most animals due to its richness in fragrant essential oils (Ulziikhutag N., 1985).

In traditional Mongolian medicine, the leaves and inflorescences are used as a remedy for sore throats and toothaches, and the jam is used to relieve fever. In Tibetan hospitals, the above-ground part is included in prescriptions for antipyretics (Ligaa U., 2015). Recent studies indicate that *Artemisia adamsii* is considered a supportive plant for other palatable plants on the Mongolian rangeland due to its low phytotoxicity, which facilitates the germination of other grassland species (Kinugasa T., 2019).

Fig. 18. *Artemisia adamsii*

Until recently, comprehensive scientific information about the phytochemicals in this plant has been lacking. As a result, the reputed traditional uses of the plant have not been adequately supported by scientific evidence. Our findings, which reveal the

phytochemicals of *A. adamsii*, may contribute to further research aimed at understanding ecological interactions with animals and other plant species, as well as exploring the therapeutic applications of this plant.

3.2 **Result and discussion**

3.2.1 **Identification of isolated compounds**

Three new sesquiterpenoids (**67**−**69**) (Fig. 19) and known compounds (**70**−**92**) were isolated from the extracts of dried *A. adamsii* areolas via HP-20 column chromatography and HPLC. The known compounds were identified as 1-keto-6β,7α,11β-H-selin-4(5)-en-6,12-olide (**70**) (Antonio G., 1981), artemin (**71**) (Antoaneta B., 1996), 1α-acetoxyeudesm-4-en-6β H,11β H-12,6-olide (**72**) (Kazuyosgi K., 2003), martimin (**73**) (Antonio G., 1981), santolinifolide A (**74**) (Jakupovich J., 1991), (*Z*)-5′-hydroxyjasmone-5′-*O*-β-*D*-glucopyranoside (**75**) (Junichi K., 2004), (6*R*,9*R*)-3-oxo- α-ionol-9-*O*-β-*D*-glucopyronoside (**76**) (Kuang Hx., 2008), vicenin-2 (**77**) (Chen Xie., 2003), quercetin-3-*O*-β-D-glucopyranoside (**78**) (Enaam Y. Backheet., 2003), kaempferol 3-*O*-β-D-glucopyranoside (**61**) (Enam Y., 2003), rutin (**79**) (Zor M., 2017), kaempferol 3-*O*-rutinoside (**80**) (Osw S., 2020), tomentin (**81**) (Hammodal H.M., 2007), isofraxetin (**82**) (Haoxin Li., 2018), isofraxidin (**83**) (Cho JY., 2016), umbelliferone (**84**) (Haoxin Li., 2018), eleutheroside B_1 (**85**) (Ozawa M,. 1983), cichoriin (**86**) (Kisiel W., 1983), 3,5-di-*O*-caffeyol quinic acid (**87**) (Purusatam B., 1996), methyl 3,5-di-*O*-caffeoyl quinate (**88**) (Choi S.Z., 2004), chlorogenic acid methyl ester (**89**) (Zhu X., 2005), picein (**90**) (Jeon S.H., 2008), benzyl 2-*O*–β-D-glucopyranosyl-2,6-dihydroxybenzoate (**91**) (Brigida D'Abroska., 2001), pleoside (**92**) (Etemadi-Tajbakhsh N., 2020).

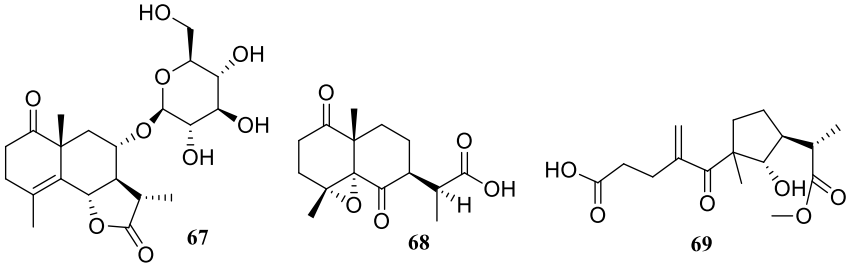


Fig. 19. Molecular sturucture of new sesquiterpenoids from *Artemisia adamsii*

Compound 67: $[\alpha]^{20.1}_{D}$ −48.57 (*c* 1.4, MeOH) was acquired in the form of a white amorphous powder. A sodium adduct ion $(M+Na)^+$ at *m/z* = 449.1787 (calcd for $C_{21}H_{30}O_9Na^+$: 449.1787) in the (+)-high-resolution fast atom bombardment mass spectrometry indicated a molecular formula of $C_{21}H_{30}O_9$.

The resonances for 3 methyl at δ_H 1.38 (3H, *d*, *J*=7.5 Hz, H-13), 1.25 (3H, s, H-14), and 1.95 (3H, s, H-15) and oxygenated protons in the aliphatic region (δ_H 4.35, H, d, *J* = 8.0 Hz, 3.09, H, dd, *J* = 9.0, 8.0 Hz, 3.24, H, dd, *J* = 9.0, 8.0 Hz, 3.25, 2H, overlapped, 3.65, H, dd, *J*=12.0, 5.0 Hz, 3.83, H, dd, *J*=12.0) observed in the ^{1}H-NMR spectrum. Moreover, H-6 (δ_H 5.66, H, d, *J* = 6.0 Hz) is doublet wich indicates *cis*-lactone fusion junction (Pinhey JT,. 1965). A total of 21 resonances in the ^{13}C NMR spectrum assigned as three methyl, four methylene, two methine and six oxygenated carbons by the HMQC spectrum. Among them, signals for carbonyl (δ_C 216.7), double bond (δ_C 140.0 and 128.1) and oxygenated sugar carbons (δ_C 105.0, 75.3, 78.2, 71.4, 77.8 and 62.7) were assigned via their chemical shifts.

The HMBC spectrum of **67** showed long-range coupling between H-13 and C-7 (δ_C 49.8), C-11 (δ_C 42.3), and C-12 (δ_C 182.4), which resonances were indicated lactonic functional group, H-14 correlated with C-1 (δ_C 216.7), C-5 (δ_C 128.1), and C-9 (δ_C 38.9) and C-10 (δ_C 47.5), while H-15 coupled with C-3 (δ_C 30.9), C-4 (δ_C 140.1), and C-5 (δ_C 128.1). (Table 13, Fig. 20). ^{1}H-^{1}H COSY showed correlations between H-2/H-3; H-6/H-7; H-7/H-8 and H-6; and H-8/H-9 and H-7. (Fig. 20). The relative configuration of **67** was established by the NOESY correlations. The main correlations of aglycone was compared with data of 8-hydroxytaurin (Mericli, A., 1987). Distinguished characteristic NOE cross-peak between the anomeric sugar proton (δ_H 4.35, H, d, *J* = 8.0 Hz) and H-8 of the aglycone corroborating the connection of the glucopyronosyl moiety to the C-8 position. (Fig. 20)

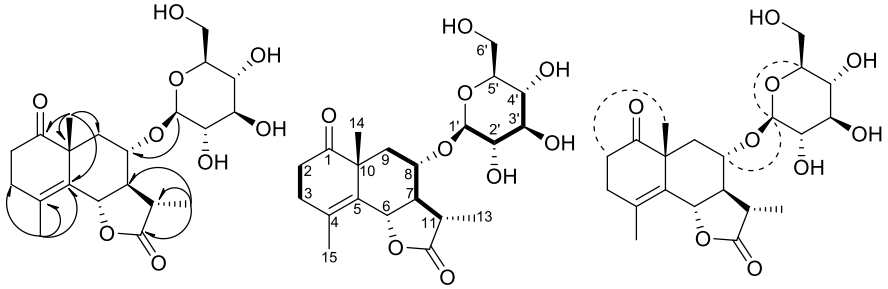


Fig. 20. HMBC, ^{1}H-^{1}H COSY and NOESY correlations of compound **67**

Table 13 ^{1}H and ^{13}C NMR data for compound **67**

Position	δ_H (*J* in Hz)	δ_C	Position	δ_H (*J* in Hz)	δ_C
1		216.7	**12**		182.4
2	2.35 2.26	36.3	**13**	1.38 (*d*, 7.5)	15.6
3	2.45 2.62	30.9	**14**	1.25 (s)	25.5
4		140.1	**15**	1.95 (s)	19.0
5		128.1	**16**		
6	5.66(*d*,6.0)	78.9	**1'**	4.35 (*d*, 8.0)	105
7	2.22 (*dd*, 7.0, 6.0)	49.8	**2'**	3.09(*dd*, 9.8)	75.3
8	3.80 (m)	77.8	**3'**	3.34 (*dd*, 9.8)	78.2
9	1.83(m) 2.09 (m)	38.9	**4'**	3.25 (over)	71.4
10		47.5	**5'**	3.25 (over)	77.8
11	2.85 (*d*, 7.5)	42.3	**6'**	3.65 (dd, 12.5) 3.83 (brd, 12)	62.7

[a]In CD_3OD-d_6 solution

Compound 68: $[\alpha]^{20.1}_D$ –48.57 (*c* 1.4, MeOH), was obtained as a colorless white amorphous powder and its molecular formula was determined as $C_{15}H_{20}O_5$ by the (+)-high-resolution fast atom bombardment mass spectrometry deprotonated ion $(M - H)^-$ at *m/z* = 281.1389 (calcd for $C_{15}H_{21}O_5$ 281.1389).

A total of 15 resonances were observed in the ^{13}C NMR spectrum which are the resonances of carbonyl δ_C 210.4, 204.4 and 180.7, three methyl carbons at δ_C 14.2, 18.2 and 17.9, an aliphatic quaternary and four methylene carbons deduced the aid of HMQC experiment. The two oxygenated carbons C-4 (63.9) and C-5 (71.4) were elucidated to form of an epoxy moiety by the HMBC correlations of H_3-15/C-4/C-3/C-5, H_3-14/C-5 and H_2-3/ C-4/C-5. The HMBC correlations of δ_H 1.23/C-11/C-12 and C-7 shows isobutyric acid was attached to C-7. (Fig. 21). In the ^{1}H-NMR spectrum three methyl signals at δ_H 1.23 (3H, *d*, *J* = 7.0 Hz, H-13), 1.14 (3H, s, H-14), and 1.29 (3H, s, H-15) observed. Other methine and methylene groups resonances are presented in the table 14.

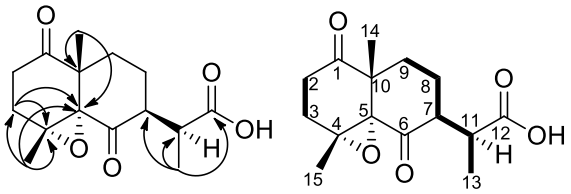


Figure 21. The key HMBC and ^{1}H-^{1}H COSY correlations of **68**

Table 14 ^{1}H and ^{13}C NMR data for **68**

Positions	δ_H (*J* in Hz)	δ_C	Positions	δ_H (*J* in Hz)	δ_C
1		210.4	**9**	1.92 2.15	32.7
2	2.65 (over) 2.30	34.2	**10**		49.2
3	2.30 2.18	27.9	**11**	2.84(m)	38
4		63.9	**12**		180.7
5		71.4	**13**	1.23 (d, 7.0)	14.2
6		204.4	**14**	1.14(s)	18.2
7	2.65(m)	53.2	**15**	1.29(s)	17.9
8	1.72 2.15	23.1			

Compound 69: $[\alpha]^{20.1}_{D}$ –48.57 (*c* 1.4, MeOH), obtained as a colorless white amorphous powder and its molecular formula of $C_{16}H_{24}O_6$ was determined by a sodium adduct ion $(M + Na)^+$ at *m/z* = 335.1470 (calcd for 335.1470) and its ^{1}H and ^{13}C NMR data (table 15).

The compound described previously (Shuai-Hua T., 2018), has a close molecular structure to arvestonate C, but differs by absence of an methoxy group and some relative configurations were doesn't match in the NOESY experiment (Fig. 22). Particularly, in comparison to arvestonate C (Shuai-Hua T., 2018) non-appearance of correlations between H_3-14/H-8 and H-11 expecting H_3-14 and H-6 are *β*–oriented. Sixteen carbon resonances were detected in the ^{13}C NMR spectrum and further classified into 3 methyl, five methylene, three methine and five quaternary carbons by the HMQC spectrum. The ^{1}H - ^{1}H COSY spectrum showed the correlations H_2-2/H_2-3, H-6/H-7, H_2-8/H_2-9, and H-11/H_3-13 (Fig. 22).

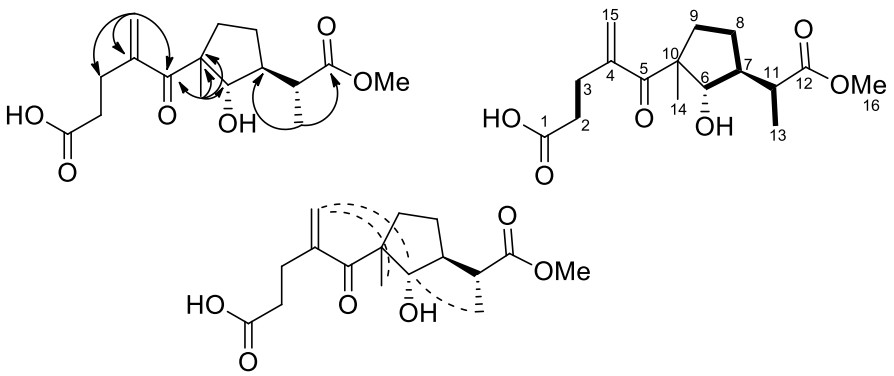


Fig. 22. The key HMBC, ^{1}H-^{1}H COSY and NOESY correlations of compound **69**

Table 15 ^{1}H and ^{13}C NMR data for **69**

C	δ_H (*J* in Hz)	δ_C	**C**	δ_H (*J* in Hz)	δ_C
1		176.6	**9**	1.39 (*m*)	35.4
2	2.4 (t, 7.6, 7.2)	33.7	**10**		59.6
3	2.52 (*m*)	29.7	**11**	2.43	44.7
4		147.8	**12**		178.3
5		209.4	**13**	1.14 *d* (7.0)	15.5
6	3.78 *d* (5.6)	85	**14**	3.67 (*s*)	52.6
7	2.18 (*m*)	52.1	**15**	1.27 (*s*)	24.3
8	**1.87 (*m*)**	**26.3**	**16**	**5.70 (*s*) 5.78 (*s*)**	**122.5**

3.3 **Conclusion**

We found 26 different compounds in *Artemisia adamsii*, including three new sesquiterpenoids. This is the first time these compounds have been discovered in this plant. This highlights the need for more research to understand the other phytochemicals in *Artemisia adamsii*. It's important to scientifically confirm how this plant has been traditionally used for medicine. Additionally, besides its possible medical uses, it's crucial to study how *Artemisia adamsii* affects livestock animals and the environment based on its chemical constituents. Continuing to explore the biological activities of these isolated compounds may reveal their possible advantages.

3.4 **Experimental**

The instruments used in the study, as well as the plant materials and experimental procedures related to the trypanocidal activity, are explained in detail on page 50−52.

3.4.1 **Extraction, isolation and purification process of aerial parts of *A.adamsii***

The dried aerial parts of *A. adamsii* (250 g) were extracted with 4:1 acetone/H_2O (2L x 72h x 3) at room temperature. The concentrated extract 26.6g was subjected to column chromatography on HP-20 porous polymer gel using water and methanol (MeOH) for elution. Seven fractions were collected: Fraction 1A (5.7 g) in water; Fraction 1B (0.56 g) in 4:1 H_2O/MeOH; Fraction 1C (1.25 g) in 3:2 H_2O/MeOH, Fraction 1D (4.2 g) in 2:3 H_2O/MeOH, Fraction 1E (5.48 g) in 1:4 H_2O/MeOH, Fraction 1F (5.65 g) in MeOH, and Fraction 1G (3.78 g) in acetone. Fraction 1C to 1F were subjected to ODS-SM-50C-M open column chromatography using aforementioned solvent system as the

mobile phase. Fraction 2A−2M from 1F (3:2, 4:1 and 5:0, MeOH: H_2O), fraction 3A-3N from 1E (2:3, 3:2, 4:1 and 5:0, MeOH: H_2O), fractions 4A−4I from 1D (1.5:3.5, 2.5:2.5, 4:1 and 5:0, MeOH: H_2O), fractions 5A-5F from 1C (1.5:3.5, 2.5:2.5, and 5:0, MeOH: H_2O). Fraction 2B (785.7 mg) was subjected to HPLC separation using ODS-80Ts (1.5:3.5 acetonitrile/H_2O containing 0.2% TFA as the mobile phases) and C_{30}-UG-5 column (2.25:2.75, acetonitrile/H_2O containing 0.2% TFA as the mobile phases) to obtain **73** (2.0 mg) and **74** (6.0 mg). Fraction 2C (1498.4 mg) was obtained as a white powder which were determined as **70** (1498.4 mg). Fractions 2D and 2E (1431.2 mg) were mixed together and soluble part objected to HPLC separation using ODS-80Ts (2:3 acetonitrile/H_2O containing 0.2% TFA as the mobile phases) and C_{30}-UG-5 column (2.2:2.8, acetonitrile/H_2O containing 0.2% TFA as the mobile phases) to obtain **13** (10.9 mg). Fraction 2G (361.9 mg) was subjected to HPLC separation using ODS-80Ts (2.5:2.5 acetonitrile/H_2O containing 0.2% TFA as the mobile phases) to obtain **72** (159.6 mg). Fraction 3D (453.6 mg) was subjected to HPLC separation using ODS-50C (2:3 MeOH/H_2O as the mobile phases), ODS-80Ts (1:4 acetonitrile/H_2O containing 0.2% TFA as the mobile phases) and C_{30}-UG-5 column (1.5:3.5, acetonitrile/H_2O containing 0.2% TFA as the mobile phases) to obtain **83** (5.9 mg), **84** (7.7 mg), **87** (8.8 mg), and **91** (10.4 mg). Fraction 3E (600.4 mg) was subjected to HPLC separation using ODS-50C (2:3 MeOH/H_2O as the mobile phases), ODS-80Ts (1:4 acetonitrile/H_2O containing 0.2% TFA as the mobile phases) and C_{30}-UG-5 column (1.5:3.5, acetonitrile/H_2O containing 0.2% TFA as the mobile phases) to obtain **71** (6.0 mg). Fraction 3F (501.4 mg) was subjected to HPLC separation using ODS-80Ts (1:4 acetonitrile/H_2O containing 0.2% TFA as the mobile phases) and C_{30}-UG-5 column (1.75:3.25, acetonitrile/H_2O containing 0.2% TFA as the mobile phases) to obtain **88** (17.0 mg). Fraction 4B (165.3 mg) was subjected to HPLC separation using ODS-80Ts (1:4 acetonitrile/H_2O containing 0.2% TFA as the mobile phases) and C_{30}-UG-5 column (0.75:4.25, acetonitrile/H_2O containing 0.2% TFA as the mobile phases), Mythysil RP-18GP (0.5:4.5 acetonitrile/H_2O containing 0.2% TFA as the mobile phases) to obtain **85** (3.9 mg). Fraction 4D (600.3 mg) was subjected to HPLC separation using ODS-80Ts (0.75:4.25 acetonitrile/H_2O containing 0.2% TFA as the mobile phases) and C_{30}-UG-5 column (1:4 acetonitrile/H_2O containing 0.2% TFA as the mobile phases) to obtain **92** (98.9 mg), **82** (72.9 mg), **81** (32.9 mg), **77** (5.7 mg) and **75** (8.5 mg). Fraction 4E (326.8 mg) was subjected to HPLC separation

using ODS-80Ts (1.5:3.5 acetonitrile/H_2O containing 0.2% TFA as the mobile phases) and C_{30}-UG-5 column (1.25:3.25, acetonitrile/H_2O containing 0.2% TFA as the mobile phases) to obtain **76** (10.2 mg), **78** (5.3 mg) and **79** (5.4 mg). Fraction 4F was subjected to HPLC separation using ODS-80Ts (2:3 acetonitrile/H_2O containing 0.2% TFA as the mobile phases) and C_{30}-UG-5 column (1.25:3.25, acetonitrile/H_2O containing 0.2% TFA as the mobile phases) to obtain **61** (16.6 mg) and **80** (19.6 mg). Fraction 5B (240.7 mg) was subjected to HPLC separation using ODS-50C (1.5:3.5, MeOH/H_2O as the mobile phases), ODS-80Ts (1.5:3.5 acetonitrile/H_2O containing 0.2% TFA as the mobile phases) and C_{30}-UG-5 column (0.5:4.5, acetonitrile/H_2O containing 0.2% TFA as the mobile phases) to obtain **86** (5.2 mg) and **90** (5.6 mg). Fraction 5C (302.4 mg) was subjected to HPLC separation using ODS-50C (1.5:3.5, MeOH/H_2O as the mobile phases), ODS-80Ts (1.5:3.5 acetonitrile/H_2O containing 0.2% TFA as the mobile phases) and C_{30}-UG-5 column (0.5:4.5, acetonitrile/H_2O containing 0.2% TFA as the mobile phases) to obtain **89** (6.4mg).

Instrumental

These instruments were used for the analysis and characterization of compounds, including optical rotations, UV spectra, ECD spectra, NMR, mass spectrometry, and chromatography during the research study.

The experimental procedures for characterizing the compounds involved various analytical techniques: Melting Points determined using a Yanaco Micro melting-point apparatus, polarimeter: P-2300 (Jasco Co., Tokyo, Japan), UV Spectrometer: MPS-2450 (Manufacturer: Shimadzu, Kyoto, Japan), Electronic Circular Dichroism (ECD) Spectropolarimeter: J-720 (Manufacturer: JASCO Inc., Japan), Nuclear Magnetic Resonance (NMR) Spectroscopy: ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a JEOL JNM-AL400 FT-NMR spectrometer, chemical shifts reported as δ values with TMS as an internal standard and measurements were performed in methanol-d_4, DMSO-d_6, and chloroform-d, heteronuclear correlations: Inverse-detected heteronuclear correlations were measured using HMQC (optimized for $^{1}J_{C\text{-}H}$ =145 Hz) and HMBC (optimized for $^{n}J_{C\text{-}H}$ =8 Hz) pulse sequences with a pulsed Feld gradient, Nuclear Overhauser Effect (NOE) Spectroscopy: NOE spectra were recorded using a JEOL JNM-EX270 FT-NMR spectrometer, rotating overhauser effect spectroscopy (ROESY): ROESY (600 MHz) spectrum was recorded using a JEOL JNM-ECZ600R/S1 spectrometer, Mass Spectrometry: High-Resolution Electron Impact Mass Spectrometry (HREIMS) and High-Resolution Fast Atom Bombardment Mass Spectrometry (HRFABMS with a glycerol matrix) data were obtained using a JEOL JMS700 mass spectrometer, Column Chromatography Resin: Diaion HP-20 (Mitsubishi Chemical Co., Tokyo, Japan), Preparative High-Performance Liquid Chromatography (HPLC): Jasco 2089, UV Detector: 210 nm, Columns: TSKgel ODS-120T (Tosoh, Tokyo, Japan, 21.5 mm × 300 mm), Mightysil RP-18 GP (Kanto Chemical, Tokyo, Japan, 10 mm × 250 mm), Develosil C_{30}-UG-5 (Nomura Chemical, Aichi, Japan, 20 mm × 250 mm), Capcell Pak C_8 (Shiseido, Japan, 20 mm × 250 mm), ODS-SM-50C-M (Yamazen Co., Osaka, Japan, 37 mm × 300 mm), Chiral Pak AS-H (Daicel, Japan, 20 mm × 250 mm).

In vitro activity of the purified compounds against *Trypanosoma congolense*

The samples of purified compounds from *Scutellaria scordiifolia* and *Artemisia sieversiana* were prepared at Tohoku Medical and Pharmaceutical University, and the procedure of the activity was performed by Keisuke Suganuma at National research center for protozoan diseases, Obihiro University of Agriculture and Veterinary Medicine. The trypanocidal activity assessment followed a protocol outlined in a prior study (Banzragchgarav et al., 2016). Here's a summary of the procedure:

Trypanosome Culture:

- Bloodstream trypomastigote form of the *T. congolense* IL3000 strain was cultivated in Hirumi's modified Iscove's medium (HMI)-9.

Initial Screening:

- All the purified compounds were screened at a concentration of 25 μg/mL.
- Based on the screening, potent trypanocidal active compounds were chose further investigation.

Subsequent Assay:

- Concentrations of selected compounds were adjusted through a five-fold serial dilution, ranging from 25 μg/mL to 1.6 ng/mL.
- Trypanosomes were incubated with the chemicals for 72 hours.

ATP Concentration Measurement:

- Intracellular ATP concentration was assessed using the Cell Titer-Glo Luminescent Cell Viability Assay reagent from Promega.
- After shaking and further incubation, luminescence measurements were taken using a Glo Max-Multi + Detection System plate reader from Promega.

IC_{50} Determination:

- To determine the IC_{50} value for each compound, a plot was generated in GraphPad PRISM 8 software.
- The y-axis represented % inhibition, and the x-axis represented the log concentration.
- Diminazene and pentamidine from Fujifilm Wako, Osaka, Japan, served as positive controls.

This methodology aimed to identify compounds with potent trypanocidal activity against the *T. congolense* IL3000 strain and determine their IC_{50} values for further evaluation.

Plant materials

The aerial parts of *Scutellaria scordiifolia* Fisch. ex Schrank were harvested from a fallow land in Tuv province, Arkhust soum, Narst 2nd bag, Mongolia, August 8, 2019.

Aerial parts of *Artemisia sieversiana* were collected on October 8, 2016, August 13, 2017, and May 5, 2018, from Argalant soum, Tuv province, Mongolia. The roots of *Artemisia sieversiana* were collected in May 4, 2020 from the same place with aerial parts.

The aerial parts of *Artemisia adamsii* were gathered in October 2020 from Bayan Soum, Tuv province, Mongolia.

Voucher specimens have been preserved in the Laboratory of Bioorganic Chemistry and Pharmacognosy, National University of Mongolia (90.02.01.18). The identification of the plant species was expertly conducted by Dr. Shagdar Dariimaa of the Mongolian State University of Education, Ulaanbaatar, Mongolia.

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