

Anti-urease Secondary Metabolites from *Seriphidium quettense*

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Abstract: Ethyl acetate layer of the methanolic extract of *Seriphidium quettense* was subjected to silica gel column chromatography to isolate one new; seriphiloid (**1**), and four known compounds; ilicic acid (**2**), 6 α -hydroxy-8(10)-oplopen-14-one (**3**), 2-(4-hydroxyphenyl)-5,6,7-trimethoxy-4H-chromen-4-one (**4**) and 2-(3,4-dihydroxyphenyl)-5,6,7-trimethoxy-4H-chromen-4-one (**5**). The chemical structure of the new isolate was established with the help of 1D, 2D NMR techniques and high resolution mass spectrometry. Known compounds were identified because of 1D NMR and mass spectrometric analysis and in comparison with the literature values. Compounds **1-5** were evaluated for their acetylcholinesterase, butyrylcholinesterase, α -glucosidase and urease inhibitory activities. Most of the metabolites were found inactive; however, compounds **2** and **3** showed good antiurease activity with IC₅₀ value 21.5 \pm 0.1 and 20.8 \pm 0.1 μ g/mL, respectively.

Keywords: *Seriphidium quettense*; secondary metabolites; structure elucidation; enzyme inhibition. © 2016 ACG Publications. All rights reserved.

1. Plant Source

Seriphidium quettense of the plant family Asteraceae is a woody shrub growing in Hazarganji, Baluchistan, Pakistan [1, 2]. The chromatographic purification of the methanolic extract of this plant yielded one new compound seriphiloid (**1**) and four known metabolites; ilicic acid (**2**), [3] 6 α -hydroxy-8(10)-oplopen-14-one (**3**), [4, 5] 2-(4-hydroxyphenyl)-5,6,7-trimethoxy-4H-chromen-4-one (**4**) [5] and 2-(3,4-dihydroxyphenyl)-5,6,7-trimethoxy-4H-chromen-4-one (**5**) [6] (Figure 1).

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The whole plant material (stem, leaves and branches) of *Seriphidium quettense* was collected from Ziarat, Baluchistan, in September 2010. The botanical identification was carried out by Prof. Dr. Rasool Bakhsh Tareen, Department of Botany, University of Baluchistan, Quetta, Pakistan, where a voucher specimen (RBT-SQ-11) has been deposited in the herbarium.

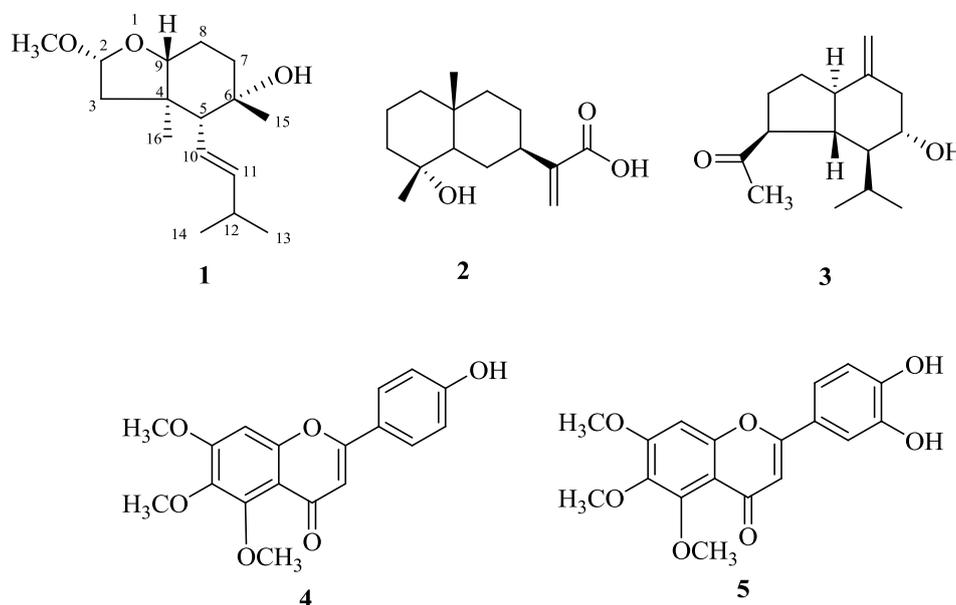


Figure 1. Structures of compounds isolated from *Seriphidium quettense*

2. Previous Studies

Literature search revealed that other members of the genus *Seriphidium* are used as antihelmintics [7], gastrointestinal disorders, diabetes and high blood pressure [8]. *S. annua* and *S. indica* are source of artemisinin, isolated from the aerial parts of these plants, whereas, *S. dubia* has been used as anti-ulcer and purgative agent; it is also used to cure asthma and skin diseases like scabies [9]. *S. absinthium* L. is source of anthelmintic, antifungal and antimicrobial agents. *S. brevifolia* is widely used as anthelmintic agent in ethno-veterinary medicinal system of Pakistan [9]. *S. kurramense* is commercially used for extraction of santonin, therefore, it is transported to many countries for this purpose [10].

3. Present Study

The plant material was dried under shade for two weeks and was ground to semi-powder (5.0 kg). It was then extracted with methanol (8.0 L) at room temperature for 6 days (twice). The combined concentrated extract (150 gm) was suspended in water (1.0 L) and was extracted with *n*-hexane and ethyl acetate to get 65 and 40 g respectively. The remaining water fraction was calculated as 35 g. The ethyl acetate fraction was then subjected to silica gel column chromatography eluting with *n*-hexane, *n*-hexane–chloroform, chloroform, chloroform–methanol in increasing order of polarity to obtain eight fractions (SQ1-SQ8) based on their TLC profiles. The fractions SQ1 (6 g) and SQ2 (4 mg) mostly contain oil and fat; thus were not processed further. The fraction SQ3 (4 g) obtained with *n*-hexane:chloroform (7:3) on silica gel chromatography gave two subfractions; S3-1 and S3-2, which on eluting with *n*-hexane:chloroform in increasing order of their polarity yielded compound 3 (30 mg). The fraction SQ4 (3 g) obtained from the main column with *n*-hexane:chloroform (6:4) was further purified on silica gel column eluted with *n*-hexane:chloroform (4.5:5.5) to get compound 2 (25 mg). Another fraction SQ6 (3.4 g) from the main column obtained with *n*-hexane:chloroform (3:7) was re-chromatographed on silica gel column using the same eluting system to get compound 1 (20 mg). The

other main fraction SQ7 (4.2 g) eluted with *n*-hexane:chloroform (2:8), on further silica gel column chromatography using a mobile phase of *n*-hexane:chloroform (3:7) yielded three subfractions S7-1-S7-3. The subfractions S7-2 and S7-3 were further purified on silica gel column eluting with *n*-hexane:chloroform in increasing order of polarity to get compounds **4** (25 mg) and **5** (23 mg).

Seriphiloid (**1**): white amorphous powder (20 mg); IR ν_{\max} (KBr) cm^{-1} : 3410, 1645; $^1\text{H-NMR}$ (CDCl_3 ; 600 MHz): δ (ppm) 5.53 (1H, dd, $J = 15.5, 9.5$ Hz, H-10), 5.45 (1H, dd, $J = 15.5, 7.0$ Hz, H-11), 5.05 (1H, t, $J = 5.0$ Hz, H-2), 3.93 (3H, s, 2-OMe), 3.41 (1H, br s, H-9), 2.30 (1H, m, H-12), 1.89 (1H, d, $J = 5.5$ Hz, H-3), 1.85 (1H, d, $J = 4.5$ Hz, H-7), 1.78 (2H, m, H-8), 1.75 (1H, d, $J = 9.5$ Hz, H-5), 1.51 (1H, dd, $J = 5.4, 1.8$ Hz, H-7), 1.37 (1H, d, $J = 5.5$ Hz, H-3), 1.08 (3H, s, Me-15), 1.02 (3H, s, Me-16), 0.97 (3H, d, $J = 6.5$ Hz, Me-13) and 0.96 (3H, d, $J = 6.5$ Hz, Me-14); $^{13}\text{C-NMR}$ (CDCl_3 ; 150 MHz): δ (ppm) 142.0 (C-11), 122.5 (C-10), 105.5 (C-2), 83.5 (C-9), 72.3 (C-6), 57.2 (C-5), 47.8 (C-3), 46.0 (C-4), 39.5 (C-7), 31.4 (C-12), 31.0 (C-15), 22.7 (C-13, 14), 21.6 (C-8), 15.8 (C-16) and 56.0 (2-OMe); EIMS: m/z 268 $[\text{M}]^+$; HR-EIMS: m/z 268.2036 $[\text{M}]^+$ (calcd. 268.2038 for $\text{C}_{16}\text{H}_{28}\text{O}_3$).

Enzyme Inhibitory Assays:

Acetylcholinesterase assay: A solution of 100 μL reaction mixture was prepared which contain 60 μL of buffer Na_2HPO_4 with pH 7.7, 10 μL (0.5 mM) test compound and 10 μL (0.005 unit well) enzyme was added and then pre-read at 405 nm. It was incubated for 10 minutes at 37°C. The reaction was initiated by the addition of 10 μL of 0.5 mM substrate (acetylthiocholine iodide), followed by the addition of 10 μL DTNB (0.5 mM). Eserine (0.5 mM) was used as a positive control. Then absorbance was measured at 405 nm on Synergy HT (BioTek, USA) microplate reader [11]. The percentage inhibition of the enzyme was calculated using the following formula:

$$\text{Inhibition (\%)} = \frac{\text{Control} - \text{Test} \times 100}{\text{Control}}$$

Butyrylcholinesterase assay: The butyrylcholinesterase inhibition activity was performed according to the same method as reported for Acetylcholinesterase with small difference as butyrylcholinesterase enzyme was used here. [11]

α -Glucosidase inhibition assay: A Total volume of 100 μl of the reaction mixture was prepared. It contained 70 μl 50 mM phosphate buffer saline with pH 6.8, 10 μl (0.5 mM) test compound, followed by the addition of 10 μl (0.057 units) enzyme. All these contents were mixed, preincubated for 10 min at 37°C and pre-read at 400 nm. The reaction was initiated by the addition of 10 μl of 0.5 mM substrate (*p*-nitrophenyl glucopyranoside) and acarbose was used as positive control. After 30 min of incubation at 37°C, absorbance was measured at 400 nm using Synergy HT microplate reader. All experiments were carried out in triplicates [12]. The percentage inhibition of the enzyme was calculated using the following formula:

$$\text{Inhibition (\%)} = \frac{\text{Control} - \text{Test} \times 100}{\text{Control}}$$

Antiurease assay (Berthelot method): The 6 mL of phosphate buffer was prepared and added into 20 mL of urease enzyme. It was dispensed in wells of plates. It was incubated for 10 minutes at 25 °C and 5 mL of the test compound was added in it. This mixture was further incubated at room temperature and after that 15 mL of 20mM urea was added. It was again incubated for 10 minutes at 25 °C and 100 mL RGT 2 was added. After incubation at room temperature for 25 minutes, absorbance was measured on ELISA reader by using Gen 5 software at 630 nm and percentage inhibition was calculated by using the following formulae [13].

$$\text{Inhibition (\%)} = \frac{\text{Control} - \text{Test} \times 100}{\text{Control}}$$

*Control: No sample was added.

*Negative control: No urea was added.

IC_{50} values of the compounds were calculated using EZ-Fit Enzyme Kinetics Software (Perrella Scientific Inc., Amherst, MA, USA).

Compound **1** was isolated as white amorphous powder, which displayed IR absorption bands at 3410 and 1645 cm^{-1} for hydroxyl and olefinic functions respectively. The EIMS spectrum of compound **1** displayed molecular ion peak at m/z 268, whereas, the molecular formula could be depicted by the HR-EIMS spectrum, which showed molecular ion at m/z 268.2036 corresponding to the molecular formula as $\text{C}_{16}\text{H}_{28}\text{O}_3$ with three index of hydrogen deficiency (IHD).

The ^1H -NMR spectrum of **1** displayed two signals for an olefinic system at δ 5.53 (1H, dd, $J = 15.5, 9.5$ Hz) and 5.45 (1H, dd, $J = 15.5, 7.0$ Hz). The larger J value of these two nuclei revealed the *trans* nature of the double bond. Four more methine resonances were observed at δ 5.05 (t, $J = 5.0$ Hz), 3.41 (br s), 2.30 (m) and 1.75 (d, $J = 9.5$). These protons were correlated in HSQC spectrum with the carbons at δ 105.5, 83.5, 31.4 and 57.2 respectively. A methoxyl signal displayed its position at δ 3.93. This data revealed that the methine resonated at δ 5.05 could be attached to two oxygen atoms. The spectrum further displayed signals at δ 1.89 (1H, d, $J = 5.5$ Hz), 1.85 (1H, d, $J = 4.5$ Hz), 1.78 (2H, m), 1.51 (1H, dd, $J = 5.4, 1.8$ Hz) and 1.37 (1H, d, $J = 5.5$ Hz), which could be attested for three methylene groups, based on the COSY, ^{13}C -DEPT and HSQC experiments. The resonances due to four methyl groups were also observed in the ^1H NMR spectrum at δ 1.08 (s), 1.02 (s), 0.97 (d, $J = 6.5$ Hz) and 0.96 (d, $J = 6.5$ Hz). An isoprene unit was identified in the molecule due to the COSY correlation of the two doublet methyl with the methine multiplet at δ 2.30, which in turn was correlated with olefinic methine proton at δ 5.45.

The ^{13}C -NMR spectrum of **1** was in complete agreement with the ^1H -NMR and mass data as it showed six signals for methine carbon (δ 142.0, 122.5, 105.5, 83.5, 57.2 and 31.4), three signals for methylene carbon (δ 47.8, 39.5 and 21.6), one for methoxyl group at δ 56.0 and four for methyl moieties (δ 31.0, 22.7, 22.7 and 15.8), while two quaternary carbon atoms showed their resonances at δ 72.3 and 46.0.

Most of the part of this molecule could be established due to several COSY correlations (Figure 2), whereas, the C-H connectivities were accomplished due to HSQC experiment. One IHD in molecular formula could be attested for double bond; while, remaining two IHD could be attributed to two cyclic systems. The HMBC correlation (Figure 2) of H-9 at δ 5.05 with the oxymethine carbon at δ 83.5 (C-9) and aliphatic methine carbon at δ 46.0 (C-4) led to the formation of a furan ring, therefore, the second ring could be established as six membered. The positions of other substituents on the two ring systems were established through further analysis of the HMBC spectrum, in which two methyl groups (δ 0.97, Me-13 and 0.96, Me-14) showed HMBC correlations with C-12 (δ 31.4), C-11 (δ 142.0). The HMBC correlation of methyl proton at δ 1.02 (Me-16) with the carbon at δ 83.5 (C-9), 57.2 (C-5), 46.0 (C-4) and 47.8 (C-3) and HMBC interaction of H-5 with Me-16 (δ 15.8) and C-11 (δ 142.0) confirmed the position of Me-16 and isopropyl moiety at C-5 (δ 57.2). Other diagnostic HMBC correlations are shown in figure 2.

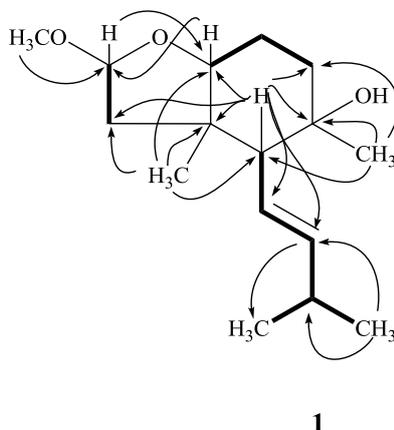


Figure 2. Key HMBC ($^1\text{H} \rightarrow ^{13}\text{C}$) and ^1H - ^1H COSY (—) correlations for compound **1**.

The combination of all spectroscopic data guided to the structure of **1** as a sesquiterpenoid, in which the relative stereochemistry at various chiral centers was established through careful examination of NOESY spectrum and construction of deriding model (Figure 3). In the NOESY

spectrum, H-2 (δ 5.05) interacted with H-9 (δ 3.41) as an indication of the orientation in the same plane. Accordingly, Me-15 showed NOESY correlation with H-5 (δ 1.75), which in turn exhibited NOESY interaction with H-9 (δ 3.41) set the orientation of these atoms or groups on the same side of the molecule. The combination of all spectroscopic data helped us to establish structure of **1** as a sesquiterpenoid, which was identified as new natural product, to which, we proposed the name seriphiloid.

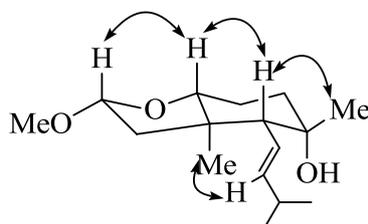


Figure 3. Key NOESY (\longleftrightarrow) correlations of compound **1**, and substantiated due to deriding model.

The isolated compounds **1-5** were evaluated for enzyme inhibitory activity against the enzymes acetylcholinesterase (AChE), butyrylcholinesterase (BChE), [11] α -glucosidase [12] and urease [13]. Compound's percent inhibition (%) was determined at a concentration of 0.5 mM. All the tested compounds **1-5** were found inactive against AChE, BChE and α -glucosidase whereas, compounds **2** and **3** exhibited significant activity against the enzyme urease with IC_{50} values of 21.51 ± 0.1 and 20.82 ± 0.1 $\mu\text{g/mL}$, respectively.

Table 1. Biological activity of the pure compounds

Sample Tested	AChE		BChE		α -Glucosidase		Urease	
	% inhibition	IC_{50} (μM)	% inhibition	IC_{50} (μM)	% Inhibition	IC_{50} (μM)	% Inhibition	IC_{50} ($\mu\text{g/mL}$)
1	25.12 \pm 0.16	Nil	24.12 \pm 0.17	Nil	24.13 \pm 0.14	Nil	28.71 \pm 0.44	Nil
2	29.14 \pm 0.16	Nil	27.18 \pm 0.16	Nil	46.12 \pm 0.16	Nil	97.83 \pm 0.26	21.51 \pm 0.1
3	23.79 \pm 0.12	Nil	9.78 \pm 0.17	Nil	38.26 \pm 0.14	Nil	96.64 \pm 0.76	20.82 \pm 0.1
4	15.18 \pm 0.14	Nil	23.23 \pm 0.14	Nil	31.28 \pm 0.11	Nil	30.45 \pm 0.43	Nil
5	20.39 \pm 0.13	Nil	18.17 \pm 0.13	Nil	26.25 \pm 0.19	Nil	29.65 \pm 0.24	Nil
Eserine	91.27 \pm 1.17	0.04 \pm 0.001	82.82 \pm 1.09	0.85 \pm 0.001	-	-	-	-
Acarbose	-	-	-	-	92.23 \pm 0.14	38.25 \pm 0.12	-	-
Vit.C/ Thiourea	-	-	-	-	-	-	46.45 \pm 0.21	11.25 \pm 0.1

*% inhibition was determined at a concentration of 0.5mM

Supporting Information

Supporting Information accompanies this paper on <http://www.acgpubs.org/RNP>

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