SHORT REPORT

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Phytochemical and Bioactivity Evaluation of *Scrophularia amplexicaulis* Benth.

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Abstract: *Scrophularia amplexicaulis* Benth. is an Iranian endemic species of the genus *Scrophularia*, which comprises ca. 200 medicinally important herbaceous flowering plants. Phytochemical investigation of the methanol extract of the aerial parts of this species afforded two iridoid glycosides, scropolioside D (**1**) and scrophuloside B_4 (**2**), and two phenylalkanoid glycosides, salidroside (**3**) and verbascoside (**4**). Structures of these compounds were determined by comprehensive spectroscopic analyses. Free-radical-scavenging activity, potential antimalarial property, and contact toxicity as well as general toxicity of the extract and fractions were assessed.

Keywords: *Scrophularia amplexicaulis*; Scrophulariaceae; iridoid; phenylalkanoid; glycoside; DPPH (2,2-diphenyl-1-picrylhydrazyl); antimalarial.

1. Plant Source

In continuation of our phytochemical and bioactivity studies on the medicinal plants from the Iranian flora [1-6], *Scrophullaria amplexicaulis* Benth. (Scrophulariaceae) has been investigated. This short communication reports on the isolation and identification of two iridoid glycosides, scropolioside D (**1**) and scrospioside B_4 (**2**), and two phenylalkanoid glycosides, salidroside (**3**) and verbascoside (**4**) (Figure 1), and the bioactivity of the extract and fractions (Tables 1 and 2).

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The aerial parts of *S. amplexicaulis* were collected from East Azerbaijan, Iran, 30 km to Sarab town, Sabalan montion, Gareh gol village, during the flowering stage, and a voucher specimen (AP-2817) has been deposited at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Tabriz University, East Azerbaijan, Iran.

2. Previous Studies

Chemical composition, antibacterial (against *Staphylococcus aureus*) and free-radical-scavenging activities of the essential oils of *S. amplexicaulis* were reported previously [1].

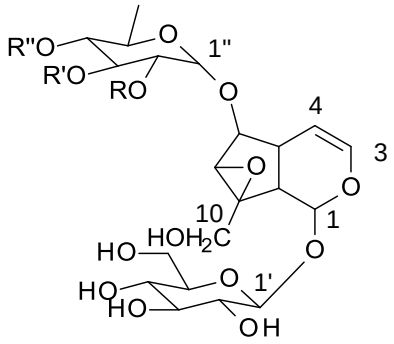
3. Present Study

The air-dried and ground aerial parts of *S. amplexicaulis* (1.8 kg) were Soxhlet-extracted, successively, with *n*-hexane, dichloromethane (DCM) and methanol (MeOH) (2 L each). All these extracts were separately concentrated using a rotary evaporator at a maximum temperature of 45°C. A portion of the MeOH extract (2 g) was subjected to solid-phase-extraction (SPE) on a Sep-Pak 10g C_{18} cartridge using a step gradient of MeOH-water mixture (10:90, 20:80, 40:60, 60:40, 80:20 and 100:0). The preparative HPLC (Dr. Mainsch GmbH ODS column, 20 μm, 250 mm × 20 mm; a liner gradient 0-48 min 50-90% MeOH in water, an isocratic elution 90% MeOH in water during 48-50 min, a liner gradient 50-51 min 90-100% MeOH in water, an isocratic elution 51-53 min 100% MeOH, a liner gradient 100-50% MeOH during 55-57 min; flow rate = 8 mL/min, detection at 190-400 nm) analyses yielded scropolioside D (**1**, 12.3 mg, t_R= 26.3 min) [7] and scrophuloside B_4 (**2**, 7.5 mg, t_R= 28.1 min) [8], from the 60% MeOH SPE fraction. A similar HPLC purification (a liner gradient 0-40 min 40-90% MeOH in water. an isocratic elution 90% MeOH in water during 40-50 min, a liner gradient 50-51 min 90-100% MeOH in water, an isocratic elution 51-53 min 60-100% MeOH in water, a liner gradient 100-40% MeOH during 53-55 min, flow rate =8 mL/min, detection at 190-400 nm) afforded salidroside (**3**, 9.1 mg, t_R= 14.4 min) [9] and verbascoside (**4**, 7.2 mg, t_R= 21.4 min) [2-4, 10] from the 10% MeOH SPE fraction (Figure 1). The structures of these compounds were elucidated by comprehensive spectroscopic analyses, i.e., UV, MS and NMR (1D and 2D), and also by comparison of spectroscopic data with respective published data.

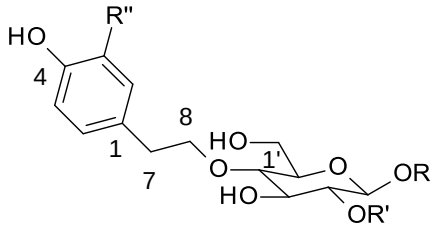
*Scropolioside D **(1)**:* Yellowish amorphous solid; UV (MeOH): λ_{max}: 205, 224 and 311; ^{1}H NMR (400 MHz, CD_3OD): δ (ppm) = 4.89 (1H, d, *J* =9.4 Hz, H-1), 6.42 (1H, dd, *J* =1.4, 6 Hz, H-3), 5.04 (1H, dd, *J* =6.0, 4.5 Hz, H-4), 2.51 (1H, m, H-5), 4.09 (1H, d, *J* = 8.0 Hz, H-6), 3.79 (1H, brs, H-7), 2.60 (1H, dd, *J* =9.4, 8 Hz, H-9), 4.20,3.85 (2H, dd, *J* =13.0 Hz, H-10 α and β), 4.83 (1H, d, *J* = 7.8 Hz, H-1', glucose anomeric), 3.36-3.49 (4H, signal pattern unclear due to overlapping, H-2', H-3', H-4', H-5'), 3.77_4.2 (2H, overlapped, H-6'), 5.13 (1H, d, *J* =1 .5, H-1", rhamnose anomeric), 5.31 (1H, brs, H-2"), 5.39 (1H, d, *J* = 9.6 Hz, H-3"), 5.16 (1H, d, *J* = 5.0 Hz, H-4"), 1.21 (3H, d, *J* = 6.0 Hz, H-6"), 2.10 (3H, s, H-8"), 1.99 (3H, s, H-10"), 7.48 (2H, signal pattern unclear due to overlapping, H-2"', H-6"'), 7.34 (3H, signal pattern unclear due to overlapping, H-3"', H-4"', H-5"'), 7.69 (1H, d, *J* = 16.0 Hz, H-7"' or H-α), 6.48 (1H, d, *J* = 16.0 Hz, H-8"' or H-β); ^{13}C NMR (100 MHz, CD_3OD): δ (ppm) = 93.7 (C-1), 141.0 (C-3), 101.7 (C-4), 35.7 (C-5), 83.4 (C-6), 57.9 (C-7), 65.1 **(**C-8), 41.8 (C-9), 59.9 (C-10), 98.2 (C-l'), 73.4 (C-2'), 77.2 (C-3'), 70.8 (C-4'), 76.2 (C-5'), 61.5 (C-6'), 96.2 (C-1"), 69.3 (C-2"), 70.3 (C-3"), 68.8 (C-4"). 66.6 (C-5"), 16.3 **(**C-6"), 170.2, 170.4 **(**C-7",C-9" carbonyl in COMe), 19.3 (C-8"and C-10" methyl in COMe), 130.4 (C-l"'), 128.0 (C-2"'and C-6"'), 128.6 (C-3"'and C-5"), 134.0 (C-4"), 116.4 (C-α), 145.9 (C-β), 165.8 (C-9"') 17.1 (C-6"'); HRESIMS: *m/z* 723.2511 (calculated 723.2500 for $C_{34}H_{43}O_{17}$, $[M + H]^+$); all data were in agreement with the published data [7].

*Scrophuloside B_4 **(2)**:* yellowish powder; UV (MeOH): λ_{max}: 216, 222 and 282; ^{1}H NMR (400 MHz, CD_3OD): δ (ppm) = 4.80 (1H, d, *J* = 7.8 Hz, H-1), 6.43 (1H, dd, *J* = 1.4, 6.0 Hz, H-3), 5.13 (1H, dd, *J* = 1.4, 6.0 Hz, H-4), 2.57 (1H, m, H-5), 4.08 (1H, d, *J* =8.0 Hz, H-6), 3.73 (1H, brs, H-7), 2.65 (1H, dd, *J* = 8.0, 9.5 Hz, H-9), 4.21 (2H, dd, *J* = 11.0,1.4 Hz, H-10), 4.80 (1H, d, *J* = 7.8 Hz, H-1', glucose

anomeric), 3.36-3.87 (6H, signal pattern unclear due to overlapping, H-2', H-3', H-4', H-5', H-6'), 5.14 (1H, d, *J* =1.5 Hz, H-1", rhamnose anomeric), 5.41(1H, dd, *J* =1.8, 3.4 Hz, H-2"), 5.52 (1H, dd, *J* = 10.0, 6.8 Hz, H-3"), 5.34 (1H, t, *J* = 9.8 Hz, H-4"), 4.10 (1H, m, H-5"), 1.28 (3H, d, *J* = 6.2 Hz, H-6"), 2.20 (3H, s, Acetyl), 7.48 (2H, overlapped peak, H-2"', H-6"'), 7.34 (3H, overlapped peak, H-3"', H-4"' and H-5"'), 6.56 (1H, d, *J* = 16.0 Hz, H-7"' or H-α), 7.76 (1H, d, *J* = 16.0 Hz, H-8"' or H- β), 7.46 (2H, overlapped peak, H-2"", H-6""), 6.89 (2H, overlapped peak, H-3"", H-5""), 6.43 (1H, d, *J* = 16.0 Hz, H-7"" or H-α), 7.66 (1H, d, *J* = 16.0 Hz, H-8"" or H- β), 3.89 (3H, s, OCH_3); ^{13}C NMR (100 MHz, CD_3OD): δ (ppm) = 95.1 (C-1), 141.7 (C-3), 102.4 (C-4), 36.0 (C-5), 84.2 (C-6), 57.8 (C-7), 65.3 (C-8), 42.7 (C-9), 58.5 (C-10), 99.4 (C-1'), 73.2 (C-2'), 77.5 (C-3'), 70.9 (C-4'), 76.3 (C-5'), 61.4 (C-6'), 95.6 (C-1"), 69.4 (C-2"), 70.1 (C-3"), 68.7 (C-4"), 66.5 (C-5"), 16.4 (C-6"), 132.6 (C-1'"), 128.0 (C-2'" and C-5'"), 128.4 (C-3'"), 130.5 (C-4'"), 128.9 (C-6'"), 116.3 (C-7'" or C-α), 144.8 (C-8'" or C-β), 166.8 (COO), 126.8 (C-1""), 129.6 (C-2"" and C-6""), 116.3 (C-3"" and C-5""), 166.4 (C-4""), 57.3 (OCH_3), 165.3(COO); HRESIMS: *m/z* 841.2911 (calculated 841.2919 for $C_{42}H_{49}O_{18}$, $[M + H]^+$); all data were in agreement with the published data [8].



Compound	R	R'	R''
1	Acetyl	*trans*-Cinnamoyl	Acetyl
2	Acetyl	*trans*-Cinnamoyl	*p*-Methoxycinnamoyl



Compound	R	R'	R''
3	H	H	H
4	*trans*-Caffeoyl	rhamnosyl	OH

Figure 1. Structures of compounds **1**-**4** isolated from *S. amplexicaulis.*

*Salidroside **(3)**:* Withe amorphous solid; UV (MeOH): λ_{max}: 236 and 279; ^1H NMR (400 MHz, CD_3OD): δ (ppm) = 7.09 (2H, d, *J* = 8.0 Hz, H-2 and H-6), 6.73 (2H, d, *J* = 8.0 Hz, H-3 and H-5), 2.77 (2H, t, *J* = 7.4 Hz, H-7), 4.10 (2H, m, H-8), 4.33 (1H, d, *J* = 7.8 Hz, H-1', glucose anomeric), 3.20-3.80 (6H, signal pattern unclear due to overlapping, H-2', H-3', H-4', H-5', H-6'), 129.0 (C-1), 128.8 (C-2 and C-6), 113.9 (C-3 and C-5), 152.4 (C-4), 32.8 (C-7), 69.5 (C-8), 100.7 (C-1'), 74.0 (C-2'), 74.2 (C-3'), 71.6 (C-4'), 74.4 (C-5'), 68.1 (C-6'); HRESIMS: *m/z* 301.1272 (calculated 301.1287 for $C_{14}H_{21}O_7$, $[M + H]^+$); all data were in agreement with the published data [9].

*Verbascoside **(4)**:* Brown amorphous solid; UV (MeOH): λ_{max}: 220, 290 and 332; ^1H NMR (400 MHz, CD_3OD): δ (ppm) = 7.64 (1H, d, *J* = 16.0, H-7''), 7.06 (1H, d, *J* = 1.8, H-2''), 6.98 (1H, dd, *J* = 1.8, 8.0 Hz, H-6''), 6.84 (1H, d, *J* = 8.0 Hz, H-5''), 6.70 (1H, d, *J* =1.6 Hz, H-2), 6.68 (1H, d, *J* =8.0 Hz, H-

5), 6.59 (1H, dd, *J* = 1.6, 8.0 Hz, H-6), 6.32 (1H, d, *J* = 16.0, H-8''), 5.19 (1H, d, *J* =1 .5, H-1''', rhamnose anomeric), 4.33 (1H, d, *J* = 8.0 Hz, H-1', glucose anomeric), 3.70-4.20 (1H, overlapped peak, H-8), 3.20-3.80 (10H, signal pattern unclear due to overlapping, H-2', H-3', H-4', H-5', H-6', H-2''', H-3''', H-4'''and H-5'''), 2.83 (2H, t, *J* =7.2 Hz, H-7) and 1.11 (3H, d, *J* = 6.0 Hz, H-6'''); ^{13}C NMR (100 MHz, CD_3OD): δ (ppm) = 166.9 (C-9''), 148.4 (C-4''), 146.6 (C-7''), 145.4 (C-3''), 144.7 (C-3), 143.3 (C-4), 130.0 (C-1), 126.2 (C-1''), 121.8 (C-6''), 119.8 (C-6), 115.7 (C-5), 115.7 (C-5''), 115.1 (C-8''), 113.8 (C-2''), 113.8 (C-2), 102.8 (C-1'''), 101.6 (C-1'), 80.3 (C-3'), 74.8 (C-2'), 74.6 (C-5'), 72.4 (C-4'''), 70.9 (C-8), 70.9 (C-2'''), 70.6 (C-3'''), 69.1 (C-5'''), 69.1 (C-4'), 60.9 (C-6'), 35.2 (C-7) and 17.1 (C-6'''); HRESIMS: *m/z* 642.2392 (calculated 642.2397 for $C_{29}H_{40}NO_{15}$, $[M + NH_4]^+$); all data were in agreement with the published data [2-4,10].

This is the first report on the isolation of scropolioside D (**1**), scrophuloside B_4 (**2**), salidroside (**3**) and verbascoside (**4**) from *S. amplexicaulis.* However, iridoid and phenylalkanoid glycosides, including compounds **1, 2** and **4**, are known to be present in other species of the genus *Scrophularia*. For example, scropolioside D (**1**) was reported from *S. deseti* [11] and *S. ilwensis* [7], scrophuloside B_4 (**2**) from *S. ningpoensis* [8] and verbascoside (**4**) was from *S. trifoliata* [12], *S. scorodonia* [13] and *S. auriculata* [14]. Salidroside (**3**) is common in the genera *Veronica* from the family Plantaginaceae [15] and *Rhodiola* from the Crassulaceae [9].

Bioactivity test- free-radical-scavenging assay (DPPH assay): The free-radical-scavenging activity of the MeOH extract and SPE fractions of the aerial parts of *S. amplexicaulis* were evaluated *in vitro* by the DPPH assay [10, 16, 17]. The method is based on the reduction of the methanolic DPPH solution in the presence of a hydrogen-donating scavenger through the formation of the non-radical form DPPDH [17]. All tested extracts and fractions reduced the stable radical DPPH (Table 1). A concentration-dependent activity pattern was observed. Quercetin was used as a positive control. Among the SPE fractions, 40% aqueous methanolic fraction showed the highest level of free-radical-scavenging activity with an RC_{50} value of 0.0104 mg/mL, which was better than that of the positive control, quercetin (RC_{50} value 0.0294 mg/mL), followed by the 60% aqueous methanolic fraction with a RC_{50} value of 0.0314 mg/mL (Table 1). The significant level of free-radical-scavenging activity of the MeOH extract as well as the SPE fractions is mainly because of the presence of well know free-radical-scavengers like verbascoside (**4**) and related phenylalkanoids [10].

Brine-shrimp-lethality assay (general toxicity assay): The MeOH extract and the 60% aqueous methanolic SPE fraction displayed low to mild level of general toxicity (Table 1) in the brine-shrimp-lethality assay [18]. The toxicity could be attributed to the iridoid glycosides (**1** and **2**), as similar compounds, scropolioside B and scrospioside A were previously shown to posses certain level of cytotoxicity [8].

Table 1. Free-radical-scavenging activity and general toxicity of the MeOH extract of *S. amplexicaulis* and its SPE fractions.

Test samples	Free-radical-scavenging activity (DPPH assay) RC_{50} value in mg/mL	General toxicity (Brine-shrimp-lethality assay) LD_{50} in mg/mL
MeOH extract	0.8390	0.4581
SPE fractions		
10% MeOH in water	0.0957	0.9534
20% MeOH in water	0.1620	0.7203
40% MeOH in water	0.0104	0.5911
60% MeOH in water	0.0314	0.1542
80% MeOH in water	0.2670	0.2675
100% MeOH	0.5740	0.3681
Positive control		
Quercetin	0.0294	-
Podophyllotoxin	-	0.00289

Contact toxicity assay: The SPE fractions, 40% and 60% MeOH in water, displayed potent toxicity towards the test insects (adults of *Oryzeaphilus mercator*) in the contact toxicity assay [19] (Table 2). Other SPE fractions did not show any detectable toxicity at tested concentrations. This finding is in line with the previous finding where, the fractions with high amounts of phenylethanoids and acylated iridoids were shown to possess potent insecticidal activity [19].

Heme biocrystallization assay for potential antimalarial property: Malaria-infected erythrocytes are characterized by a high rate of production of ferriprotoporphyrin IX (heme) as a result of the ingestion and digestion of host cell hemoglobin [20]. Parasite utilizes hemoglobin as food source during intra erythrocytes development and proliferation [21]. Released heme is a toxic compound [22]. For the detoxification of heme, crystals of hemozoin in the food vacuoles of malaria parasites are formed that commonly known as malaria pigment [23-25]. Chloroquine and most of other antimalarial compounds inhibit β-hematin formation under different conditions [24]. The heme biocrystallization inhibition assay is based on the above facts [26, 27]. In this study, the MeOH extract of *S. amplexicaulis* and its fractions were assessed for potential antimalarial activity by the heme biocrystallization inhibition assay [27, 28]. The 80% and 60% MeOH in water SPE fractions showed significant activity compared to other fractions; the IC_{50} values were 0.827 and 0.431 mg/mL, respectively, while the IC_{50} of the positive control (chloroquine) was 0.064 mg/mL.

Statistical analysis: SPSS 16 software was used for statistical analysis. Analysis of variance (ANOVA) followed by Duncan test was performed to find out the differences amongst various replicates. The significance level was set at $p<0.05$.

Table 2. Contact toxicty of the methanol extract of *S. amplexicaulis* and its SPE fractions against *Oryzeaphilus mercator.*

Hours	Concentration of test samples (in mg)	% Mortality)						
		MeOH extract	SPE fractions, % MeOH in water					
			10%	20%	40%	60%	80%	100%
4	1	0	0	0	10	20	0	0
	5	10	0	0	20	0	0	0
	10	10	0	0	20	0	0	0
	15	20	0	0	10	10	0	0
8	1	10	0	0	10	20	0	0
	5	20	0	0	20	10	0	0
	10	20	0	0	20	0	0	0
	15	20	0	0	20	10	0	0
24	1	20	0	0	20	30	0	0
	5	20	0	0	40	40	0	0
	10	20	0	0	40	20	0	0
	15	40	0	0	40	20	0	0
48	1	20	0	0	30	30	0	0
	5	20	0	0	40	40	0	0
	10	30	0	0	40	20	0	0
	15	40	0	0	40	30	0	0

References

[1] A. Pasdaran, A. Delazar, H. Nazemiyeh, L. Nahar and S. D. Sarker (2012). Chemical composition, and antibacterial (against *Staphylococcus aureus*) and free-radical-scavenging activities of the essential oils of *Scrophularia amplexicaulis* Benth., *Rec. Nat. Prod.* **6**, 350-355.

[2] A. Delazar, S. Gibbons, Y. Kumarasamy, L. Nahar, M. Shoeb and S. D. Sarker (2005). Antioxidant phenylethanoid glycosides from the rhizomes of *Eremostachys glabra* (Lamiaceaea), *Biochem. Syst. Ecol.* **33**, 87-90.

[3] A. Delazar, M-R. Delnavazi, N. Yassa, S. Parkhideh, N. Delazar, L. Nahar and S. D. Sarker (2012). Essential oil composition and isolation of free-radical-scavenging phenolic glycosides from the aerial parts of *Ajuga chamaepitys* (L.) Schreb. (Lamiaceae) growing in Iran, *Braz. J. Pharmacog.* **22**, 299-305.

[4] H. Nazemiyeh, M. M. Rahman, S. Gibbons, L. Nahar, A. Delazar, M-A. Ghahramani, A-H. Talebpour and S. D. Sarker (2008). Assessment of the antibacterial activity of phenylethanoid glycosides from *Phlomis lanceolata* against multiple-drug-resistant strains of *Staphylococcus aureus*, *J. Nat. Med.* **62**, 91-92.

[5] E. Magharri, S. M. Razavi, E. Ghorbani, L. Nahar and S. D. Sarker (2015). Chemical composition, some allelopathic aspects, free-radical-scavenging property and anti-fungal activity of the volatile oil of the flowering tops of *Leucanthemum vulgare* Lam., *Rec. Nat. Prod.* **9**, 538-545.

[6] A. Pasdaran, L. Nahar, S. Aanaashari, S. D. Sarker and A. Delazar (2013). GC-MS analysis, free-radical-scavenging and insecticidal activities of essential oil of *Scrophularia oxysepala* Boiss, *Pharm. Sci.* **19**, 1-5.

[7] I. Calis, M. Zor, A. A. Basaran, A. D. Wright and O. Sticher (1993). Karsoside and scropolioside D, two new iridoid glycosides from *Scrophularia ilwensis*, *J. Nat. Prod.*, **56**, 606-609.

[8] A-T. Nguyen, J. Fontaine, H. Malonne, M. Claeys, M. Luhmer and P. Duez (2005). A sugar ester and an iridoid glycoside from *Scrophularia ningpoensis*, *Phytochem.* **66**, 1186-1191.

[9] Z-W. Ma, G-F. He and L-L. Wu (2001). Studies on chemical constituents of *Rhodiola phariensis* (H. Ohla) S. H. Fu, *Acta Bot. Sin.* **37**, 574-580.

[10] N. K. Chima, L. Nahar, R. R. T. Majinda, S. Celik and S. D. Sarker (2014). Assessment of free-radical scavenging activity of the extracts and fractions of *Gypsophila pilulifera*: Assay-guided isolation of the active component, *Braz. J. Pharmacog.* **24**, 38-43.

[11] B. Ahmed, A. J. Al-Rehaily, T. A. Al-Howininy, K. A. El-Sayed and M. S. Ahmad (2003). Scropolioside-D-2 and harpagoside-B: Two new iridoid glycosides from *Scrophularia deserti* and their antidiabetic and antiinflammatory activity, *Biol. Pharm. Bull.* **26**, 462-467.

[12] A. Ramunno, A. M. Semili, F. Piccioni, M. Serafini and M. Ballero (2006). Taxonomical markers in two endemic plants of Sardinia: *Verbascum conocarpum* and *Scrophularia trifoliata*, *Nat. Prod. Res.* **20**, 511-516.

[13] L. Fernandez, J. de Santos, A. M. Diaz-Lanza, E. Perez and L. Villaescusa (2005). Quantitative determination of verbascoside in *Scrophularia scorodonia* by high performance liquid chromatography, *Pharm. Biol.* **43**, 226-229.

[14] R. M. Giner, M. L. Villalba, M. D. C. Recio, S. Menez, A. I. Gray and J. L. Rio (1998). New iridoid from *Scrophularia auriculata* ssp. *pseudoauriculata*, *J. Nat. Prod.* **61**, 1152-1163.

[15] R. M. Taskova, C. H. Gotfredsen and S. R. Jensen (2006), Chemotaxonomy of Veroniceae and its allies in the Plantaginaceae, *Phytochem.* **67**, 286-301.

[16] T. Takao, F. Kitatani, N. Watanabe, A. Yagi and K. Sakata (1994). A simple screening method for antioxidants and isolation of several antioxidants produced by marine bacteria from fish and shellfish, Bioscience, *Biotechnol. Biochem.* **58**, 1780-1783.

[17] T. Kumarasamy, M. Byres, P. J. Cox, M. Jaspars, L. Nahar and S. D. Sarker (2007). Screening seeds of some Scottish plants for free radical scavenging activity, *Phytotherap. Res.* **21**, 615-621.

[18] B. N. Meyer, N. R. Ferringi, J. E. Putnam, L. B. Jacobsen, D. E. Nicholas and J. L. McLaughlin (1982). Brine shrimp: A convenient general bioassay for active plant constituents, *Planta Medica* **45**, 31-34.

[19] S. Asnaashari, A. Delazar, S. S. Alipour, L. Nahar, A. S. Williams, A. Pasdaran, M. Mojarab, F. Fatih-Azad and S. D. Sarker (2010). Chemical composition, free-radical-scavenging and insecticidal activities of the aerial parts of *Stachys byzantina*, *Arch. Biol. Sci.* **62**, 653-662.

[20] O. Famin, M. Krugliak and H. Ginsburg (1999). Kinetics of inhibition of glutathione-mediated degradation of ferriprotoporphyrin IX by antimalarial drugs. *Biochem. Pharmacol.* **58**, 59-68.

[21] S. E. Francis, D. J. Sullivan Jr. and D. E. Goldberg (1997). Hemoglobin metabolism in the malaria parasite *Plasmodium falciparum*, *Ann. Rev. Microbiol.* **51**, 97-123.

[22] A. K. Tripathi, A. Gupta, S. K. Garg and Tekwani, B. L. (2001). *In vitro* beta-hematin formation assays with plasma of mice infected with *Plasmodium yoelii* and other parasite preparations: comparative inhibition with quinoline and endoperoxide antimalarials, *Life Sci.* **69**, 2725-2733.

[23] M. Kalkanidis, N. Klonis, L. Tilley and L. W. Deady (2002). Novel phenothiazine antimalarials: synthesis, antimalarial activity, and inhibition of the formation of β-haematin, *Biochem. Pharmacol.* **63**, 833-842.

[24] T. J. Egan and K. K. Ncokazi (2005). Quinoline antimalarials decrease the rate of beta-hematin formation, *J. Inorg. Biochem.* **99**, 1532-1539.

[25] D. Rathore (2006). Heme detoxification and antimalarial drugs – Known mechanisms and future prospects. *Drug Discovery Today* **3**, 153-158.

[26] C. D. Fitch, G. Z. Cai, Y. F. Chen and J. D. Shoemaker (1999). Involvement of lipids in ferriprotoporphyrin IX polymerization in malaria, *Biochim. Biophys. Acta*. **1454**, 31-37.

[27] A. K. Tripathi, S. I. Khan, L. A. Walker and Tekwani, B. L. (2004). Spectrophotometric determination of de novo hemozoin/beta-hematin formation in an *in vitro* assay. *Analytical Biochem.* **325**, 85-91.

[28] A. I. Hussain, F. Anwar, S. Rasheed, P. S. Nigam, O. Janneh and S. D**.** Sarker (2011). Composition and potential antibacterial, anticancer, antimalarial and antioxidant properties of the essential oils from two *Origanum* species growing in Pakistan, *Braz. J. Pharmacog.* **21**, 943-952.