Two New Antioxidant Triterpenoids from *Lonicera quinquelocularis*

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Abstract: Two new triterpenoids namely, 3α-hydroxyferna-7, 9(11),22-trien-12-one (**1**) and 3α-acetoxyferna-7, 9(11)-dien-22-ol (**2**) along with two known compounds $3\alpha,16\alpha$-dihydroxyferna-7,9(11)-dien-12-one (**3**) and $3\alpha,16\alpha$-dihydroxyferna-8-en-11-one (**4**) were isolated from *Lonicera quinquelocularis.* Their structures were elucidated by chemical and modern spectroscopic techniques. All the compounds showed good antioxidant activities in the DPPH radical scavenging assay.

1. Introduction

The genus *Lonicera* belongs to the family Caprifoliaceae and comprises of about 12 genera and 450 species [1], occurring mainly in temperate region of Northern Hemisphere. In Pakistan, it is represented by 4 genera and 27 species [2]. Various species of this genus are used for the treatment of acute fever, headache, respiratory infections [3], antibacterial [4], antioxidant [5,6], cytoprotective [7], hepatoprotective [8, 9], antiviral [10], antitumor [11, 12] and anti-inflammatory activities (13). Previous studies on this genus have resulted in the isolation of a variety of constituents including iridoids, bis iridoids, sulphur containing monoterpenoids, alkaloid glycosides, tritepenoids, saponines, coumarenes glycosides and flavones glycosides [14–17]. *Lonicera quinquelocularis* is a member of this genus widely distributed in dry sunny places between 750-3000 m in many countries of Asia [18]. In Pakistan, it is found in Baluchistan, Kurram, Chitral, Swat, Astor, Hazara, Murree hills, Poonch and Kashmir [19]. Previous phytochemical study on this plant resulted in the isolation of triterpenoid, benzoates, lonicerin, loganin, coumarine and iridoid glycosides [6, 20]. It is widely used in the treatment of hypotensive, sedative, antipyretic and antioxidant activities [6, 21, 22].
The diverse medicinal applications of genus *Lonicera* have prompted us to investigate the constituents of *L.quinquelocularis*.

Herein, we reported the isolation, separation and identification two new tritepenoids known as 3α-hydroxyferna-7,9(11),22-trien-12-one and 3α-acetoxyferna-7,9(11)-dien-22-ol along with two known compound known as $3\alpha,16\alpha$-dihydroxyferna-7, 9(11)-dien-12-one and $3\alpha,16\alpha$-dihydroxyferna-8-en-11-one (Figure 1)

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3α, Hydroxyferna-7, 9(11),22-trien-12-one (**1**)

3α -acetoxyferna-7, 9(11)-dien-22-ol (**2**)

3a, 16a-dihydroxyferna-7,9(11)-dien-12-one (**3**)

3α,16α-dihydroxyfern-8-en-11-one (**4**)
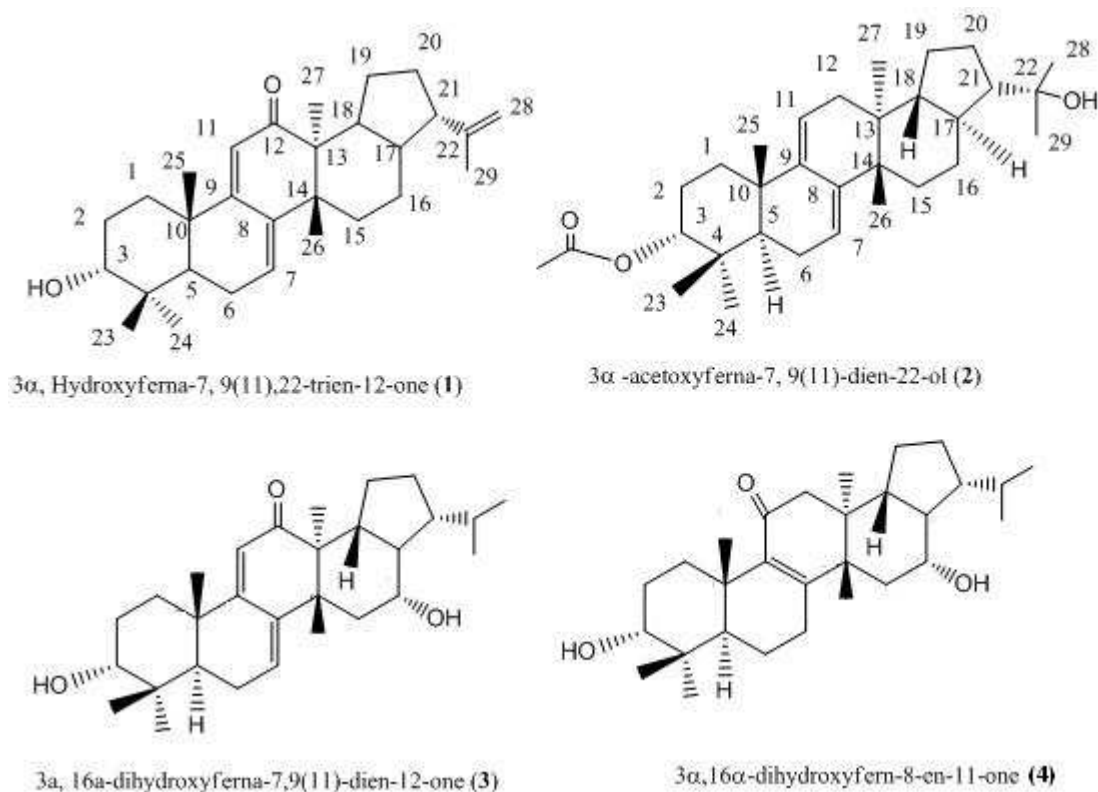
Figure 1. Structures of Compounds **1-4**

2. Materials and Methods

2.1. Plant Material

The whole plant of *Lonicera quinquelocularis* was collected from Bara Galli, Hazara division, District Mansehra, in June 2009. It was identified by Professor Dr. Manzoor Ahmad, Plant Taxonomist, Department of Botany, Government Degree College Abbotabad, Pakistan, where a voucher specimen has been deposited in herbarium (Accession No. C-0013).

2.2 Extraction and Isolation

The whole shade dried plant of *Lonicera quinquelocularis* (13 kg) was ground and extracted with ethanol at room temperature (3 x 25 L). The combined ethanolic extract was evaporated under reduced pressure to obtain a thick greenish gummy material. It was suspended in water and successively partitioned with *n*-hexane (151 g), chloroform (147 g), ethyl acetate (109 g), and *n*-butanol (53 g), respectively.

The chloroform soluble fraction was subjected to column chromatography over silica gel (70-230 mesh) eluting with *n*-hexane (A), *n*-hexane-CHCl₃ (B), CHCl₃ (C), CHCl₃-EtOAc (D), EtOAc (E), EtOAc-MeOH (F) and MeOH (G) in increasing order of polarity to obtain seven fractions A-G.

The fraction C (15 g) was again chromatographed over silica gel eluting with *n*-hexane (D$_A$), CHCl₃ (D$_B$), CHCl₃- EtOAc (D$_C$), EtOAc (D$_D$), EtOAc-EtOH (D$_E$) and EtOH (D$_F$). The sub-fraction D$_E$ (4.1 g) was re-chromatographed over silica gel, eluting with mixture of *n*-hexane: CHCl₃ and CHCl₃: EtOH in increasing order of polarity. The eluates obtained from *n*-hexane: CHCl₃ (7 : 3) were combined and subjected to preparative TLC using *n*-hexane : EtOAc (3 : 2) as solvent system to provided **1** (25 mg) and **2** (21 mg). The fractions obtained from *n*-hexane: CHCl₃ (1: 4) were

combined and again chromatographed over silica gel using *n*-hexane: CHCl$_3$ (3.5: 6.5) as eluent to obtain **3** (23 mg) from the top fractions and compound **4** (19 mg) from the bottom fraction.

2.3. 3α-hydroxyferna-7,9(11),22-trien-12-one (1)

Colourless needles; UV (MeOH) λ$_{max}$ nm (log λ): 230 (4.39), 238 (4.75), 245 and 270 (5.10); IR (dry KBr) ν$_{max}$ cm^{-1}: 3425 (OH), 3070 (C=C-H), 2910 (C-C-H) and 1715 (enone); ^{1}H NMR (400 MHz, CDCl$_3$) and ^{13}C NMR (100 MHz, CDCl$_3$) spectral data see table 1. HR-EIMS *m/z* 422.46 16[M+H]$^+$ (calcd. for C$_{29}$H$_{42}$O$_2$, 422.3247). EI-MS *m/z*: 423, 407, 383, 271, 218 and 179.

2.4. 3α-acetoxyferna-7,9(11)-dien-22-ol (2)

Colourless needles; UV (MeOH) λ$_{max}$ nm (log λ): 225 (4.35), 235 (4.60) and 248 (4.90); IR (KBr) ν$_{max}$ cm^{-1}: 3620 and 3425 (OH), 3035 (C=C-H), 2910 (C-C-H); ^{1}H NMR (400 MHz, CDCl$_3$) and ^{13}C NMR (100 MHz, CDCl$_3$) spectral data see table 1. HR-EIMS *m/z* 468.5273 [M+H]$^+$ (calcd. for C$_{31}$H$_{48}$O$_3$, 468.3645). EI-MS *m/z*: 469, 453, 427, 353, 271, 218 and 179.

3. Results and Discussion

3.1. Structure elucidation

The chloroform soluble fraction was subjected to series of silica gel column chromatography and preparative TLC to afford compounds **1-4**. The compounds **3** and **4** were known as by comparison of their spectra and physical data with the available literature [23].
The structure of compounds **1** and **2** were elucidated by physical data and spectroscopic analysis.
Compound **1** was isolated as colorless needles mp 160-163. The HRMS gave molecular ion peak at 422.46 corresponding to the molecular formula C$_{29}$H$_{42}$O$_2$. The IR spectra exhibited peaks at 3425 (for OH group), 3070 (for C=C-H stretching), 2910 (for C-C-H) and 1715 (for enone). The UV spectra showed absorption at 230 (sh), 238 (sh), 245 and 270 (sh) nm indicated the presence of Δ$^{7,\ 9(11)}$ – dienone system in a triterpene skeleton. The electron ionization EI-MS gave fragment ion peaks at *m/z* 407 (M-H$_2$O) indicating the presence of one hydroxyl groups. Further EIMS peaks were observed at *m/z* 383, 271and 253 due to cleavage of D-ring. ^{1}H-NMR (400 MHz, CDCl$_3$) showed the presence of six tertiary methyl groups displayed at 1.03 (3H, s, H-23), 0.96 (3H, s, H-24), 0.98 (3H, s, H-25), 0.89 (3H, s, H-26), 1.01 (3H, s, H-27) and 1.62 (3H, d, *J* = 3.1 Hz, H-28), eight methylen groups obsrved at 2.01 (1H, t, *J* = 14.2, 7.1, H-1b), 1.51 (1H, dt, *J* = 14.2, 7.1 Hz, H-1a), 1.75 (1H, m, H-2b), 1.64 (1H, m, H-2a), 2.24 (1H, ddd, *J* = 14.2, 7.1, 4.1 Hz, H-6b), 2.13 (1H, ddd, *J* = 14.2, 7.1, 4.1 Hz, H-6a), 1.68 (1H, dt, *J* = 13.8, 6.9 Hz, H-15b), 1.53 (1H, dt, *J* = 13.8, 6.9 Hz, H-15a), 1.92 (1H, m, H-16b), 1.40 (1H, m, H-16a), 1.75 (1H, m, H-19a), 1.29 (1H, m, 19b), 1.85 (1H, m, H-20a), 1.63 (1H, m, H-20b) and 4.86 (1H, dd, *J* = 3.2, 2.8 Hz, H-28b), 4.76 (1H, dd, J = 3.2, 2.8 Hz, H-28a), four methine protons at δ 2.45 (1H, d, *J* = 4.7 Hz, H-5), 2.07 (1H, m, H-17), 2.54 (1H, ddd, *J* = 8.9, 8.1, 7.1 Hz, H-18) and 2.64 (1H, m, H-21), one hydroxyl methine groups at δ 3.48 (1H, dd, *J* = 6.8, 5.3 Hz, H-3) and two trisubstituted olefinic protons found at δ 5.54 (1H, dd, *J* = 10.1, 4.3 Hz, H-7) and 5.66 (1H, s, H-11). The ^{13}C NMR spectrum displayed 29 carbon signals which were assigned by DEPT as six methyl carbons, seven methylene carbons, eight methine carbons and eight quarternary carbons table 1. The assignment of ^{1}H NMR and ^{13}C NMR were confirmed by 2D experiments. The heteronuclear multiple bond correlation (HMBC) spectrum (Figure 2) showed correlation between H-23 and C-5/C-3; H-7 and C-5/C-9 and C-14; H-11 and C-12, C8, C10; H-25 and C-1, C-5 and C-9; H-26 and C-8, C-13 and C-15; H-27 and C-12, C-14 and C-18; H-28 and C-21; and H-29 and C-21. The above spectral data indicated that the compound **1** was fernane type triterpenoid with a Δ$^{7,\ 9(11)}$-dienophile system with a hydroxyl groups at C-3 and a carbonyl function at C-12. The configuration of hydroxyl groups at C-3 was determined by the coupling pattern and constants in ^{1}H NMR spectrum which displayed signals for H-3(OH) at δ 3.48 (1H, dd, *J* = 6.8, 5.3, Hz, H-3). On the basis of these spectral assignments the structure of compound **1** was determined to be 3α-hydroxyferna-7, 9(11),22-trien-12-one.

Compound **2** was isolated as colourless needles mp 168-170. The HRMS gave molecular ion peak at 468.52 corresponding to the molecular formula $C_{31}H_{48}O_3$. The IR spectra exhibited peaks at 3620 and 3425 (for OH groups), 3035 (for C=C-H stretching), 2910 (for C-C-H). The UV spectra showed absorption at 225, 235 and 248 nm indicated the presence of $\Delta^{7,\,9(11)}$ – diene system in a triterpene skeleton. The electron ionization EI-MS gave fragment ion peaks at *m/z* 453 (M-H$_2$O) indicating the presence of one hydroxyl groups. Further EIMS peaks were observed at *m/z* 427, 271and 253 due to fragmentation of acyl group and D-ring. ^{1}H-NMR (400 MHz, CDCl$_3$) exhibited signals for five singlet methyl groups at δ 1.05 (3H, s, H-23), 0.99 (3H, s, H-24), 1.07(3H, s, H-25), 0.86 (3H, s, H-26) and 0.75 (3H, s, H-27), two doublet methyl groups at δ 1.25 (3H, d, *J* = 3.4 Hz, H-28) and 1.21 (3H, d, J = 3.4 Hz, H-29), eight methylene groups observed at δ 2.07 (1H, dt, *J* = 14.1, 7.1, H-1b), 1.51 (1H, dt, *J* = 14.1, 7.1, H-1a), 1.71 (1H, ddd, *J* = 12.3, 7.0, 4.8 Hz, H-2b), 1.58 (1H, dd, *J* = 12.3, 7.0, 4.8 Hz, H-2a), 2.18 (1H, ddd, *J* = 15.1, 7.8, 6.2 Hz, H-6b), 1.90 (1H, ddd, *J* = 15.1, 7.8, 6.2 Hz, H-6a), 1.99 (1H, dd, *J* = 4.6, 4.2 Hz, H-12b), 1.80 (1H, dd, *J* = 4.6, 4.2 Hz, H-12a), 1.82 (2H, dt, *J* = 12.7, 6.9 Hz, H-15), 1.92 (1H, m, H-16b), 1.40 (1H, m, H-16a), 1.64 (2H, m, H-19b), 1.53 (2H, m, H-19a), 1.85 (1H, m, H-20a) and 1.63 (1H, m, H-20b), four methine groups displayed at δ 2.40 (1H, d, *J* = 4.7 Hz, H-5), 1.60 (1H, m, H-17), 2.48 (1H, m, H-18) and 1.82 (1H, m, H-21), one acetoxy methine proton resonated at δ 4.60 (1H, dd, *J* = 7.3, 5.1 Hz, H-3), two trisubstituted olefinic protons found at δ 5.56 (1H, t, *J* = 6.3 Hz, H-7) and 5.51 (1H, t, *J* = 6.7 Hz, H-11), and the acetyl protons were resonated at δ 2.05 (3H, s, OAc). The ^{13}C NMR spectrum displayed 31 carbon signals which were assigned by DEPT as eight methyl carbons, eight methylene carbons, seven methine carbons and eight quarternary cabons (Table 1). The assignment of ^{1}H NMR and ^{13}C NMR were confirmed by HMBC experiments (Figure 3). HMBC correlation was found between H-3 and OAc and C-5; H-23 and C-5/C-3; H-24 and C-5; H-7 and C-5/C-9 and C-14; H-11 and C-8, C-13 and C-10; H-25 and C-1, C-5 and C-9; H-26 and C-8, C-13 and C-15; H-27 and C-12, C-14 and C-18; H-28 and C-21; and H-29 and C-21. The above spectral data indicated that the compound **2** was fernane type triterpenoid with a $\Delta^{7,\,9(11)}$ -diene system with an acetyl group at C-3. On the basis of these spectral assignments, the structure of compound **2** was determined to be 3α-acetoxyferna-7,9(11)-dien-22-ol.
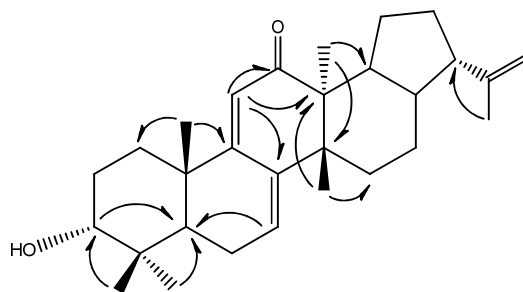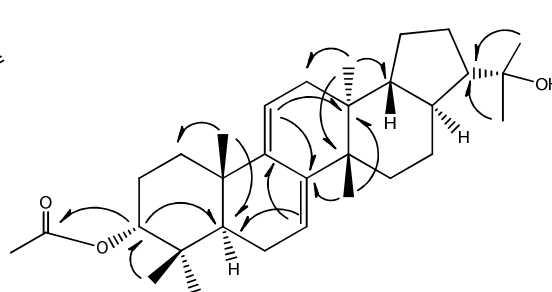

Figure 2. HMBC correlation of compound **1**   **Figure 3.** HMBC correlation of compound **2**

Table 1. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) of Compounds **1** and **2** (*J* in Hz, δ in ppm)

Position	Compound 1		Compound 2	
	^{1}**H NMR**	^{13}**C NMR**	^{1}**H NMR**	^{13}**C NMR**
1	2.01 (1H, dt, *J* = 14.2, 7.1, H-1b), 1.51 (1H, dt, *J* = 14.2, 7.1, H-1a)	34.57	2.07 (1H, dt, *J* = 14.1, 7.1, H-1b), 1.51 (1H, dt, *J* = 14.1, 7.1, H-1a)	36.05
2	1.75 (1H, m, H-2b), 1.64 (1H, m, H-2a)	25.39	1.71 (1H, ddd, *J* = 12.3, 7.0, 4.8, H-2b), 1.58 (1H, dd, *J* = 12.3, 7.0, 4.8, H-2a)	23.51
3	3.48 (1H, dd, *J* = 6.8, 5.3, H-3)	76.54	4.60 (1H, dd, *J* = 7.3, 5.1, H-3)	84.42
4	_	37.81	_	35.92
5	2.45 (1H, d, J = 4.7, H-5)	48.20	2.40 (1H, d, J = 4.5, H-5)	47.63
6	2.24 (1H, ddd, *J* = 14.2, 7.1, 4.1 H-6b), 2.13 (1H, ddd, *J* = 14.2, 7.1, 4.1, H-6a)	26.69	2.18 (1H, ddd, *J* = 15.1, 7.8, 6.2, H-6b), 1.90 (1H, ddd, *J* = 15.1, 7.8, 6.2, H-6a)	25.02
7	5.54 (1H, dd, *J* = 10.1, 4.3, H-7)	117.84	5.56 (1H, t, *J* = 6.3, H-7)	174.79
8	_	140.46	_	145.36
9	_	165.72	_	140.75
10	_	38.23	_	35.49
11	5.66 (1H, s, H-11)	113.75	5.51 (1H, t, *J* = 6.7, H-11)	117.12
12	_	205.10	1.99 (1H, dd, *J* = 4.6, 4.2, H-12b), 1.80 (1H, dd, *J* = 4.6, 4.2, H-12a)	35.37
13	_	36.47	_	38.43
14	_	42.43	_	42.55
15	1.68 (1H, dt, *J* = 13.8, 6.9, H-15b), 1.53 (1H, dt, *J* = 13.8, 6.9, H-15a)	33.16	1.82 (2H, dt, *J* = 12.7, 6.9, H-15)	27.65
16	1.92 (1H, m, H-16b), 1.40 (1H, m, H-16a)	27.25	1.92 (1H, m, H-16b), 1.40 (1H, m, H-16a)	25.59
17	2.07 (1H, m, H-17)	42.62	1.60 (1H, m, H-17)	42.17
18	2.54 (1H, ddd, *J* = 8.9, 8.1, 7.1, H-18)	51.32	2.48 (1H, m, H-18)	48.22
19	1.75 (1H, m, H-19a), 1.29 (1H, m, 19b)	29.67	1.64 (2H, m, H-19b), 1.53 (2H, m, H-19a)	23.50
20	1.85 (1H, m, H-20a), 1.63 (1H, m, H-20b)	26.77	1.85 (1H, m, H-20a) and 1.63 (1H, m, H-20b)	26.15
21	2.64 (1H, m, H-21)	50.65	1.82 (1H, m, H-21)	55.27
22		148.55		73.32
23	1.03 (3H, s, H-23)	19.56	1.05 (3H, s, H-23)	17.46
24	0.96 (3H, s, H-24)	26.91	0.99 (3H, s, H-24)	27.95
25	0.98 (3H, s, H-25)	26.48	1.07(3H, s, H-25)	21.50
26	0.89 (3H, s, H-26)	22.35	0.86 (3H, s, H-26)	20.29
27	0.67 (3H, s, H-27)	18.26	0.75 (3H, s, H-27)	19.34
28	4.86 (1H, dd, *J* = 3.2, 2.8, H-28b), 4.76 (1H, dd, *J* = 3.2, 2.8, H-28a)	111.89	1.25 (3H, d, *J* = 3.4, H-28)	26.42
29	1.01 (3H, s, H-29)	24.11	1.21 (3H, d, *J* = 3.4, H-29)	30.38
C=O	_	_	_	171.40
OAc	_	_	_	20.84

3.2. DPPH radical scavenging activity

The DPPH radical scavenging activity assay was performed to evaluate the antioxidant property of the isolated compounds with positive reference (ascorbic acid (10µg/mL)) according to the standard procedure of with some modifications [24].
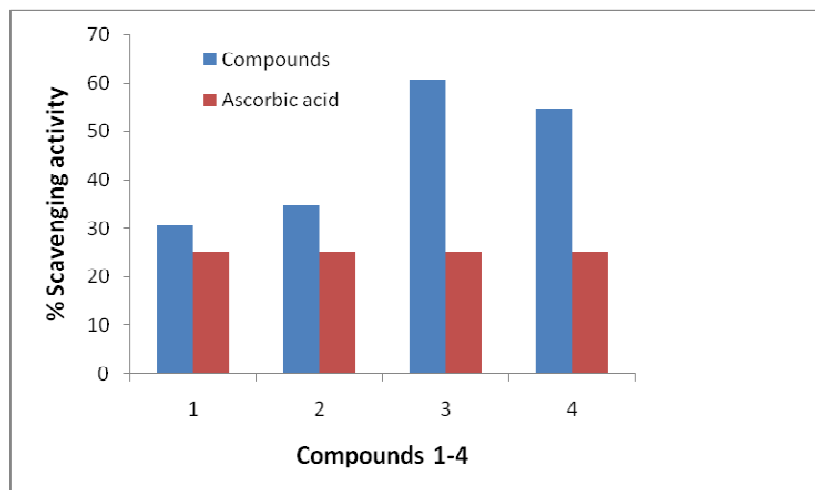

Figure 4. Antioxidant activity of compounds 1-4

The fresh stock solution was prepared by dissolving 3 mg DPPH with 100 mL of methanol and then stored at 20 °C. The working solution was obtained by diluting DPPH solution with methanol to obtain an absorbance of about 0.920 (± 0.03) at 517 nm using the spectrophotometer. A 500 µL aliquot of this solution was mixed with 100 µL of the compounds (10 µg/mL). The same was repeated with positive reference i.e. ascorbic acid (10 µg/mL). The solution in the test tubes were shaken well and incubated in the dark for 25 min at room temperature. Then the absorbance was taken at 517 nm. The scavenging activity was calculated from the percentage of DPPH radical scavenged as the following equation.

Scavenging effect (%) = [(control absorbance-sample absorbance) / (control absorbance)] ×100.

All isolated compounds in (Figure 4) showed higher antioxidant property than ascorbic acid. Furthermore, the compound **3** was found more active than the others.

Acknowledgments

Supporting Information

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

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