

## Antioxidants and $\alpha$ -Glucosidase Inhibitors from *Lactuca serriola* L.

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(Received May 11, 2020; Revised June 22, 2020; Accepted June 24, 2020)

**Abstract:** Our main objective in the present study was investigation of potential antidiabetic and antioxidant compounds from the methanol extract of *Lactuca serriola* L. Phytochemical investigation allowed the isolation of seventeen known compounds and their structures were identified based on IR, 1D and 2D NMR techniques and mass spectrometry. Lupeol (**1**),  $\beta$ -sitosterol (**2**), taraxast-20-ene- $3\beta,30$ -diol (**3**), glycerol monopalmitate (**4**) (2*S*,3*S*,4*R*,2'*R*,14*E*)-2-(2'-hydroxytetracosanoylamino)-14-octadecene-1,3,4-triol (**5**), lactucopicrin (**6**), daucosterol (**7**), (*E*)-*p*-coumaric acid (**8**), 11 $\beta$ ,13-dihydroxylactucin (**9**), 4-hydroxybenzoic acid (**10**), protocatechuic acid (**11**), kaempferol (**12**), quercetin (**13**), lactuside A (**14**), luteolin-7-*O*- $\beta$ -D glucoside (**15**), lactuside B (**16**) and cichorioside B (**17**) were isolated from the species. The antioxidant activity was evaluated using ABTS, DPPH radical scavenging and FRAP assays. The ethyl acetate fraction of the aerial parts of species showed remarkable  $\alpha$ -glucosidase inhibitory activity (IC<sub>50</sub>, 9.16 $\pm$ 0.17  $\mu$ g/mL) and antioxidant activity among the tested fractions. The *in vitro*  $\alpha$ -glucosidase inhibitory assay is reported in this study for the first time for *L. serriola* L. The results strongly suggest that *L. serriola* L. can be used as a potential good natural remedy targeting oxidative stress-related diabetes mellitus.

**Keywords:** Asteraceae; *Lactuca serriola*; antioxidant;  $\alpha$ -glucosidase inhibitors; oxidative stress. © 2020 ACG Publications. All rights reserved.

### 1. Plant Source

The aerial parts of *Lactuca serriola* L. (Asteraceae) were collected from Nile Valley area, Dakahlia Governorate, Egypt in March 2015. The species was identified by Dr. Ibrahim Mashaly, Professor of Ecology, Faculty of Science, Mansoura University, Egypt. A voucher specimen 03-15-LS-Mansoura was deposited at the Herbarium of Faculty of Science, Mansoura University.

### 2. Previous Studies

In traditional medicines, *L. serriola* L. was used as sedative, anti-spasmodic, expectorant, purgative, diuretic [1], for treatment of headache, hypertension and fever [2]. Previous pharmacological studies were

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conducted to evaluate the therapeutic significance of the crude extract of *L. serriola* L. which showed its analgesic [3], cytotoxic [4], antibacterial [5], antidiabetic [6] and antioxidant properties [7]. Former phytochemical investigations allowed isolation of several sesquiterpene lactones and flavonoids as well [7,8].

Diabetes mellitus (DM) is a major health problem worldwide. One of the most effective drugs for management of type 2 DM are  $\alpha$ -glucosidase inhibitors, including acarbose and voglibose [9], which control postprandial hyperglycemia (a sharp rise in blood sugar after meals) caused by the  $\alpha$ -glucosidase enzyme. This intestinal enzyme breaks down carbohydrates such as disaccharides, starch and glycogen into glucose by hydrolyzing the terminal non-reducing 1 $\rightarrow$ 4 linked  $\alpha$ -glucose residues to release a single  $\alpha$ -glucose unit [10]. However, those drugs may cause mild gastrointestinal side effects. Therefore, there is an increasing concern for discovery of new compounds from natural sources having potential  $\alpha$ -glucosidase inhibitory activity without any side effects [9,11,12]. In addition, it is currently theorized that oxidative stress accumulation is the common pathogenic factor leading to impaired glucose tolerance, insulin resistance,  $\beta$ -cell dysfunction promoting type 2 DM [13]. Chronic hyperglycemia in diabetes increases the oxidative stress in the body of diabetic patient as well through generated reactive oxygen species (ROS) [11,13]. This enhances the initiation and progression of diabetes-associated secondary complications such as neuropathy, nephropathy and retinopathy [14,15].

Eventually, it became obvious that searching for natural products combining both  $\alpha$ -glucosidase inhibitory and antioxidant activities can be a potential safe strategy for the prophylaxis against diabetes or for the protection against the worse complications of type 2 DM patients together with DM conventional therapy.

### 3. Present Study

In the present study, chromatographic fractionation and purification of the methanol extract of the aerial parts of *L. serriola* L. resulted in the isolation and identification of seventeen compounds (Figure 1) based on various spectroscopic techniques and by comparing their spectral data with those reported in literature (see supporting information for spectra). It is worth mentioning that taraxast-20-ene-3 $\beta$ ,30-diol (**3**), (2*S*,3*S*,4*R*,2'*R*,14*E*)-2-(2'-hydroxytetracosanoylamino)-14-octadecene-1,3,4-triol (**5**), and 4-hydroxybenzoic acid (**10**) are isolated in the present study for the first time from genus *Lactuca*. Meanwhile, (*E*)-*p*-coumaric acid (**8**), protocatechuic acid (**11**), lactuside B (**16**) and cichorioside B (**17**) are reported for the first time from the titled plant.

Moreover, on a trial to find new natural therapeutic agents for management of diabetes mellitus, the studied plant extract, fractions and selected metabolites (**10-15**) were investigated for their  $\alpha$ -glucosidase inhibitory and antioxidant activity.

**Glucosidase Inhibitory Activity:** The assay was performed according to the previously reported method (see supporting information for details) [16]. From the results (Table 1), it is obvious that the total methanol extract of *L. serriola* L. exhibited good inhibition for  $\alpha$ -glucosidase activity with IC<sub>50</sub> value 46.16 $\pm$ 0.26  $\mu$ g/mL. The ethyl acetate fraction showed remarkable inhibition among other tested fractions with IC<sub>50</sub> value 9.16 $\pm$ 0.17  $\mu$ g/mL compared to acarbose (IC<sub>50</sub>, 6.11 $\pm$ 0.22  $\mu$ g/mL). A quite moderate  $\alpha$ -glucosidase inhibitory activity was recorded for the methylene chloride fraction with IC<sub>50</sub> value 16.88 $\pm$ 0.28  $\mu$ g/mL. As to petroleum ether fraction, it displayed low  $\alpha$ -glucosidase inhibition with IC<sub>50</sub> value 24.88 $\pm$ 0.12  $\mu$ g/mL. It is worth mentioning that the lowest  $\alpha$ -glucosidase inhibitory activity among the tested fractions was recorded for the *n*-butanol fraction of *L. serriola* L. (IC<sub>50</sub> >100  $\mu$ g/mL).

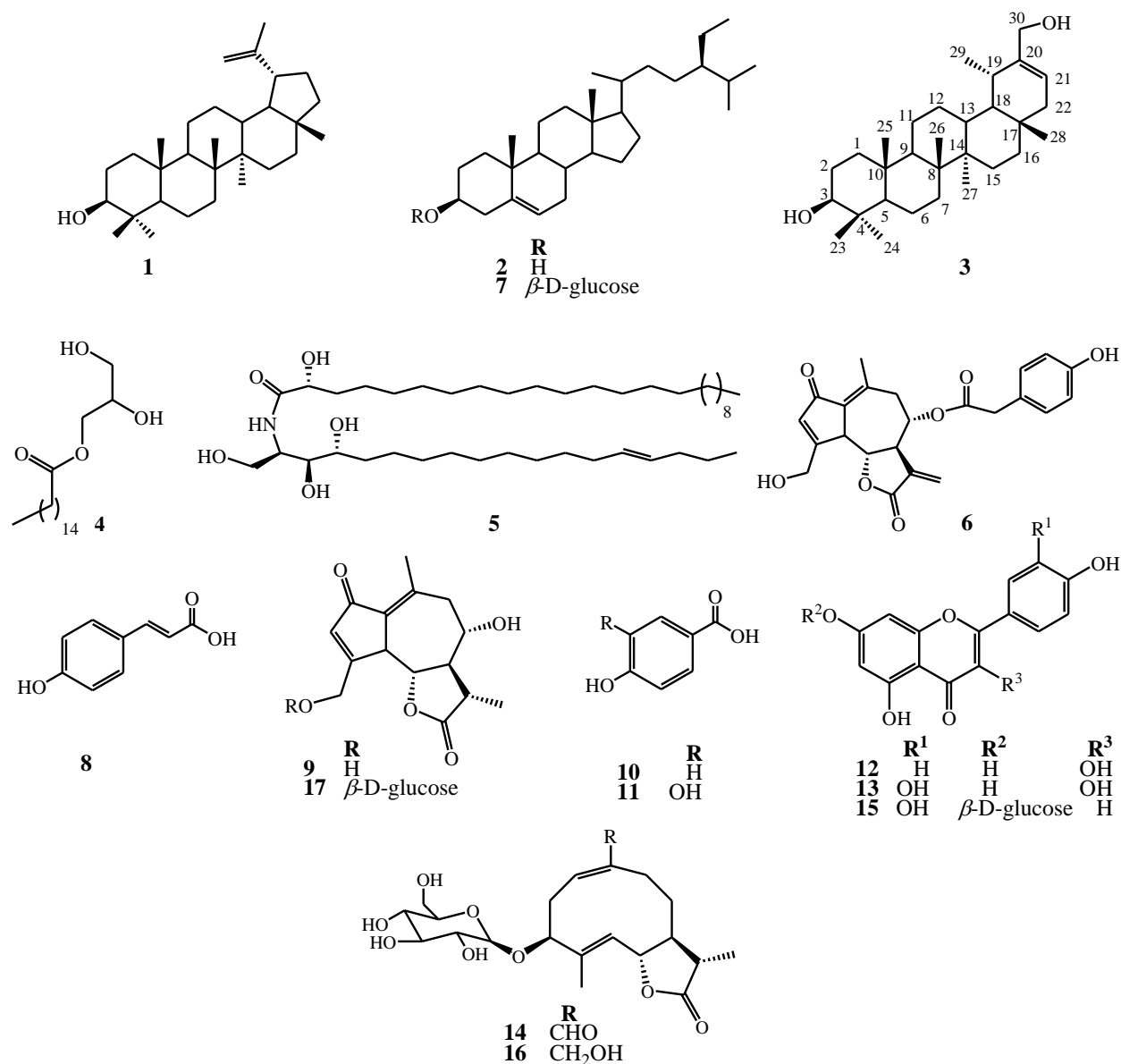
The compounds isolated from ethyl acetate fraction were further investigated for their  $\alpha$ -glucosidase inhibitory activity. From Table 2, it is clear that kaempferol (**12**) and quercetin (**13**) showed the highest inhibitory action against  $\alpha$ -glucosidase with IC<sub>50</sub> 39.72 $\pm$ 0.43 and 39.82 $\pm$ 1.12  $\mu$ M, respectively. The  $\alpha$ -glucosidase inhibitory activity of both kaempferol and quercetin compounds was supported by previous studies which reported them as good potential candidates for Type 2 DM treatment [11]. As for

protocatechuic acid (**11**) and luteolin-7-*O*- $\beta$ -D glucoside (**15**), they showed moderate inhibitory activity against  $\alpha$ -glucosidase enzyme. Relatively low  $\alpha$ -glucosidase inhibitory activity was recorded for lactuside A (**14**) and 4-hydroxybenzoic acid (**10**) in comparison to the standard compound, acarbose. The presence of an extra hydroxyl group in protocatechuic acid (**11**) has observably increased the inhibitory activity against  $\alpha$ -glucosidase enzyme ~ 6.4-fold higher than 4-hydroxybenzoic acid (**10**) [17]. According to SAR of the three active flavonoids **12**, **13** and **15**, the presence of hydroxyl substitution at position-3 of the C-ring in both kaempferol (**12**) and quercetin (**13**) seems to be advantageous for the intended activity whereas it is absent in luteolin-7-*O*- $\beta$ -D-glucoside (**15**) [18].

These results suggested that the high  $\alpha$ -glucosidase inhibitory activity of the ethyl acetate fraction of *L. serriola* L. was partly attributable to **12**, **13** and **11** as potential candidates allowing the plant to be used as a probable natural remedy for diabetes prevention and treatment.

**Antioxidant Activity:** The antioxidant capacity of *L. serriola* L. was assessed using three different techniques: ABTS radical scavenging assay, DPPH radical scavenging assay and ferric reducing antioxidant power (FRAP) assay. The radical scavenging assay measures the ability of an antioxidant in inhibiting radicals from attacking proteins, fatty acids, DNA, amino acids in biological or food systems [19]. In the ABTS radical scavenging assay, the ethyl acetate fraction (Table 1) showed the strongest radical scavenging activity among the other tested fractions with IC<sub>50</sub> value 34.88±0.22  $\mu$ g/mL. This could be explained by the high phenolic content of the ethyl acetate fraction which plays an important role in the observed antioxidant activity [11]. Followed by the methylene chloride with IC<sub>50</sub> value 37.11±0.28  $\mu$ g/mL. The *n*-butanol fraction exhibited moderate antioxidant activity with IC<sub>50</sub> value 46.06±0.27  $\mu$ g/mL. Though, the petroleum ether fraction showed poor radical scavenging activity compared to standard ascorbic acid. Concerning the major compounds isolated from the ethyl acetate fraction (Table 2), quercetin (**13**) showed the highest radical scavenging activity with IC<sub>50</sub> value 33.53±0.21  $\mu$ M, which is almost comparable to ascorbic acid, followed by kaempferol (**12**) which exhibited remarkable antioxidant activity as well with IC<sub>50</sub> value 35.16±0.24  $\mu$ M. This may be due to the presence of a catechol group in ring B of quercetin which appeared to be crucial for high antioxidant activity [20]. Both protocatechuic acid (**11**) and luteolin-7-*O*- $\beta$ -D-glucoside (**15**) showed noticeable antioxidant activity with IC<sub>50</sub> value of 36.56±0.23 and 37.64±0.25  $\mu$ M respectively. The sesquiterpenoid structure, lactuside A (**14**) exhibited moderate antioxidant action compared to ascorbic acid. However, 4-hydroxybenzoic acid (**10**) showed weak radical scavenging activity. In the DPPH radical scavenging assay, the highest scavenging activity against the stable DPPH radical was recorded for ethyl acetate fraction (26.80±2.43  $\mu$ g/mL, Table 1). Quercetin (**13**) (Table 2) showed remarkable antioxidant activity with IC<sub>50</sub> value of 19.15±0.89  $\mu$ M followed by luteolin-7-*O*- $\beta$ -D-glucoside (**15**) and kaempferol (**12**) with IC<sub>50</sub> value of 41.47±0.71 and 41.60±2.2  $\mu$ M, respectively compared to that recorded for the standard, trolox (56.82±0.87  $\mu$ M). However, protocatechuic acid (**11**) showed moderate radical scavenging activity.

The FRAP assay evaluates the capability of the antioxidant to reduce ferric tripyridyltriazine complex (Fe<sup>3+</sup>-TPTZ) into a colored ferrous tripyridyltriazine complex (Fe<sup>2+</sup>-TPTZ) and this reduction potential indicates acting by breaking down the free radical chain reaction through hydrogen donation [21]. The ferric reducing ability of the samples is presented as trolox equivalent antioxidant capacity,  $\mu$ M TEAC/mg of extracts or as  $\mu$ M TEAC/mM of pure compounds. The ethyl acetate fraction (Table 1) exhibited the highest ferric reduction potential among the tested fractions with 1288.6±43.8  $\mu$ M TEAC/mg. The highest reducing power among the isolated compounds (Table 2) was recorded for quercetin (**13**) as 2333.5±88.77  $\mu$ M TEAC/mM followed by luteolin-7-*O*- $\beta$ -D-glucoside (**15**) and kaempferol (**12**) with reducing powers of 1304.5±82.3 and 883.18±65.26  $\mu$ M TEAC/mM, respectively. Moderate reduction potential was observed with protocatechuic acid (**11**). From the above findings, we can conclude that the strong antioxidant activity observed for the ethyl acetate fraction of *L. serriola* L. was probably attributable to compounds **12**, **13**, **15** and **11** which shared the highest observed  $\alpha$ -glucosidase inhibitory activities as well (see supporting information for details).



**Figure 1.** Structures of the isolated compounds (1-17)

In conclusion, the present study showed that the aerial parts of *L. serriola* L. possess promising  $\alpha$ -glucosidase inhibitory and antioxidant activities as a dual target. These activities can be partly attributed to kaempferol (12), quercetin (13), protocatechuic acid (11) and luteolin-7-*O*- $\beta$ -D-glucoside (15) as they shared the highest  $\alpha$ -glucosidase inhibitory activities and antioxidant activities among the isolated compounds. In the light of these findings, the aerial parts of *L. serriola* L., which can be consumed as a salad, proved its ability as a probable natural remedy for diabetes prevention and treatment with low possibility of developing secondary complications.

**Table 1.**  $\alpha$ -Glucosidase inhibitory and antioxidant activities of different fractions of *L. serriola* L.:

Extract/ Fraction	$\alpha$ -Glucosidase inhibition	ABTS	DPPH	FRAP
	IC <sub>50</sub> <sup>a</sup> ( $\mu$ g/mL)	IC <sub>50</sub> <sup>a</sup> ( $\mu$ g/mL)	IC <sub>50</sub> <sup>b</sup> ( $\mu$ g/mL)	TEAC <sup>c</sup> ( $\mu$ M/mg)
MeOH extract	46.16 $\pm$ 0.26	80.32 $\pm$ 0.39	520.60 $\pm$ 23.58	171.03 $\pm$ 9.56
Pet. ether fraction	24.88 $\pm$ 0.12	58.11 $\pm$ 0.31	319.0 $\pm$ 15.41	191.12 $\pm$ 18.42
CH <sub>2</sub> Cl <sub>2</sub> fraction	16.88 $\pm$ 0.28	37.11 $\pm$ 0.28	69.83 $\pm$ 3.14	649.9 $\pm$ 14.2
EtOAc fraction	9.16 $\pm$ 0.17	34.88 $\pm$ 0.22	26.80 $\pm$ 2.43	1288.6 $\pm$ 43.8
<i>n</i> -BuOH fraction	>100	46.06 $\pm$ 0.27	156.80 $\pm$ 6.3	325.60 $\pm$ 16.37
Acarbose*	6.11 $\pm$ 0.22	---	---	---
Ascorbic-acid**	---	5.32 $\pm$ 0.03	---	---
Trolox**	---	---	14.22 $\pm$ 0.22	---

<sup>a,b,c</sup>The data are expressed as means  $\pm$ SD, <sup>a</sup>(n=3), <sup>b,c</sup>(n=6), <sup>c</sup>TEAC, trolox equivalent antioxidant capacity.

\*Acarbose is used as a standard  $\alpha$ -glucosidase inhibitor with IC<sub>50</sub> 6.11 $\pm$ 0.22  $\mu$ g/mL

\*\*Ascorbic acid (ABTS), trolox (DPPH, FRAP) are used as standard antioxidants with IC<sub>50</sub> 5.32 $\pm$ 0.03, 14.22 $\pm$ 0.22  $\mu$ g/mL, respectively.

**Table 2.**  $\alpha$ -Glucosidase inhibitory and antioxidant activities of selected compounds of *L. serriola* L.:

Compound	$\alpha$ -Glucosidase inhibition	ABTS	DPPH	FRAP
	IC <sub>50</sub> <sup>a</sup> ( $\mu$ M)	IC <sub>50</sub> <sup>a</sup> ( $\mu$ M)	IC <sub>50</sub> <sup>b</sup> ( $\mu$ M)	TEAC <sup>c</sup> ( $\mu$ M/ mM)
4-hydroxybenzoic acid ( <b>10</b> )	810.31 $\pm$ 1.03	50.61 $\pm$ 0.29	>500	58.82 $\pm$ 6.5
protocatechuic acid ( <b>11</b> )	126.65 $\pm$ 1.82	36.56 $\pm$ 0.23	154.60 $\pm$ 11.9	295.0 $\pm$ 12.28
kaempferol ( <b>12</b> )	39.72 $\pm$ 0.43	35.16 $\pm$ 0.24	41.60 $\pm$ 2.2	883.18 $\pm$ 65.26
quercetin ( <b>13</b> )	39.82 $\pm$ 1.12	33.53 $\pm$ 0.21	19.15 $\pm$ 0.89	2333.5 $\pm$ 88.77
lactuside A ( <b>14</b> )	468.98 $\pm$ 0.45	73.80 $\pm$ 0.34	>500	106.37 $\pm$ 11.78
Luteolin-7- <i>O</i> - $\beta$ -D-glucoside ( <b>15</b> )	161.29 $\pm$ 0.31	37.64 $\pm$ 0.25	41.47 $\pm$ 0.71	1304.5 $\pm$ 82.3
Acarbose*	9.48 $\pm$ 0.34	---	---	---
Ascorbic-acid**	---	30.21 $\pm$ 0.19	---	---
Trolox**	---	---	56.82 $\pm$ 0.87	---

<sup>a,b,c</sup>The data are expressed as means  $\pm$ SD, <sup>a</sup>(n=3), <sup>b,c</sup>(n=6), <sup>c</sup>TEAC, trolox equivalent antioxidant capacity.

\*Acarbose is used as a standard  $\alpha$ -glucosidase inhibitor with IC<sub>50</sub> 9.48 $\pm$ 0.34  $\mu$ M.

\*\*Ascorbic acid (ABTS), trolox (DPPH, FRAP) are used as standard antioxidants with IC<sub>50</sub> 30.21 $\pm$ 0.19, 56.82 $\pm$ 0.87  $\mu$ M, respectively.

## Supporting Information

Supporting Information accompanies this paper on <http://www.acgpubs.org/journal/records-of-natural-products>

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