Gundelia rosea seed: Evaluation of biopharmaceutical potential and bioactive composition

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A R T I C L E   I N F O

Article history:
Received 10 May 2019
Received in revised form 3 July 2019
Accepted 16 August 2019
Available online xxxx

Edited by AR Ndhlala

Keywords:
Gundelia rosea
4-Caffeoylquinic acid
Fatty acids
Biopharmaceuticals

A B S T R A C T

Gundelia rosea species are among significant key medicinal plants extensively utilized in folk medicine of Middle Eastern countries. This study focused on researching the biopharmaceutical potency and bioactive compounds of Gundelia rosea seed. Hereby, traditional knowledge-based preparing methods (infusion and decoction) and ethanol-based lyophilized extracts obtained from Gundelia rosea seeds were assessed for (i) antioxidant capacities, (ii) enzyme inhibitory activities, (iii) HPLC-MS/MS and (iv) GC–MS studies. Phytochemical analysis revealed that ethanol extract which primarily compromised of mainly phenolics (4-Caffeoylquinic acid and luteolin hexoside) and several fatty acids (palmitic, stearic, oleic and linoleic acids), was superior to those of infusion and decoction extracts. Antioxidant activities findings revealed that ethanol extract contained a high level of total phenolics (55.3 mg Gallic acid Eq./g extract) and had high capacities of reducing (1683 μmol Fe²⁺ and 214.1 mg Trolox Eq./g extract for FRAP and CUPRAC respectively) and radical scavenging (ORAC: 2241.9 μmol, DPPH: 91.7 mg, ABTS: 141.2 mg Trolox Eq./g extract) and total antioxidant (Phosphomolybdenum: 1.39 mmol Trolox Eq./g extract) properties. The suppressive abilities of the extracts against selected isolated enzymes revealed that ethanol extract had pronounced levels of inhibitory activities against AChE (4.3 mg Galanthamine Eq.), BChE (3.4 mg Galanthamine Eq.), tyrosinase (120 mg Kojic acid Eq.), amylase (0.61 mmol Acarbose Eq.), glucosidase (11.91 mmol Acarbose Eq.) and lipase (53.4 μmol Orlistat Eq.) per gram extract. Findings obtained within this study confirmed the traditional utilization of Gundelia rosea and suggest its potential as a novel candidate of biopharmaceutical agents for public health problems.

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1. Introduction

Gundelia taxa known as tumbleweed belong to Asteraceae are perennial medicinal plants native to Middle Eastern countries. They have been utilized for a wide range of diseases treatment in traditional medicine such as chest pain, heart stroke, diabetes, laxative, gastric pain, bronchitis, inflammations, dental abscess, epilepsy and kidney stones (Halabi et al., 2005; Jarald et al., 2008; Sarper et al., 2009; Dalar et al., 2018). Scientific studies regards to Gundelia species such as in vitro antioxidant and enzyme inhibitory (Sekergolu et al., 2012), antitumor in cell culture (Abu-Lafi et al., 2019), in vivo antidiabetic (Mohammadi et al., 2018; Kadan et al., 2018) activities and phytochemical composition (Haghi et al., 2011; Sekergolu et al., 2012; Asgari et al., 2015; Kadan et al., 2018; Abu-Lafi et al., 2019) were principally focused on Gundelia tournefortii and scientific data with regard to biopharmaceutical potential of other Gundelia species were limited.

In Turkey, Gundelia species have been commonly used for a wide range of utilization including medicine, food, forage, chewing gum and coffee (Sekergolu et al., 2012; Dalar et al., 2018). Among them, Gundelia rosea locally known as kengerres were grown in West Iran, Northern of Iraq and Eastern parts of Turkey. It has been traditionally used in the treatment of diabetes and epilepsy in Turkey. Additionally, a chewing gum has been obtained and sold by local healers from its latex, which is used in the treatment of digestive problems. Moreover, in rural areas of Eastern Anatolia such as Gürpinar provinces, a local coffee is prepared from seeds of Gundelia rosea. Infusion and/or decoction prepared from Gundelia rosea has been extensively used traditionally for the treatment of epilepsy, diabetes and digestive ailments in Eastern Anatolia. This study focused on the answer of the following question: What are the biologically important chemical compounds of the Gundelia rosea? and is there any association between biological activities and traditional usage of Gundelia rosea? Therefore, this study...
aimed to (i) compare traditional preparing methods (infusion and decoction) and commonly used scientific extraction method (ethanol-based) in terms of extraction efficiency, (ii) analyze the presence of biologically active compounds of the extracts, (iii) evaluate total phenolics and antioxidant capacities comprehensively through FRAP, ORAC, DPPH, ABTS, CUPRAC, Phosphomolybdenum and metal chelation methods, and (iv) measure the enzyme inhibitory abilities against isolated enzymes including AChE, BChE, tyrosinase, amylase, glucosidase, and lipase.

2. Materials and methods

2.1. Plant materials

Seed samples of Gundelia rosea Al-Taey & Hossain were harvested from Konalga village, Çatak/Van city, in the Eastern Anatolia Region of Turkey, on August 8, 2018 (GPS coordinates 37° 5’ 255”N 043° 09’ 857”E). The identity of plant materials were confirmed at Van Pharmaceutical Herbarium, Pharmacy Faculty, Van Yuzuncu Yil University, Van/Turkey by Abdullah Dalar, PhD and Muzaffer Mukemre, PhD and the voucher sample was kept properly (Herbarium code: VPH-379; Collector code:AD-802). The study materials were dried suitably in the dark and subsequently subjected to grinding process, and stored at −20 °C until extraction procedure.

2.2. Chemicals

All chemicals were obtained from Sigma–Aldrich, Inc. (St Louis, MO, USA) and were of analytical or HPLC grade.

2.3. Preparation of extracts

2.3.1. Ethanol-based lyophilized extract

Ethanol-based lyophilized extract was prepared as described previously (Dalar and Konczak, 2013).

2.3.2. Lyophilized infusion extract

Lyophilized infusion extract was prepared according to Baytop (1999). Briefly, the ground air-dried seed samples were mixed with a 10-fold volume of boiled mineral water (gr/ml), incubated for 10 min. Subsequently, the mixture was filtered through cotton and vacuum filtration (45 μm) with the supernatant collected. The supernatant of infusion was evaporated under reduced pressure at 37 °C using a rotary evaporator (Rotavapor R-205; Buchi, Switzerland). The derived concentrated infusion fraction was freeze-dried under a vacuum at −51 °C to obtain fine lyophilized infusion powder.

2.3.3. Lyophilized decoction extract

Lyophilized decoction extract was prepared according to Baytop (1999). Briefly, the ground air-dried seed samples were mixed with a 10-fold volume of cold mineral water (gr/ml), and heated until boiling. The mixture kept in boiled water for 3 min and then the mixture was re-centered in 10-fold volume of cold mineral water (gr/ml), and heated until boiling. The mixture was moved from heat, and stood for 10 min to be re-centered. The mixture kept in boiled water for 3 min and then the mixture was re-centered to be 10-fold volume of cold mineral water (gr/ml), and heated until boiling.

2.4. Antioxidant capacity

Folin–Ciocalteu reducing (Total phenolic content), total reducing (FRAP), and oxygen radical scavenging (ORAC) capacities of the extracts were measured as described previously by Dalar and Konczak (2013). The total antioxidant (phosphomolybdenum method), DPPH radical scavenging, ABTS radical cation scavenging, the cupric ion reducing (CUPRAC), and metal chelating activities of the extracts were determined as described previously by Uysal et al. (2017).

2.5. Enzyme inhibitory activities

Cholinesterase (ChE), α-amylase, α-glucosidase, and tyrosinase inhibitory activities of the extracts were determined according to Zengin (2016). The pancreatic lipase activity was assayed as described previously (Dalar and Konczak, 2013).

2.6. HPLC-MS/MS analysis

Phenolic compounds of the extracts were identified and quantified using high liquid chromatography–diode array–mass spectrometry (LC-DAD-MS/MS) as reported previously (Dalar and Konczak, 2013).

2.7. GC–MS analysis

Fatty acids present in extracts were analyzed by gas chromatography–mass spectrometry (GC/MS) using a Headspace Solid phase micro extraction as described previously (Uzun et al., 2017).

2.8. Data analysis

The mean values were calculated based on at least three determinations (n = 3). One-way ANOVA followed by the Bonferroni post-hoc test was performed to assess differences between the samples at the level of p < .05 through Graphpad Prism 5 (Graphpad Software, CA, USA).

3. Results and discussion

3.1. Phytochemical profiling

Phytochemical profiling of Gundelia rosea extracts were analyzed via HPLC-MS/MS (Table 1 and Fig. 1) and GC–MS (Table 2 and Fig. 2). Based on molecular weight, neutral loss, fragment ions, spectrum properties and co-chromatography analyses, two major phenolic compounds were detected in the extracts. The dominated compound showed a negatively charged molecular ion ([M − 1]−) at m/z 353 and produced MS/MS fragment ions of 191, 179 and 173 m/z respectively. According to MS/MS data (neutral loss, molecular weight, fragmentation pattern and absorbance spectrum) and differential ion mobilities (Willems et al., 2016), this compound was tentatively identified as 4-O-Caffeoylquinic acid.

### Table 1

<table>
<thead>
<tr>
<th>Individual phenolic compounds</th>
<th>MS/MS</th>
<th>Concentration (mg/g extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-Caffeoylquinic acid</td>
<td>−/−353</td>
<td>−/−171, 173, 191</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>−/−179</td>
<td>−/−133</td>
</tr>
<tr>
<td>Luteolin</td>
<td>−/−285</td>
<td>T</td>
</tr>
<tr>
<td>Luteolin hexoside</td>
<td>449/447</td>
<td>287/285</td>
</tr>
</tbody>
</table>

Means with different letters in the same raw were significantly different at the level (p < .05); n = 3. T: trace level.
acids were identified in the extracts (Table 2, Fig. 2) with the dominancy of linoleic acid, followed by oleic, palmitic and stearic acids. Additionally, traces of arachidic, linolenic and oxiraneoctanoic acids were also detected in the extracts (Table 2).

Chlorogenic acids, which are the ester of quinic and caffeic acids are among common phenolic acids present in medicinal plants and therefore their biological activities including antioxidant, antidiabetic, anti-inflammatory activities were investigated (Kartini et al., 2014; Oboh et al., 2015; Alam et al., 2016; Willems et al., 2016). The dominated phenolic compound of the extracts was 4-O-Caffeoylquinic acid that contributed 83%, 82.6% and 84.7% of the phenolic composition of ethanol, infusion and decoction extracts respectively. Our chromatographic findings are in concordance with the scientific literature of *Gundelia* species. For instance, Asgari et al. (2015) reported the presence of caffeic and chlorogenic acids as major phenolics and linolenic acid as major fatty acids of *Gundelia tournefortii*. In a study conducted by Sekeroglu et al. (2012) palmitic, stearic, oleic and linoleic acids were found as dominant fatty acids of *Gundelia tournefortii*. The presence of chlorogenic acid and its isomers including cryptochlorogenic acid (4-O-Caffeoylquinic acid) in *Gundelia* species was reported previously by Haghi et al. (2011). These findings suggest that, chlorogenic acid and its isomers are among the significant marker phenolic compounds of *Gundelia* species.

Application of an organic solvent such as ethanol or boiling water might extract some toxic compounds, and therefore the application of cold water is proposed in order to minimize the extraction of possible toxic compounds present in plant matrix (Farzaneh and Carvalho, 2015). Based on our chromatographic studies, not any toxic compounds were detected in ethanol extract, heat treatment used infusion, and decoction extracts which suggest that the level of toxic substance might be at trace or negligible levels and can explain the extensive utilization of *Gundelia rosea* in folk medicine.

### Table 2

<table>
<thead>
<tr>
<th>No</th>
<th>Retention time</th>
<th>Compound</th>
<th>Fragment ions</th>
<th>Relative concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ethanol</td>
<td>Infusion</td>
</tr>
<tr>
<td>1</td>
<td>36.77</td>
<td>Palmitic acid</td>
<td>18.60</td>
<td>11.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>60, 73, 83, 97, 129, 143, 157, 171, 185, 199, 213, 227, 239, 256</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>40.40</td>
<td>Stearic acid</td>
<td>3.68</td>
<td>3.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>55, 60, 73, 87, 115, 129, 143, 157, 171, 185, 193, 213, 227, 241, 255, 267, 284</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>41.17</td>
<td>Oleic acid</td>
<td>20.31</td>
<td>30.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>55, 69, 83, 97, 111, 125, 151, 165, 180, 195, 207, 222, 246, 264</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>42.47</td>
<td>Linoleic acid</td>
<td>53.33</td>
<td>52.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>55, 67, 81, 95, 110, 123, 136, 150, 164, 185, 209, 223, 241, 262, 280</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>43.14</td>
<td>Arachidic acid</td>
<td>T</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>41, 55, 57, 69, 73, 85, 97, 129, 157, 171, 185, 213, 230, 269, 312</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>43.77</td>
<td>α-Linolenic acid</td>
<td>T</td>
<td>ND</td>
</tr>
<tr>
<td>7</td>
<td>49.75</td>
<td>Oxiraneoctanoic acid</td>
<td>T</td>
<td>T</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>41, 55, 57, 69, 83, 97, 113, 120, 124, 139, 155, 167, 185</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 1. HPLC profile of Gundelia rosea seed.**

3.2. Antioxidant capacities

Antioxidants which are mainly act to deactivate the free radicals can be categorized according to their activity (enzymatic or non-enzymatic), solubility (hydrophilic or lipophilic), and size (Nimse and Pal, 2015). Therefore, various methods based on different reaction mechanisms should be utilized in order to reveal the comprehensive antioxidant capabilities of the extract tested. The antioxidant activities of *Gundelia rosea* extracts were evaluated comprehensively through complementary methods (Table 3). Ethanol extract contained a high level of total phenolics (55.3 mg Gallic acid Eq./g extract) and had pronounced levels of reducing (1683 μmol Fe²⁺) and 214.1 mg Trolox Eq./g extract for FRAP and CUPRAC respectively) and radical scavenging (ORAC: 2241.9 μmol, DPPH: 91.7 mg, ABTS: 141.2 mg Trolox Eq./g extract) and total antioxidant (Phosphomolybdenum: 1.39 mmol Trolox Eq./g extract) activities. These results showed that ethanol-based extract had superior levels of antioxidant activities than those of the water-based infusion and decoction extracts, which suggest the superior extraction potential of ethanol solvent in terms of major bioactive compounds of *Gundelia rosea*. This hypothesis was partly confirmed via the highest total phenolic content of the ethanol extraction. In addition,
the results showed that the major compounds of the extracts were less sensible to the heat treatment, which was resulted in lesser total phenolics and antioxidant capacities in decoction extracts (Table 3). The total phenolic content and antioxidant activities of Gundelia rosea were superior to ethanol extract obtained from Gundelia tournefortii (Sekeroglu et al., 2012). Fraisse et al. (2011) reported that caffeoyl derivatives were the major antioxidant compounds of wild herbs of several species belong to Asteraceae family which propounded that chlorogenic acids were among the major key antioxidant compounds of Asteraceae family specifically Gundelia species.

3.3. Inhibitory effects on tested enzymes

The increase of global health problems across the world conducted the enzymes to be one of the most significant pharmacological patterns (Copeland et al., 2007). For instance, WHO (2018) reported that approximately 1.9 billion people which 18% of them were children have been affected by obesity (WHO, 2018). Moreover, a dramatical prevalence (from 4.7% to 8.5%) of diabetes mellitus has been arised between the periods of 1980 and 2014 (Pesce et al., 2019). Therefore, modest strategies are needed for the management of such diseases. Such an approach can alleviate the symptoms of public health diseases. Among them, key enzyme inhibitory strategy effectively applied as pharmaceutically. For instance, cholinesterase is a target for preventing Alzheimer’s disease, which hydrolyzes acetylcholine in the synaptic of gap (Jiang and Gao, 2019). Similarly, pancreatic lipase is the main enzyme of hydrolysis of triglycerides in gastrointestinal tract, thus inhibiting its activity may be linked to reduced lipid absorption and obesity (Hamdan et al., 2019). In this context, several drugs (tacrine for cholinesterases; kojic acid for tyrosinase; voglibose for amylase and orlistat for lipase) have been produced as inhibitor agents in pharmaceutical industry. However, the side effects including diarrhea, abdominal disturbance or toxicity (Buchholz and Melzig, 2015; Thakur et al., 2019; Palacios et al., 2019) of such inhibitors limited their utilization properly. Thus, novel alternative inhibitors from natural sources with minimum side effects are needed.

The enzyme inhibitory effects of G. rosea seed extracts were investigated on several enzymes including cholinesterases, tyrosinase, amylase, glucosidase and lipase (Table 4). Solely the ethanol extract was active on cholinesterases (4.30 mg GALAE/g for AChE and 3.40 mg GALAE/g for BChE), while infusion and decoction were not active on these enzymes. The best tyrosinase inhibitory activity was observed in ethanol extract with the value of 120.25 mg KAE/g, followed by infusion and decoction. Antidiabetic potential of the extracts were assayed through amylase and glucosidase enzymes and similar to cholinesterases and tyrosinase, the superior inhibitory abilities were detected in ethanol extract. Moreover, infusion and decoction did not exhibit inhibitor effect on glucosidase. With regard to lipase inhibition, the ethanol extract was the best inhibitor among all extracts. Generally, the antioxidant and enzyme inhibitory effects for G. rosea extracts followed the same pattern of ethanol > infusion > decoction. This finding is in agreement with Yao et al. (2009), Reza et al. (2018), Moein et al. (2017), and Sun et al. (2017), who reported the positive correlations between total phenolics and enzyme inhibitory effects. Also, the highest level of 4-cafeoylquinic acid and luteolin hexoside was found in ethanol extract and these

**Fig. 2.** GC–MS profile of Gundelia rosea seed.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Total phenolic contents and antioxidant activities of Gundelia rosea seed extracts.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ethanol</td>
</tr>
<tr>
<td>Antioxidant activity</td>
<td>Total phenolics content-FCR&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>55.3 ± 3.2a</td>
</tr>
<tr>
<td></td>
<td>32.9 ± 0.5c</td>
</tr>
<tr>
<td></td>
<td>23.1 ± 0.02c</td>
</tr>
</tbody>
</table>

Means with different letters in the same raw were significantly different at the level (p < 0.05); n = 3.

<sup>1</sup> Folin–Ciocalteu values – mg Gallic acid Equivalent/g extract,
<sup>2</sup> Ferric reducing antioxidant power – μ mol Fe<sup>2+</sup>/g extract,
<sup>3</sup> Oxygen radical absorbance capacity - μ mol Trolox Equivalent/g extract,
<sup>4</sup> DPPH radical scavenging activity – mg Trolox Equivalent/g extract,
<sup>5</sup> ABTS radical scavenging activity – mg Trolox Equivalent/g extract,
<sup>6</sup> Cupric ion reducing antioxidant capacity – mg Trolox Equivalent/g extract,
<sup>7</sup> Phosphomolybdenum total antioxidant capacity - mmol Trolox Equivalent/g extract,
<sup>8</sup> Metal chelation activity – mg EDTA Equivalent/g extract.
components have been already reported as antioxidant, antiadipic, anti-Alzheimer or antiobesity agents (Akhisa et al., 2013; Iwai et al., 2004; Hu et al., 2015; Szwajger et al., 2017; Zang et al., 2016; Choi et al., 2014; Kawser Hossain et al., 2016). From this point forth, these components could be among the main contributors of the biopharmaceutical properties of Gundelia rosea extracts.

4. Conclusions

- This work is the first report of chemical composition, antioxidant and enzyme inhibitory properties of G. rosea extracts.
- Ethanol extract exhibited the highest biological activities.
- 4-Caffeoylquinic acid, luteolox hexoside, and fatty acids were detected as major phytochemical compounds of Gundelia rosea.
- Our findings indicate that chlorogenic acids might be among the key phenolic compounds of Gundelia species.
- Ethanol solvent was more efficient than that of the water solvent in terms of extraction capability of bioactive compounds.
- Our findings suggest a pronounced biopharmaceutical potential of Gundelia rosea for pharmaceutical industry.

Funding source

This research didn’t receive any grant from public, commercial, or private sectors.

Declaration of Competing Interest

The authors declare that there is no conflict of interest.

Acknowledgements

None.

References


