Exploring regional variation: Antioxidant potential of *Lycium barbarum* L. samples collected across Iraq

Omaima Saddam Zirjawi | Ula M. Noor Almousawi | Amjed Haseeb Khamees

Abstract *Lycium barbarum*, popularly known as Goji berries, is revered for its exceptional properties as a superplant, owing to the abundance of minerals and bioactive chemicals present in its fruits and leaves. Throughout history, several Asian countries, particularly China, have integrated *Lycium barbarum* (hereafter referred to as *L. barbarum*) into their traditional medicinal and nutritional practices. This widespread adoption is primarily due to the vital role played by the active components found in *L. barbarum* extracts, such as carotenoids, flavonoids, and polysaccharides, which exhibit notable efficacy in preventing various chronic ailments, including age-related macular degeneration. This study seeks to explore the impact of ultrasound-assisted extraction, in comparison to the remaceration extraction method, on the antioxidant activity of *L. barbarum* fruits and leaves obtained from three distinct cities in Iraq. The findings of this investigation hold significant promise for expanding our understanding of the optimal extraction technique for maximizing the antioxidant potential of *L. barbarum*, thus contributing to the potential development of novel applications in the medical and nutritional sectors.

Keywords: *Lycium barbarum*, medicinal plants, antioxidant, herbal medicine, Iraq flora, wild plant

1. Introduction

The use of alternative medicine, particularly herbal medicine, has experienced a substantial surge in popular and academic settings in both developed and developing nations. This remarkable surge is attributed to the fact that alternative medicines are derived from natural sources, rendering them considerably safer than prescribed, chemically derived medications. (Ekor, 2014; Bahall et al., 2015).

Herbal extracts contain high concentrations of phytochemicals and bioactive ingredients, which can collectively exert their therapeutic effects through several pathways (Eid et al, 2023).

The herbal medicine *L. barbarum* belongs to the Solanaceae family. This plant is widely planted and common in both subtropical and tropical regions of the Southern and Northern Hemispheres, including Africa, Asia, North America, South America, and Australia (Wang et al, 2022). *L. barbarum* and *L. brevipes* Benth. are two common species in Iraq (Chakravarty, 1976).

In Iraq and other countries, *L. barbarum* is known as Awsaj, Sireem, Box-Thorn, and Goji berries (Al-Mayah et al, 2016). *L. barbarum* is a thorny woody shrub that can grow to heights as high as four meters. In terms of anatomy, the leaves of *L. barbarum* are long or oblong and are spathulate to linear in shape. The flowers of *L. barbarum* were arranged in numbered groups (1-3 flowers). The fruit has an oval, bright orange core with a 1-to-2 cm diameter and tiny brown seeds. The plant has a large root system, as it grows in alkaline soils that can withstand surrounding temperatures as high as 45°C (Hussein and Farhoud 2021).

Goji berries are known to be popular medicines owing to their role in the treatment of fever, headache, night sweats, heart disease, diabetes, kidney dysfunction, gynecological problems, neurasthenia, abdominal pain, dry cough, and blurred vision (Wang et al., 2022).

Over the past few years, more than 200 different components of *L. barbarum* have been identified, described, and examined. These various components included carotenoids, phenylpropanoids, flavonoids, other polysaccharides, and polyphenols. Each of these genes possesses a positive biological trait. The broad spectrum of pharmacological properties of *L. barbarum* is believed to be caused by the large concentrations of polysaccharides present in that plant. Thus, a variety of polysaccharides, carotenoids, flavonoids, alkaloids, amides, peptides, anthraquinones, lignanoids, steroids, sterols, terpenoids, organic acids, anthocyanins, glycolipids, and essential oils have been identified in the leaves, fruits, and root bark of *L. barbarum* (Gao et al., 2017).

Thus, *L. barbarum* (Figure 1) has several pharmacological activities, most of which are commonly related to the prevention and treatment of different diseases. These medical activities include increased metabolism; high antioxidant and...
anti-aging effects; immune system regulation; neuroprotection; reduced incidence of cardiovascular disease; and antidiabetic, anticancer, antiglaucoma, and immunomodulatory effects (Žitek et al., 2020).

Figure 1 Iraqi L. barbarum collected from Basrah city.

Definitionally, for any atom (such as oxygen or nitrogen) within the topmost shell, at least one unpaired electron can exist independently. When a covalent link breaks, a radical is easily produced. Leaving one electron with each new atom, species of free radicals are extremely volatile and reactive, becoming stable only by absorbing electrons from lipids, proteins, nucleic acids, carbohydrates, or any other surrounding molecule, leading to a chain reaction of harm and disease (Wood, 2012). Oxidative stress has the potential to generate peroxides and free radicals, leading to detrimental effects on many biological components, such as DNA, proteins, and lipids (Eid et al., 2023). Antioxidants stop the oxidation process by neutralizing free radicals. Therefore, this study aimed to investigate the antioxidant activities of L. barbarum extracts of fruits and leaves via ultrasound-assisted extraction and maceration methods. The plant samples were collected from three Iraqi cities, Baghdad, Maysan, and Basra.

2. Materials and Methods

We prepared twelve plant samples coded from one to twelve, as shown in Table 1.

<table>
<thead>
<tr>
<th>Code number of sample</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>Leave extract of Baghdad by Ultrasound-Assisted Extraction (UAE) method</td>
</tr>
<tr>
<td>Sample 2</td>
<td>Leaves extract of Baghdad by re maceration method</td>
</tr>
<tr>
<td>Sample 3</td>
<td>Leaves extract of Basrah by UAE method</td>
</tr>
<tr>
<td>Sample 4</td>
<td>Leaves extract of Basrah by re maceration method</td>
</tr>
<tr>
<td>Sample 5</td>
<td>Fruit extract of Basrah by UAE method</td>
</tr>
<tr>
<td>Sample 6</td>
<td>Fruit extract of Basrah by re maceration method</td>
</tr>
<tr>
<td>Sample 7</td>
<td>Fruit extract of Baghdad by re maceration method</td>
</tr>
<tr>
<td>Sample 8</td>
<td>Fruit extract of Baghdad by UAE method</td>
</tr>
<tr>
<td>Sample 9</td>
<td>Leave extract of Maysan by remaceration method</td>
</tr>
<tr>
<td>Sample 10</td>
<td>Fruit extract of Maysan by remaceration method</td>
</tr>
<tr>
<td>Sample 11</td>
<td>Leaves extract of Maysan by UAE method</td>
</tr>
<tr>
<td>Sample 12</td>
<td>Fruit extract of Maysan by UAE method</td>
</tr>
</tbody>
</table>

2.1. Plant collection

L. barbarum leaves and fruits were collected from three Iraqi cities in the middle and southern regions of Iraq (Baghdad, Basrah, Mysan) between November 2022 and April 2023. The plants were then classified and diagnosed by Dr. Ula Al-Mousawi of the Department of Pharmacognosy, College of Pharmacy, University of Basra (voucher code: 50777).

2.2. Plant extraction

Both the leaves and fruits of the plants were cleaned, washed, and air-dried at room temperature for five days. These parts were subsequently pulverized in a blender for extraction, after which two extraction methods were used.

2.2.1. Method A (Maceration)

At room temperature, 5 gm of each part (of the plant) was macerated in 100 ml (85%) of a methanol-diluted solution, incubated for 14 days, and stirred using a magnetic stirrer. The extracts were filtered through filter paper (Leite et al., 2019). The yield of the leaf extracts ranged from 18% to 19%. The percentage of fruit extracted ranged from 20% to 21%.

2.2.2. Method B: (Ultrasound-assisted extraction)
Five grams of each plant part was placed in a conical flask, and then 100 ml of 85% methanol solvent was added. Then, an ultrasonic bath (DS-2510 DT) was used at a frequency of 60 kHz for 30 minutes at room temperature. Finally, all the extracts obtained were filtered, stored, and refrigerated pending analyses (Abduljalil et al., 2017). Leaf extract yields ranging from 19% to 20%. The percentage of fruit extracted ranged from 20% to 22%.

2.3. The antioxidant activity (DPPH scavenging assay [2,2-diphenyl-1-picrylhydrazyl (DPPH)])

Technique was employed to assess the radical scavenging activity of the in vitro extracts (Narazary et al. 2016), which depends on the colorimetric shift (from deep violet to bright yellow).

The antioxidant activities of different compounds isolated from the leaves and crude extracts of Lycium barbarum fruits were detected by using a DPPH radical scavenging assay according to the procedure described below.

Aliquots (0.5 mL) of twofold serial dilutions of the Lycium barbarum crude extracts and ascorbic acid (25, 50, 100 and 200 µg/mL) were added to the test tubes. Simultaneously, 3 mL of a methanol-DMSO (9:1) mixture and 0.3 mL of DPPH solution were added to each solution.

The samples were incubated at 37°C for one hour, and the radical scavenging activity of the samples against stable DPPH radicals was determined spectrophotometrically using an ELISA reader. The colorimetry changed (from deep violet to light yellow) when DPPH reduction was measured at 517 nm.

The % inhibition of radical by the samples was calculated according to the following formula:

\[
\% \text{ of scavenging} = \left( \frac{A_0 - A_1}{A_0} \right) \times 100
\]

The negative control consisted of a methanol-DMSO mixture and DPPH solution, while ascorbic acid was used as a reference (Aldoghachi et al, 2021; Haider, 2014).

2.3. Statistical analysis

The results were analyzed statistically by using the computer system MINI tap according to analysis of variance (ANOVA). The averages were compared by using the DNCA polynomial test at a probability of p≤0.05 and a significant difference of p≤0.01.

3. Results

The antioxidant test was performed using the DPPH method (Figures 1 to 13). The DPPH test relies on antioxidants to donate electrons to neutralize the DPPH radical. Discoloration indicates antioxidant activity because it cooccurs with the reaction and, additionally, changes the color of the DPPH dye measured at 517 nm (Munteanu et al., 2021).
Figure 4 The scavenging activity of sample 3.

Figure 5 The scavenging activity of sample 4.

Figure 6 The scavenging activity of sample 5.

Figure 7 The scavenging activity of sample 6.
Figure 8 The scavenging activity of sample 7.

Figure 9 The scavenging activity of sample 8.

Figure 10 The scavenging activity of sample 9.

Figure 11 The scavenging activity of sample 10.
As shown in Tables 2 and 3 show the DPPH free radical scavenging activity of the plant extracts. All the plant extracts were compared to standard ascorbic acid in this study, and the methanol extracts of the plants demonstrated DPPH free radical scavenging activity. All of the plant methanol extracts demonstrated significant free radical scavenging activity in a concentration-dependent manner, with the scavenging activity increasing with increasing concentrations of each particular plant extract.

**Table 2** Scavenging activity % (mean ±SD) of 1, 2, 3, 4, 5, and 6 compounds.

<table>
<thead>
<tr>
<th>Concentration (µg mL⁻¹)</th>
<th>Ascorbic acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>82.06±3.91</td>
<td>80.36±2.86</td>
<td>66.35±2.4</td>
<td>63.34±1.9</td>
<td>56.4±5.5</td>
<td>79.05±1.6</td>
<td>65.2±4.05</td>
</tr>
<tr>
<td>100</td>
<td>74.80±1.50</td>
<td>69.48±3.7</td>
<td>52.27±3.54</td>
<td>58.29±1.1</td>
<td>42.6±7.15</td>
<td>65.7±1.0</td>
<td>52.43±5.1</td>
</tr>
<tr>
<td>50</td>
<td>64.3±3.1</td>
<td>54.4±2.4</td>
<td>36.76±8.23</td>
<td>51.15±3.06</td>
<td>25.42±2.8</td>
<td>54.4±2.6</td>
<td>44.09±1.4</td>
</tr>
<tr>
<td>25</td>
<td>52.7±3.1</td>
<td>40.4±3.7</td>
<td>28.9±6.2</td>
<td>36.4±0.82</td>
<td>24.61±3.3</td>
<td>40.8±1.0</td>
<td>30.5±4.7</td>
</tr>
<tr>
<td>12.5</td>
<td>39.27±1.3</td>
<td>17.63±7.19</td>
<td>16.74±3.9</td>
<td>26.5±0.9</td>
<td>15.7±0.9</td>
<td>28.97±4.2</td>
<td>17.63±3.5</td>
</tr>
</tbody>
</table>

**Table 3** Scavenging activity % (mean ±SD) of 7, 8, 9, 10, 11, and 12 compounds.

<table>
<thead>
<tr>
<th>Concentration (µg mL⁻¹)</th>
<th>Ascorbic acid</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>82.7±2.7</td>
<td>73.37±0.9</td>
<td>65.58±1.46</td>
<td>78.93±1.17</td>
<td>82.71±3.2</td>
<td>53.58±3.95</td>
<td>32.02±1.5</td>
</tr>
<tr>
<td>100</td>
<td>74.80±1.4</td>
<td>64.69±6.08</td>
<td>55.36±1.6</td>
<td>73.34±2.54</td>
<td>76.23±1.7</td>
<td>41.04±2.3</td>
<td>26.19±1.5</td>
</tr>
<tr>
<td>50</td>
<td>64.3±3.41</td>
<td>49.7±6.21</td>
<td>40.93±4.06</td>
<td>65.43±1.8</td>
<td>64.93±1.91</td>
<td>31.21±2.89</td>
<td>19.40±2.66</td>
</tr>
<tr>
<td>25</td>
<td>52.74±3.1</td>
<td>40.6±±3.1</td>
<td>30.32±2.3</td>
<td>53.85±2.44</td>
<td>52.0±2.88</td>
<td>19.9±1.2</td>
<td>15.50±1.55</td>
</tr>
<tr>
<td>12.5</td>
<td>39.27±1.3</td>
<td>28.8±1.26</td>
<td>22.18±3.95</td>
<td>39.6±2.41</td>
<td>40.08±1.39</td>
<td>15.62±1.4</td>
<td>10.30±2.54</td>
</tr>
</tbody>
</table>

The IC50 value is the crude extract inhibitory concentration that may scavenge 50% of the ROS (reactive oxygen species) or inhibit oxidation by 50%. The IC50 value is inversely related to activity, and a lower IC50 value indicates greater antioxidant activity (Narzary et al., 2016).

The results revealed that the fruit extract of Maysan by the maceration method had the greatest DPPH radical scavenging activity, with an IC50 value of 32.2 µg/ml, followed by the fruit extract of Baghdad by the maceration method, with an IC50 value of 36.6 µg/ml; the leaf extract of Maysan by the UAE method, with an IC50 value of 37.1 µg/ml; the leaf extract of Baghdad by the UAE method, with an IC50 value of 39.2 µg/ml; the fruit extract of Basrah by the UAE method, with...
an IC50 value of 42.1 µg/ml; the leaf extract of Basrah by the UAE method, with an IC50 value of 50.8 µg/ml; the leaf extract of Baghdad by the maceration method, with an IC50 value of 51.4 µg/ml; the fruit extract of Basrah by the re maceration method, with an IC50 value of 54.7 µg/ml; the fruit extract of Baghdad by the UAE method, with an IC50 value of 55.9 µg/ml; the leaf extract of Maysan.

### Table 4 IC50% of the sample. Arranging in a deciding manner.

<table>
<thead>
<tr>
<th>Samples</th>
<th>IC50(µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 10</td>
<td>32.2</td>
</tr>
<tr>
<td>Sample 7</td>
<td>36.6</td>
</tr>
<tr>
<td>Sample 9</td>
<td>37.1</td>
</tr>
<tr>
<td>Sample 1</td>
<td>39.2</td>
</tr>
<tr>
<td>Sample 5</td>
<td>42.1</td>
</tr>
<tr>
<td>Sample 3</td>
<td>50.8</td>
</tr>
<tr>
<td>Sample 2</td>
<td>51.4</td>
</tr>
<tr>
<td>Sample 6</td>
<td>54.7</td>
</tr>
<tr>
<td>Sample 8</td>
<td>55.9</td>
</tr>
<tr>
<td>Sample 11</td>
<td>68.4</td>
</tr>
<tr>
<td>Sample 4</td>
<td>71.6</td>
</tr>
<tr>
<td>Sample 12</td>
<td>85.6</td>
</tr>
</tbody>
</table>

The IC50 values ranged from 32.2 µg/ml (Sample 10) to 85.6 µg/ml (Sample 12). This indicates a significant difference in the inhibitory activity of the samples. Ascorbic acid was included as a reference standard. It is a well-known antioxidant and free radical scavenger. Interestingly, Sample 10 had lower IC50 values than ascorbic acid, suggesting that it may be an even more potent inhibitor of this biological process. Conversely, Samples 4 and 12 had the highest IC50 values, indicating that they had the weakest activity. The data appear to be skewed toward higher IC50 values, with a median of 50.8 µg/ml and a mean slightly greater at 50.7 µg/ml. This suggests that most samples have moderate inhibitory activity. It is interesting to note that some extracts, such as Samples 10, 7, and 9, outperform others within the same species.

4. Discussion

The metabolism and build-up of secondary metabolites are significantly influenced by altitude, temperature, light, and moisture. The variations in the active component concentrations and antioxidant activity of medicinal plants are caused by environmental factors (such as altitude, temperature, lighting, precipitation, humidity, and soils) at different production locations (Liu et al., 2016).

Numerous investigations have made a compelling case for the idea that plant antioxidant qualities are modified by environmental factors (Yu et al., 2003).

The production and build-up of secondary metabolites in medicinal plants are likewise impacted by illumination. The amount of secondary metabolites can increase with increasing illumination time. For instance, with prolonged light exposure, the flavonoid content of Arabidopsis increased (Fuglevand et al., 1996).

The present study investigated how different geographical, climatic, and weathering environments impact the antioxidant activity of both the leaves and fruits of *L. barbarum* in three different places in Iraq, namely, Basra, Maysan, and Baghdad. These two main parts of *L. barbarum* contain active compounds that are principally responsible for antioxidation. Plants vary in the degree of oxidation, as there are different levels of antioxidant activity that resist free radicals based on their unique components (Waqas et al., 2013).

The active components in the leaves and fruit of *L. barbarum* were extracted following two protocols: maceration and sonication. These active chemicals have been found in high concentrations in plant extracts (Ex/Maceration) with the highest antioxidant power. Although the production of active constituents in medicinal plants is genetically controlled, it is also greatly affected by environmental factors. Accordingly, certain environmental changes can influence the growth of medicinal plants, as well as the quantity and quality of their constituents, including flavonoids, glycosides, alkaloids, steroids, and essential oils (Zargoosh et al., 2019).

In this study, antioxidant activity was found to be greater in Maysan fruit after maceration than after ascorbic acid treatment, which could be attributed to the harvest season, which occurred in March, indicating that *L. barbarum* is rich in antioxidant compounds when the average temperature is 17 to 20°C (Çolak et al., 2019). This result is consistent with (Nurullayeva et al., 2021), who reported that the best temperature for *L. barbarum* is 20°C and that the best temperature for the plant is March. The findings of this study are in line with those of (Mocan et al., 2019), who concluded that the active constituents of *L. barbarum* are mainly related to certain geo-factors, soil type, climate, and harvesting time.

This study also confirmed that the antioxidant activity of methanol extracts of both leaves and fruits is high, which is consistent with the findings of (Benchennouf et al., 2017).

5. Conclusions

https://www.malque.pub/ojs/index.php/msj
The active constituents that have antioxidant effects are present in the leaves and fruit parts of \textit{L. barbarium}. These constituents were isolated from three different Iraqi cities via two extraction protocols that differ in their environment and temperature. These compounds can be detected in large amounts by sonication. The antioxidant activity of the various extracts was evaluated by the DPPH scavenging test. Compared with those from the other two cities, the extracts of \textit{L. barbarium} collected from Maysan showed a significant antioxidant effect.

Sophisticated phytochemical studies are recommended to identify, extract, and purify the active constituents in \textit{L. barbarium}, especially those related to medication. These components, as laboratory and phytochemical tests have shown, were found to be effective and preventive against different diseases. With these medically proven effects of this plant, additional and further studies are recommended to investigate geographical regions in Iraq other than those surveyed in this study where \textit{L. barbarum} is common.

6. Limitations of the study

Although \textit{L. barbarum} is widely planted across Iraq, this study is limited to three main cities in Iraq, particularly in the central and southern parts of Iraq, namely, Baghdad, Maysan, and Basra. This study followed certain laboratory and chemical methods and procedures to analyze the medicinal and chemical benefits of this plant.

Acknowledgments

The authors would like to thank the Faculty of Pharmacy/Department of Pharmacognosy/Basrah University, Iraq, for making this study possible.

Ethics considerations

The authors declare no potential conflicts of interest.

Conflict of Interest

The authors declare no conflicts of interest.

Funding

No funding was received for this study.

References


Eidor, M. (2014). \textit{Synthesis and Study the Antioxidant Activity of some new Heterocyclic Compounds from chalcone.}


in Potentilla fruticosa L. and its quality assessment. Scientific Reports, 6(June 2015), 1–18. https://doi.org/10.1038/srep28591


