SHORT COMMUNICATION

Efficacy analysis of preserved great burdock essence compounds

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chlorogenic acid;
great burdock;
long-term storage test

Abstract In our previous study, we developed the great burdock essence compounds successfully. Clinical trial has proved that great burdock can function as an adjuvant therapy for patients with gastric ulcer caused by \textit{Helicobacter pylori}. This study aims to assess the storage stability of great burdock essence compounds. We used the high-performance liquid chromatography method to test the concentration of chlorogenic acids at different times in the long-term storage test and accelerated storage test, and observed their appearance. The results showed that the concentration of chlorogenic acids degraded from 29.29 $\pm$ 0.49 mg/g to 28.29 $\pm$ 0.40 mg/g during the accelerated storage tests conducted for up to 6 months. In addition, the concentration of chlorogenic acids degraded from 29.29 $\pm$ 0.49 mg/g to 28.57 $\pm$ 0.21 mg/g during the long-term storage tests conducted for a period of up to 12 months. In both these tests, no change was observed in trait, scent, and color of the great burdock essence compounds. These results suggest that these compounds are highly stable.

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Introduction

\textit{Arctium lappa}, Linn belong to the Asteraceae family and contains nutrients such as inulin, crude fiber, protein, calcium, phosphorus, iron, and multiple essential vitamins and minerals. The polyphenolic content of great burdock is primarily made up of chlorogenic acids; studies have shown that it has antioxidant properties, can prevent iron-induced radical formation effectively, and possesses antimicrobial, anti-inflammatory, analgesic, and antipyretic activities.\textsuperscript{1–4} Previous studies have indicated that great burdock possesses antimicrobial activity, provides gastric mucosal and liver protection, and has anti-inflammatory and anticancer effects.\textsuperscript{5–10} \textit{Arnebia euchroma} is a member of the
Boraginaceae family, and its main nutrients are naphthoquinone derivatives. The use of *A. euchroma* in Chinese medicine is well established. Ingestion of *A. euchroma* has been documented to provide antipyretic and detoxification effects, reduce swelling, and improve bowel movements. External applications of *A. euchroma* are used for treating macula, smallpox, burns, dermatitis, and eczema. It has been validated in the studies of Chinese medicine pharmacology that *A. euchroma* can accelerate granulation tissue proliferation, and shows anti-inflammatory, antibacterial, and anti-tumor activities. Angelica sinensis is an Umbelliferae plant; its chemical composition includes primarily volatile oils, and water-soluble alkaloids and organic acids, along with ferulic acid as the main active ingredient. It is conventionally used for treating macula, smallpox, burns, dermatitis, and anemia. In recent studies, *A. sinensis* has been shown to possess immune system modulation, antioxidation, anti-inflammatory, and anticancer properties.

The great burdock essence compounds are formulated using a nanomicell, which is similar to liposome in structure. A liposome is a dual-layered, self-closing, spherical particle that contains a single (or multiple) lipid bilayer(s) surrounding an aqueous solution. Liposomes are vectors capable of embedding hydrophobic substances in their lipid bilayer and enclosing hydrophilic substances in their aqueous phase. The great burdock essence compounds contain a lipid phase, an emulsifier, and a small amount of aqueous phase. The lipid phase carries the main ingredients of *A. euchroma* and *A. sinensis*, which are also the main ingredients of great burdock. The lipid phase is enclosed by lecithin, and the outer layer is further covered with an aqueous layer. The resulting particle offers the following advantages: they show good stability and high hydrophilicity, are free of irritants and ethanol, exhibit improved absorbance of active ingredients, and are bioavailable. After ingestion, the particle makes contact with water and disintegrates into microemulsions, approximately 100 nm in size, to cover the gastric mucosa. The nanomicell is absorbed easily because of its structural similarity with the cell membrane.

An investigation into the effect of great burdock essence compounds on *Helicobacter pylori* infection showed that the great burdock essence compounds could help suppress *H. pylori* growth in infected patients. A stability test is a critical evaluation and an important basis for the quality of nutraceutical preservation. The primary goal of the current study was to determine the temporal impact of environmental factors (temperature, humidity, and light) on nutraceuticals. Subsequently, the shelf life of great burdock essence compounds was calculated for providing quality assurance of the product.

### Materials and methods

#### Production and preparation of samples

The samples of great burdock essence compounds were assigned to a good manufacturing practice (GMP)-certified factory to produce three batches, which were then packaged in sealed glass bottles of 10 mL capacity after going through the pasteurization process. The three batches underwent the storage test, trait analysis, and high-performance liquid chromatography (HPLC) analysis.

#### Storage test and sample collection time points

The long-term storage test was conducted at a storage temperature of 25 ± 2°C and a humidity of 60 ± 5%.

### Table 1

<table>
<thead>
<tr>
<th>Assessment criteria</th>
<th>0 mo</th>
<th>1 mo</th>
<th>2 mo</th>
<th>3 mo</th>
<th>6 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trait Viscous solution</td>
<td>Mild sesame oil scent</td>
<td>Mild sesame oil scent</td>
<td>Mild sesame oil scent</td>
<td>Mild sesame oil scent</td>
<td>Mild sesame oil scent</td>
</tr>
<tr>
<td>Scent Tawny</td>
<td>Tawny</td>
<td>Tawny</td>
<td>Tawny</td>
<td>Tawny</td>
<td>Tawny</td>
</tr>
<tr>
<td>Color Chlorogenic acid (µg/g)</td>
<td>27–33</td>
<td>29.29 ± 0.49</td>
<td>29.31 ± 0.46</td>
<td>29.09 ± 0.26</td>
<td>28.73 ± 0.37</td>
</tr>
</tbody>
</table>

The values of chlorogenic acid are expressed as mean ± SD; SD = standard deviation.

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**Figure 1** Color of great burdock essence compounds was observed visually by pouring the compounds from a brown glass bottle into a 15-mL tube.
Sampling time points were at 0 months, 3 months, 6 months, 9 months, 12 months, 18 months, 24 months, and 36 months. In the accelerated storage test, the storage temperature was 40°C to 6°C and the humidity was 75% to 5%. Samples were collected at 0 month, 1 month, 2 month, 3 month, and 6 month time points.

Trait analysis

Traits and color of the great burdock essence compounds were visually observed and recorded, along with their smell. The color of great burdock essence compounds is tawny (Fig. 1).

HPLC analysis

HPLC separation was performed at room temperature using a Hewlett Packard 1100 liquid chromatography system with a DAD G1315A detector (Hewlett Packard, Palo Alto, CA, USA). Analysis was performed using a Vercopak Inertsil 5 ODS (250-4.6 mm) column (GL Sciences, Inc., USA). A constant flow rate of 1 mL/min was employed and the eluent was monitored at 245 nm by the DAD detector. The following steps were carried out: (1) preparation of the chlorogenic acid standard. A total of 10.5 g of chlorogenic acid standard (FLUKA, Cat. No. 00500590) was dissolved in 50% methanol to a final concentration of 26.25 mg/mL (namely the 400 solution); (2) preparation of the great burdock essence compounds. Great burdock essence compounds (5 g) were added to 10 mL of methanol. The mixture was subjected to vigorous agitation to ensure proper mixing, and subsequently centrifuged for 10 minutes. The supernatant was collected and filtered through a 0.45 μm membrane filter; (3) the mobile phase consisted of (A) methanol, water, and acetic acid (15:83:2, v/v) and (B) methanol, water, and acetic acid (70:28:2, v/v). The elution profile was as follows: 0–30 minutes, the mobile phase consisted of 100% A; 30–31 minutes, A decreased to 0% and B increased to 100%; 31–45 minutes, it consisted of 100% B; 45–46 minutes, B decreased to 0% and A increased to 100%; and 46–55 minutes, it consisted of 100% A. The HPLC column was run at a flow rate of 1.0 mL/min; and (4) HPLC protocol. Standard solution (10 μL) and the prepared great burdock essence compounds were injected separately into the chromatography apparatus. The chromatograms were recorded and the major peak values were determined.

Results

Result of the accelerated storage test

The traits of samples stored for 0 months, 1 month, 2 months, 3 months, and 6 months all presented viscous solutions tawny in color and released a mild aroma of sesame oil. The chlorogenic acid content measured for the 0 months, 1 month, 2 month, 3 month, and 6 month samples were 29.29 ± 0.49 mg/g, 29.31 ± 0.46 mg/g, 29.09 ± 0.26 mg/g, 28.73 ± 0.37 mg/g, and 28.29 ± 0.40 mg/g, respectively (Table 1). A comparison of the average concentration of chlorogenic acids in 1 month, 2 months, 3 months, and 6 months with that in the 0th month showed that chlorogenic acid content reduced from 100% to 96.56% during the sequence of tests covering up to 6 months (Fig. 2).

Result of the long-term storage test

Samples collected at 0 months, 3 months, 6 months, 9 months, 12 months, and 18 months all presented traits of viscous solutions tawny in color and gave off a mild scent of sesame oil. The chlorogenic acid content measured for the 0 month, 3 month, 6 month, 9 month, and 12 month samples were 29.29 ± 0.49 mg/g, 29.45 ± 0.51 mg/g, 28.81 ± 0.55 mg/g, 28.20 ± 0.14 mg/g, and, 28.57 ± 0.21 mg/g, respectively (Table 2). A comparison of the average concentration of chlorogenic acids in 1 month, 2 months, 3 months, and 6 months with that in the 0th month showed that chlorogenic acid content reduced from 100% to 96.56% during the sequence of tests covering up to 6 months (Fig. 2).

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Results of the long-term storage tests of great burdock essence compounds.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assessment criteria</td>
<td>0 mo</td>
</tr>
<tr>
<td>Trait</td>
<td>Viscous solution Mild sesame oil scent</td>
</tr>
<tr>
<td>Scent</td>
<td>Viscous solution Mild sesame oil scent</td>
</tr>
<tr>
<td>Color</td>
<td>Tawny</td>
</tr>
<tr>
<td>Chlorogenic acid (μg/g) (N = 3)</td>
<td>27–33</td>
</tr>
</tbody>
</table>

The values of chlorogenic acid are expressed as mean ± SD; SD = standard deviation.
6 months, 9 months, and 12 months with that in the 0th month showing that chlorogenic acid content reduced from 100% to 97.52% during the sequence of tests conducted over a period of up to 12 months (Fig. 3).

Discussion

The results showed that chlorogenic acid content reduced from 100% to 97.52% during long-term storage tests spanning a period of up to 12 months and reduced from 100% to 96.52% during accelerated storage tests covering up to 6 months. These results suggest that the chlorogenic acid content of the great burdock essence compound is highly stable. The product shelf life could be extrapolated from the long-term storage test results. Firstly, the feasibility of time-dependent product degradation curve needs to be assessed by the goodness-of-fit test. Extrapolation of the nutraceutical shelf life is then achieved by determining the intersection between the lower bound of the 95% confidence interval of product degradation curve and the specified limit (such as 90% of the labeled content). Finally, the product shelf life is estimated by rounding off the nutraceutical shelf life to the nearest year or half-year (such as rounding off 25 months to 2 years). In the current study, the lower bound of the 95% confidence interval of the degradation curve at 12 months was valued at 92.64%, which is yet to intersect with the specified limit of 90% of the labeled content. Therefore, the shelf life of great burdock essence compounds exceeds 12 months. However, determination of a definite shelf life requires further evaluation of the 18 month, 24 month, and 36 month samples.

References