Extraction of essential oil from the aerial parts of Artemisia frigida Willd by way of hydrodistillation

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Abstract: This study aims to determine the optimum conditions for extraction of essential oil compounds in the aerial parts of Artemisia frigida Willd. Method: the considered extraction method is hydro-distillation, using a Clevenger apparatus. The effect of particle size of raw material, soaking time, liquid to plant material ratio and extraction time on essential oil yield were investigated through both single factor and multi-factor experiments. Results: In the single factor experiment, the influences of the following factors on essential oil extraction were studied; particle size 0.825 mm, soaking time 2 h, and liquid to plant material ratio 12:1. Under the multi-factor experiment, the influences of multiple factors of extraction conditions on essential oil were considered, particularly, extraction time (C)>soaking time (A)>liquid to plant material ratio. Conclusion: For extraction of essential oil from the aerial parts of Artemisia frigida Willd, the following optimum extraction parameters were identified: 2h of soaking time, 10:1 liquid to solid ratio, and 8h of extraction time.

Keywords: Artemisia frigida; essential oil; hydrodistillation; agi;

INTRODUCTION

Artemisia frigida Willd. (Asteraceae) is a perennial herb that reaches up to 10-14 cm in height. The Artemisia frigida is widespread throughout Central Asian countries (China, Mongolia, and Russia), Europe, Siberia, and North America [1]. Artemisia frigida is an important medicinal plant in traditional Mongolian medicine for its pharmacological application on stanch and detumescence. In Mongolian traditional medicine, Artemisia frigida is locally known as “Agi” [2, 3]. There are numerous bioactive compounds in the aerial parts of Artemisia frigida, such as flavonoids, terpenoids, tannins, phenols steroids, and organic acids and the most important chemical constituent is essential oil [1, 4]. Many scientists have investigated the chemical composition of essential oil in the aerial parts of Artemisia frigida by GC/MS, and more than 100 chemical components were isolated and defined [5-7].
Essential oils are widely used in medicine, health products, and in the cosmetic industry [8]. It has various pharmaceutical properties – it is anti-inflammatory, acts as an antioxidant, and has anticancerous antimicrobial activity [9-11]. Essential oils are essentially bio-active compounds and have a complex mixture of more than 20 different components of low molecular weight, and plant extracted essential oils, in particular, contain terpenes, oxygenated derivative, and other aromatic compounds [12].

Essential oils from various plants can be isolated using several methods, such as hydro-distillation, steam distillation, and organic solvent extraction. Hydro-distillation is the most commonly used method, which has been traditionally used for removal of essential oils from medicinal plants [13]. Several factors may influence extraction efficiency, the particle size, liquid to plant material ratio, whereas soaking and extraction time are crucial factors that affect the extraction process [14, 15].

This study aims to define the optimum conditions for the extraction of essential oil compounds from the aerial parts of Artemisia frigida Willd, using the hydro-distillation method. The objective of this study is to use a single and multi-factor experiment to investigate the influence of the primary factors on the extraction of essential oil components from Artemisia frigida Willd.

MATERIALS AND METHODS

**Plant materials**: Aerial parts of Artemisia frigida Willd were collected from the Naiman province of Inner Mongolia, China in August 2018. (The plant was identified by Prof. Bukhbaatar of the Inner Mongolia University for Nationalities)

**Extraction method**: The essential oil yield of Artemisia frigida (500 g) was obtained by hydro-distillation with a Clevenger type apparatus.

**Single factor experiment**: The effects of particle size, soaking time, liquid to plant material ratio, and extraction time on essential oil yield were investigated through a single factor experiment.

**Multi-factors experiment (orthogonal test design)**: Based on the single-factor experiment, orthogonal tests were designed to include three factors and three different levels to optimize the essential oil yield of Artemisia frigida [16]. The orthogonal test L9(3)^4 parameters are shown in Table 1.

**Statistical analysis**: Conducted the range analysis and analysis of variance (ANOVA) with a significance level set at P<0.05.

RESULTS AND DISCUSSION

**Results of single-factor experiment**

In the single-factor experiment, the following four factors, particle size, extraction time, soaking time, and liquid to plant material ratio, were studied. The results of the experiments are shown in Figures 1-3.

**Effect of particle size of the plant material and the extraction time**

In the first experiment, the influence of the varying particle size on the yield of the essential oil extraction process was studied. The plant materials were passed through three different standard size sieves (numbers 10, 40, and 60) of the following sizes: >2.0, >0.825, and >0.425 mm (figure 1). The results revealed that the extraction time and particle size of plant materials influenced the yield of essential oil. The essential oil yield increased significantly by roughly 0.24-0.42% when extraction time was within the range of 4-6 h, and the particle size>0.825 mm, in other words, the yield of essential oil was at its highest. Therefore, the identified particle size of plant material is considered for further study.
Effect of soaking time

The effect of soaking time was studied with a particle size of >0.825 mm, at 10:1 liquid to plant material ratio, and with soaking time ranging from 1 to 6 hours, extracting for 6 hours. Based on the results, it has been found that the essential oil yield (0.31-0.33%) is higher when the soaking time is longer. However, beyond 4 hours of soaking time, its effect is insignificant, and the yield of essential oil is roughly the same (figure 2). Therefore, the optimal soaking time was determined as 2 hours.

Effect of liquid to plant material ratio

This study experimented with the essential oil yield in 6 h of extraction time, soaking the plant material for 2 h and extracting with above >0.825 mm size, using three different liquid to plant material ratios (8:1, 10:1, and 12:1). The lower the ratio of liquid to the plant material ratio, the lower was the yield. Figure 3 illustrates the effect of liquid to plant material ratio on the yield of essential oil (0.33-0.37%), and the yield is higher when the liquid to plant material ratio is higher. As illustrated in Figure 3, most of the essential oil was extracted at the liquid to plant material ratio of 12:1 compared to the yield distilled with two other liquid to plant material ratios.
Result of multi factor experiment

In multi-factor experiments, the effects of the following four factors were studied - soaking time - A, liquid to plant material ratio - B, extraction time - C, and blank - D, where every factor has three levels of optimization. Table 1 summarizes the results of these experiments. The selected factors were assessed by using an orthogonal L9(3^4) test design, and nine extraction experiments were performed. Table 2 contains the schedule and results of the orthogonal test.

![Graph showing the effect of liquid to plant material ratio on yield of essential oil](image)

Figure 3. Effect of liquid to plant material ratio on yield of essential oil

Table 1. Factors and levels of orthogonal test

| Levels | A, soaking time (h) | B, ratio of liquid to plant material | C, Extraction time (h) |
|--------|---------------------|------------------------------------|-----------------------|
| 1      | 2                   | 8:1                                | 4                     |
| 2      | 4                   | 10:1                               | 6                     |
| 3      | 6                   | 12:1                               | 8                     |

Table 2. Analysis of 9(3^4) orthogonal test results

| №     | A, soaking time (h) | B, ratio of solvent to raw material | C, extraction time (h) | D, blank factor | Essential oil yield (%) |
|-------|---------------------|------------------------------------|-----------------------|-----------------|-------------------------|
| 1     | 1                   | 1                                  | 1                     | 1               | 0.35                    |
| 2     | 1                   | 2                                  | 2                     | 2               | 0.405                   |
| 3     | 1                   | 3                                  | 3                     | 3               | 0.424                   |
| 4     | 2                   | 1                                  | 2                     | 3               | 0.35                    |
| 5     | 2                   | 2                                  | 3                     | 1               | 0.368                   |
| 6     | 2                   | 3                                  | 1                     | 2               | 0.295                   |
| 7     | 3                   | 1                                  | 3                     | 2               | 0.35                    |
| 8     | 3                   | 2                                  | 1                     | 3               | 0.313                   |
| 9     | 3                   | 3                                  | 2                     | 1               | 0.368                   |
| K1    | 0.393               | 0.35                               | 0.319                 | 0.362           |                         |
| K2    | 0.338               | 0.362                              | 0.374                 | 0.35            |                         |
| K3    | 0.344               | 0.362                              | 0.381                 | 0.362           |                         |
| R     | 0.055               | 0.012                              | 0.062                 | 0.012           |                         |

Table 3. Results of variance analysis

| Factor | SS    | Df | MS   | F     | p     |
|--------|-------|----|------|-------|-------|
| A      | 0.006 | 2  | 0.003| 18.25 | >0.05 |
| B      | 0     | 2  | 0    | 1     | >0.05 |
| C      | 0.007 | 2  | 0.003| 22.75 | <0.05 |
Through intuitive and variance analysis of the orthogonal experiments of the various factors of the extraction condition, it can be concluded that the extraction time has the highest impact, and the soaking time has a greater weight compared to the liquid to plant material ratio (extraction time > soaking time > liquid to plant material ratio). As shown in the analysis of variance (Table 3), extraction time has statistically significant influences. The optimal process combination for extraction of essential oil is $A_1 B_2 C_3$, which means 2 h of soaking time, liquid to solid ratio of 10:1, and 8 h of extraction time.

The quality and yield of bioactive compounds from plant material are influenced by the geographic location, harvesting season, the choice and stage of drying conditions, and extraction method [17]. In this experiment, we studied only one assortment of plant material - *Artemisia frigida* plant and determined the optimum conditions for essential oil component extraction of aerial parts of *Artemisia frigida*; focusing on particle size, soaking time, liquid to plant material ratio, and extraction time by using the hydro-distillation method. The particle size of herbal material has the most significant effect on the extraction process. When the particle size is smaller, it has a considerably larger surface area and increased the contact area with the solvent [18]. On the other hand, finer particle sizes are more prone to agglomeration, which could hinder the extraction process. Thus, the most effective particle size for extraction should be able to maximize the surface area for mass transfer, yet prevent agglomeration. This study investigated the effect of particle size ranging from 0.425 mm to 2.0 mm on yield of essential oils of *Artemisia frigida* (Figure 1). The plant material crushed to a particle size of 0.425 mm, which had the lowest extraction efficiency, while the highest extraction efficiency was at the particle size of 0.825 mm. Also, it assessed the yield of three different liquids to the plant material ratio and soaking time. The yield of essential oil increases with the increment of liquid to plant material ratio (figure 2) and soaking time (figure 3); however, based on variance analysis of multi-factor examination (Table 3), this ratio did not demonstrate a significant impact.

CONCLUSIONS

The optimum extraction parameters for extraction of essential oils from the aerial parts of *Artemisia frigida* Willd were as follows: soaking time - 2 h, liquid to solid ratio 10:1, extraction time - 8 h.

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