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Evaluation of extraction methods for essential oils in mugwort (*Artemisia montana*) using gas chromatography-mass spectrometry

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Abstract: Mugwort (Artemisia montana), which is a perennial plant mainly distributed throughout Northeast Asian regions, has been used as a preferred source of various foods and traditional medicines in Korea. In particular, as essential oils extracted from mugwort were reported to be biologically active, its steam distillate has been widely used to treat various conditions, such as itching, hemorrhoids, and gynecological inflammation. Therefore, efforts have been devoted to develop effective methods for the collection of bioactive essential oils from mugwort. In this study, five mugwort extracts were obtained using different extraction conditions, namely, 6 % ethanol at room temperature and at 80 °C, pure ethanol, n-hexane, and an adsorbent resin. To evaluate the five extracts of mugwort, area-under-the-curve values (AUCs), chemical profiles, and major bioactive essential oil contents were investigated using gas chromatography-mass spectrometry (GC-MS). An overall assessment of the volatile components, including essential oils, in the five extracts was conducted using AUCs, and the individual essential oil in each extract was identified. Furthermore, the four major essential oils (1,8-cineole, camphor, borneol, and α-terpineol), which are known to possess anti-microbial and anti-inflammatory activities, were quantified using authentic chemical standards. Based on the evaluation results, pure ethanol was the best extractant out of the five used in this study. This study provides evaluation results for the five different mugwort extracts and would be helpful for developing extraction methods to efficiently collect the bioactive oil components for medical purposes using chemical profiles of the extracts.

Key words: mugwort, *Artemisia montana*, essential oils, extraction method, chemical profile, gas chromatography, mass spectrometry

1. Introduction

Mugwort (*Artemisia montana*), known to exist more than 200 variants, is a perennial in the family Asteraceae and its stem and leaves are used in various traditional cuisine and medicine in Korea.[1] Moreover, it was known that mugwort leaves have anti-inflammatory effects and they have been widely

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used to ameliorate stomachache, chronic hepatitis, indigestion, and diarrhea.[1,2] In mugwort, essential oils consist of various volatile organic chemicals such as benzaldehyde, 1,8-cineol, camphor, coumarin, and farnesol, which can provide characteristic flavor and color.[3] Of essential oils from mugwort, bioactive compounds such as 1,8-cineole, borneol, camphor, and α-terpineol could provide anti-microbial and anti-inflammatory effects.[4,5] In particular, it was reported that α-terpineol can significantly reduce viability of *Gardnerella vaginalis* and *Candida albicans*, compared to other essential oils.[6] Therefore, bioactive chemicals in essential oils obtained from mugwort could be used as potential drugs or pharmacophores for anti-microbes and anti-inflammation.

It is important to obtain useful essential oils in mugwort for further research and development of anti-inflammatory and anti-microbial agents. However, since chemical compositions and contents of essential oils are varied with methods and conditions for extraction and storage,[7,8] an appropriate extraction method should be employed to attain essential oils with high bioactive compound content. To extract essential oils in mugwort, various advanced extraction methods such as hydrodistillation,[9,10] steam distillation,[11] Soxhlet extraction,[12] microwave extraction,[10] and supercritical fluid extraction[13] have been developed. Although the extracts obtained by these extraction methods included major essential oil components such as 1,8-cineole, borneol, and camphor, they demanded additional apparatus and intricate procedure compared to conventional solvent extraction.

In this study, five extraction methods were employed to efficiently extract major essential oil components in mugwort based on conventional extraction conditions (such as 6 % ethanol at room temperature and 80 °C, pure ethanol, n-hexane, and an adsorbent resin). Since an individual chemical profile for extracts could present a characteristic chemical content, chemical profiles have been widely utilized to evaluate not only efficiency of extraction methods but also potency and safety of herbal medicine.[14-17] Therefore, the extraction protocols for essential oils in mugwort were evaluated with chemical profiles using gas chromatography-mass spectrometry (GC-MS). Furthermore, major essential oil components such as 1,8-cineole, camphor, borneol, and α-terpineol in mugwort were quantified using their authentic chemical standards. This study provides a chemical evaluation method based on chemical profiles and quantified major components of essential oils in mugwort using GC-MS and would be helpful to choose an efficient extraction method to obtain essential oils with high bioactive compound content for potential dietary supplements and pharmaceutical ingredients.

2. Experiments

2.1. Chemicals and materials

To identify and quantify major essential oils, four authentic chemical standards (such as 1,8-cineole, camphor, borneol, and α-terpineol) were utilized. Borneol, α-terpineol, phenanthrene-d_{10}, and Amberlite® XAD7HP were obtained from Sigma-Aldrich (St. Louis, MO, USA). Camphor and 1,8-cineole were purchased from TCI chemical industry (Tokyo, Japan). Methanol (≥ 99.5 %), ethanol (≥ 99.5 %), ethyl acetate (≥ 99.5 %), and n-hexane (≥ 95.0 %) were purchased from J.T. Baker (Phillipsburg, NJ, USA) and deionized water (DW) was purified using a Puris-Evo CB Water System (Mirae ST, Gyeonggi-do, Korea).

2.2. Preparation of reference standards

Individual essential oil standard was dissolved in methanol at individual concentration level as follows: borneol, 1050 μg/mL; camphor, 1010 μg/mL; 1,8-cineole, 920 μg/mL. Deuterated internal standard solution (phenanthrene-d_{10}) was prepared in methanol at 5 μg/mL. All stock solutions were stored in an amber vial. Stock solutions of all essential oils were successively diluted with methanol to prepare working solutions. Stock and working solutions were stored at -20 °C.

2.3. Sample preparation

In this study, the following extraction methods to afford the five mugwort (*A. montana*) extracts (Extract 1 to 5) were applied, and the methodologies were

evaluated by comparison of their essential oil compositions.

Extract 1: aerial parts of mugwort (date of sample collection: June 28, 2020) were weighed using an analytical balance (Mettler Toledo, Greifensee, Switzerland) and 2 g of the sample was transferred into 100 mL beaker with 50 mL of 6 % (*v/v*) ethanol in DW. After 18 hours of extraction at room temperature, the resulting solution was filtered to remove undissolved materials.

Extract 2: the extraction method for Extract 2 was identical as that of Extract 1, except for the elevation of the temperature to 80 °C and the decrease of the extraction time to 3 hours.

Extract 3 and 4: application of the same extraction method as Extract 1, except for the replacement of the extraction solvent with pure ethanol and *n*-hexane, provided Extract 3 and 4, respectively.

Extract 5: dried Amberlite® XAD7HP (1.0 g) was added to 50 mL of Extract 4 in a 100 mL Erlenmeyer flask. The flask was sealed and shaken for 6 hours at 25 °C at 115 rpm to adsorb the organic materials. The resulting resin was collected after filtration through cheesecloth, extracted with 50 mL of ethanol. The mixture was shaken for 18 hours at 25 °C at 115 rpm, and the resulting solution was filtered to remove the resin.

All extracts were stored at -20 °C before analysis. For GC-MS analysis, 10 μL of individual extract was transferred into 2 mL vial spiked with 10 μL of internal standard solution (5 μg/mL) and 80 μL of methanol. Afterward, 1 μL of resulting solution was injected into the GC-MS. The overall analytical procedures were described in *Fig.* 1.

2.4. GC-MS analysis

A GC-MS analysis was carried out using an Agilent 5975C mass selective detector (Palo Alto, CA, USA) connected to an Agilent 6890N gas chromatograph equipped with an DB-5MS capillary column (30 m length, 0.25 mm inner diameter, 0.25 μm film thickness, J&W Scientific, Folsom, CA, USA). The initial oven temperature was set to 50 °C and held for 5 min, increased to 200 °C at a rate of 8 °C/min and held for 5 min, increased to 320 °C at a rate of 30 °C/min and held for 4 min. The flow rate of helium (as carrier gas) was at 1 mL/min. The injection port was set at 280 °C in split mode (5:1) and injection volume was 1 μL. The optimized conditions for quadrupole mass spectrometer were as follows: ionization method, electron ionization (EI); ionization energy, 70 eV; mass scan range in scan mode, *m/z* 50 – 550; ion source temperature, 230 °C, and transfer line temperature, 280 °C.

3. Results and Discussion

3.1. Comparison of extraction methods

To extract and collect major essential oil components from mugwort, five extraction methods were employed as described in 2.3. Sample preparation. The extraction methods 1 and 2 were performed using 6 % ethanol in DW (*v/v*) at room temperature and 80 °C, respectively. Since 6 % ethanol in DW (*v/v*) could effectively inhibit bacterial activity and lead bactericidal activity,[18] it has been conventionally used to produce plant extracts. To improve extraction efficiency of volatile and hydrophobic essential oils, the extraction methods 3 and 4 were performed using ethanol and n-hexane at room temperature, respectively. The extraction method 5 was utilized a nonionic macroreticular resin, which could adsorb and desorb essential oils.[19,20] The overall extraction methods were depicted in *Fig*. 1.

To evaluate five extraction methods, a GC-MS analysis was performed for individual extract. All total ion chromatograms (TICs) of volatile components were obtained from mugwort extracts and shown in *Fig.* 2. Individual area under the curve (AUC) of TIC could reflect amount of mugwort components extracted by individual extraction process, although it is difficult to identify every peak on TICs. Therefore, individual AUC was investigated to compare overall extraction efficiency between extraction methods. Investigated AUCs were relatively calculated based on the AUC of TIC for extraction method 1. As shown in *Fig.* 2, when AUC for extraction method 1 was set at 1.00, AUCs for extraction methods 2, 3, 4, and 5 were presented as 0.97, 8.43, 0.60, and 0.40,

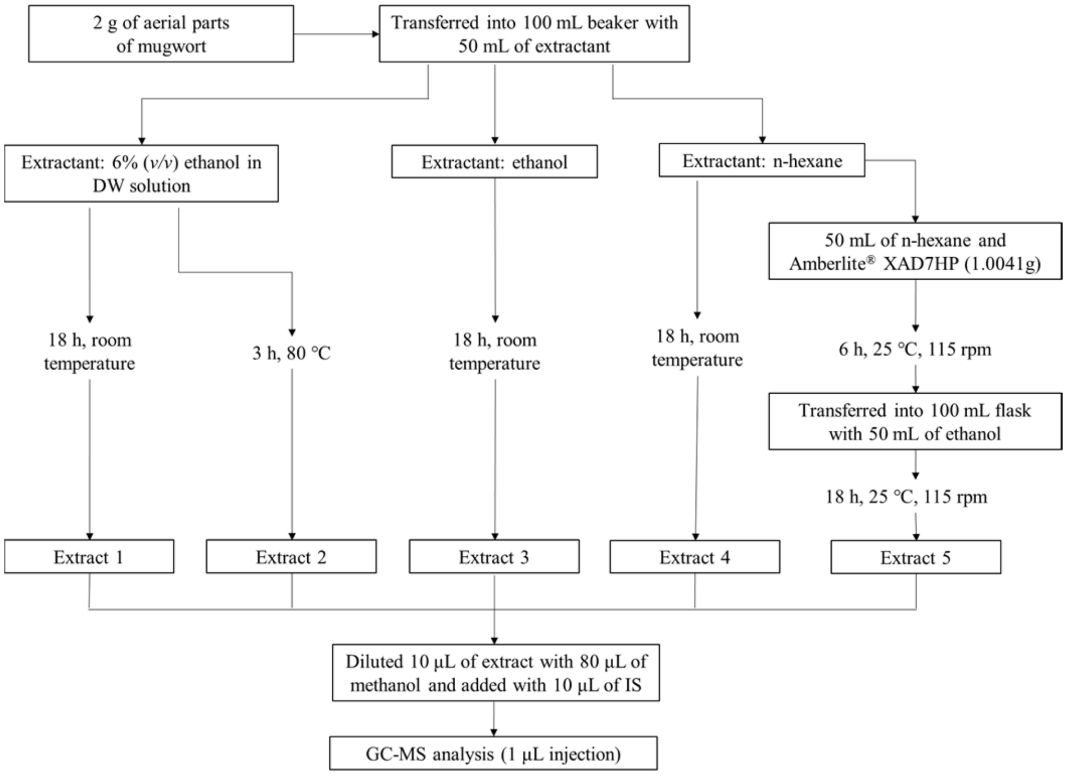

Fig. 1. Essential oil extraction procedures of mugwort.

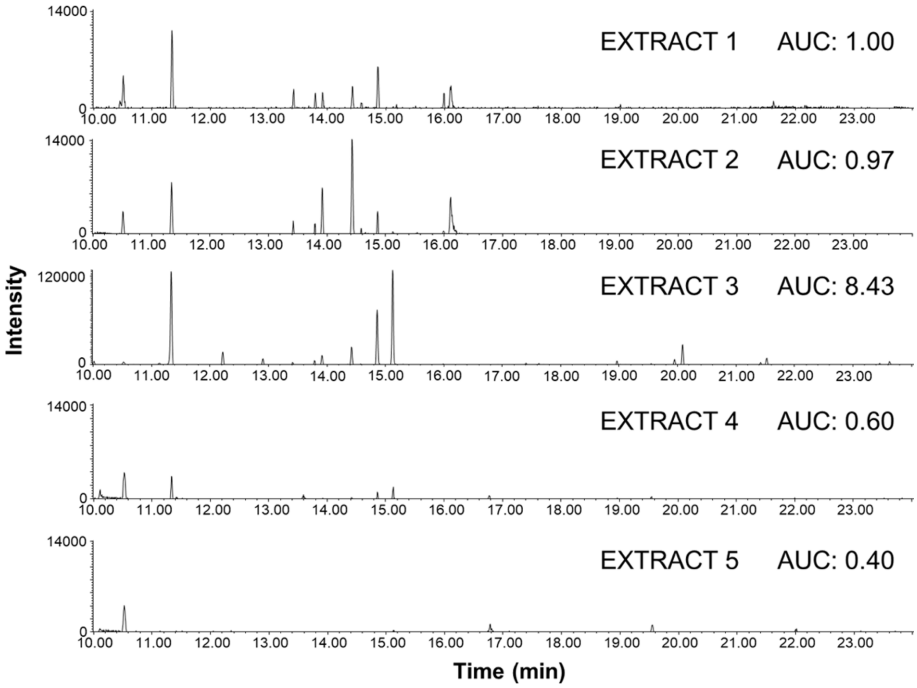

Fig. 2. Total ion chromatograms and relative area under the curves for five extraction methods.

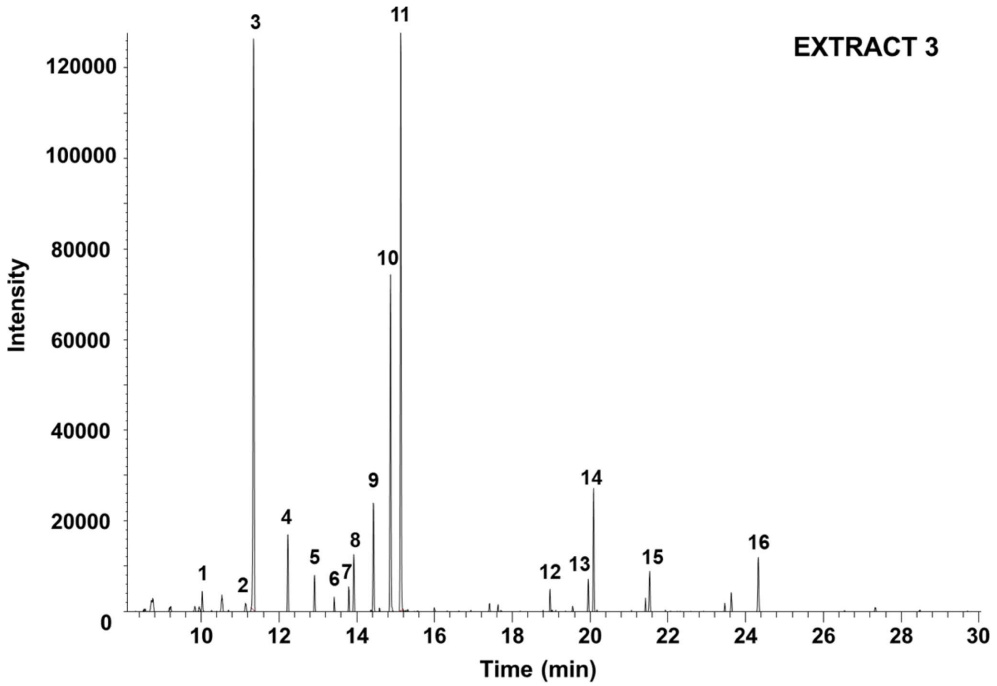

Fig. 3. Peak identities on total ion chromatograms for extraction method 3 (Peak identities are followed as: 1, 1-octen-3-ol; 2, *o,p*-cymene; 3, 1,8-cineole; 4, trans-sabinene hydrate; 5, trans-β-ocimene; 6, cis-sabinene hydrate; 7, allo-ocimene; 8, camphor; 9, borneol; 10, α-terpineol; 11, piperitol; 12, myrtenol; 13, trans-caryophyllene; 14, allo-aromadendrene; 15, caryophyllene; 16, phenanthrene-d_{10}).

respectively. According to the results, the extraction method 3 using ethanol as an extractant was shown to be most efficient extraction method to extract and collect volatile components in mugwort. On the other hand, although it is known that Amberlite could adsorb and desorb essential oils, volatile components in mugwort could not extract and collect using Amberlite.

3.2. Quantification of major essential oil components in mugwort (A. montana) extracts

Based on EI-mass spectral patterns and NIST database, abundant peaks on TIC for the extraction method 3 were identified as shown in *Fig.* 3. Fourteen volatile compounds were unveiled and mostly comprised of essential oils. Relative abundance of essential oil components in individual mugwort extracts were investigated. The identified peaks were summarized in *Table* 1.

As shown in *Table* 1, four essential compounds including 1,8-cineole, camphor, borneol, and α-terpineol were commonly found in extracts 1 – 4, except for extract 5. These four essential oil components are well known that they have anti-microbial and anti-inflammatory effects.[9,10] In particular, it was reported that α-terpineol could reduce viable *Candida albicans* and *Gardnerella vaginalis* counts, which induce bacterial vaginosis and vulvovaginal candidiasis.[7] Therefore, these four major essential oils were quantified to determine an extraction method to effectively extract and collect major essential oils, which are most potent to microbes and inflammation.

To quantify four major essential oils in mugwort extracts, their authentic chemical standards were used to establish calibration equations. All investigated calibration curves for major essential oil components were linear over each dynamic ranges. The limits of detection (LODs) and quantification (LOQs) were defined as the concentrations over signal-to-noise ratios at 3 and 10, respectively. Moreover, the LOD and LOQ ranges were within 0.061 – 0.236 and

Table 1. Representative volatile components in mugwort extracts (n = 3)

No.	Analytes	Retention time (min)	Relative abundance (%)				
			Extract 1	Extract 2	Extract 3	Extract 4	Extract 5
1	1-Octen-3-ol	10.02	-	-	0.7±0.1	-	-
2	*p*-Cymene, o-cymene	11.13	-	-	0.6±0.1	-	-
3	1,8-Cineole	11.34	43.2±0.5	11.0±3.1	33.2±0.9	62.3±2.0	-
4	Trans-sabinene hydrate	12.22	-	-	3.3±0.1	-	-
5	Trans-β-ocimene	12.91	-	-	1.3±0.1	-	-
6	Cis-sabinene hydrate	13.42	4.6±1.4	2.5±0.9	0.2±0.1	-	-
7	Allo-ocimene	13.79	4.9±0.7	2.5±0.4	0.6±0.1	-	-
8	Camphor	13.92	5.1±0.9	18.5±0.4	2.4±0.1	2.2±1.4	-
9	Borneol	14.42	12.0±0.3	58.2±4.9	4.7±0.1	5.1±1.4	-
10	α-Terpineol	14.86	30.3±2.7	7.3±0.8	16.5±0.2	5.8±2.3	-
11	Piperitol	15.13	-	-	28.8±0.4	24.5±3.5	-
12	Myrtenol	18.96	-	-	0.6±0.1	-	-
13	Trans-caryophyllene	19.95	-	-	1.0±0.1	-	-
14	Allo-aromadendrene	20.08	-	-	4.8±0.3	-	-
15	Caryophyllene	21.53	-	-	1.4±0.2	-	-
16	Phenanthrene-d_{10}	24.32	-	-	-	-	-

Table 2. Quantification results of major four essential oils in mugwort extracts (n = 3)

No.	Analytes	LOD (μg/mL)	LOQ (μg/mL)	Calibration equation	Dynamic range (μg/mL)	r^2	Contents (μg/mL)				
							Extract 1	Extract 2	Extract 3	Extract 4	Extract 5
1	1,8-Cineole	0.236	0.707	y = 0.3677x – 1.0363	1.840 – 92.0	0.9987	5.79±0.06	3.58±0.07	56.97±3.12	3.40±0.05	< LOQ
2	Camphor	0.122	0.365	y = 0.3036x – 1.041	1.010 – 101.0	0.9961	3.78±0.02	5.50±0.04	7.89±0.11	3.46±0.01	< LOQ
3	Borneol	0.061	0.182	y = 0.2809x – 1.1135	1.050 – 105.0	0.9954	5.07±0.01	11.71±0.14	13.48±0.29	4.04±0.01	< LOQ
4	α-Terpineol	0.176	0.529	y = 0.3672x – 1.7849	2.096 – 104.8	0.9955	7.13±0.08	5.48±0.04	31.35±1.28	4.90±0.01	< LOQ

0.182 – 0.707, respectively. All experiments were employed in triplicate. Major essential oils in extracts 1 – 5 were quantified and summarized in *Table* 2. Similarly with AUC results, extract 3 collected using ethanol contained the highest content of four major essential oils. Therefore, in this study, it was shown that the extraction method 3 using ethanol as an extractant were the most efficient method to extract and collect major essential oils in mugwort.

4. Conclusions

In this study, five extraction methods to extract and collect essential oils in mugwort were compared and evaluated by GC-MS. To evaluate extraction efficiencies of volatile components in mugwort, individual AUC of TICs for extraction methods were investigated. Furthermore, to determine the most efficient extraction method, four major essential oil components (1,8-cineole, camphor, borneol, and α-terpineol), which have potent anti-microbial and anti-inflammatory effects, were quantified using their authentic chemical standards. According to the results, the extraction method 3 using ethanol could provide effective extraction and collection of four major essential oils in mugwort. This study provides a useful method to effectively extract and collect essential oils from mugwort and would be helpful to evaluate extraction methods for major herbal components using chemical profiling of herbal extracts.

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