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Optimization of essential oil extraction from *Satureja sahendica* Bornm.

Jalal Khorshidi [1,2*], Seyed Hemn Amanollahi [3], Pakhshan Bayazidi Azar [3]

Assistant Professor, Department of Horticultural Science and Engineering, Faculty of Agriculture, University of Kurdistan, Sanandaj, Iran

Research Center of Medicinal Plants Breeding and Development, Faculty of Agriculture, University of Kurdistan, Sanandaj, Iran

M.Sc. student, Department of Horticultural Science and Engineering, Faculty of Agriculture, University of Kurdistan, Sanandaj, Iran

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*Corresponding Author E-Mail:
j.khorshidi@uok.ac.ir

ABSTRACT

The quantity and quality of essential oil extracted from the plant is strongly influenced by the extraction conditions, so optimizing the extraction conditions can help us to achieve the most essential oil content with the best quality. *Satureja sahendica* Bornm. is an endemic medicinal plant of Iran, which is of great importance due to its high essential oil and thymol content. In this research, the effect of plant particle size (mesh sizes of 12, 18 and 35) and the ratio of plant material to water (1:5, 1:10 and 1:15 w/v) on essential oil content and compositions of this plant were evaluated. The essential oils were extracted based on hydro-distillation method by Clevenger's apparatus and then analyzed using GC/MS and GC/FID. The results indicated that the extracted essential oil decreased with the decrease in the plant particle size. The optimal ratio of plant material to water in order to obtain more essential oil was different depending on the plant particle size. The highest of essential oil content (2.15% v/w) was obtained from plant material with mesh size of 12 in the ratio of 1:15 plant material to water. The number, type and amount of essential oil compounds were strongly affected by different treatments. The highest amount of thymol (81.79%) as the most important component of the *Satureja sahendica* essential oil was obtained from samples with mesh size of 35 and in the ratio of 1:10 plant material to water. Determining the most appropriate extraction conditions can be different depending on the purpose and desired chemical composition of the essential oil.

1. Introduction

Essential oils or volatile oils are compounds that are produced in different secretory structures of plants and have antifungal, antiviral, antibacterial, anticancer, anti-mutation, anti-diabetic, anti-inflammatory and antioxidant properties, and due to the mentioned properties have various applications in the pharmaceutical, food, cosmetic and health industries [1]. Essential oils are extracted from plants by different methods. Water distillation is the most common method for essential oil extraction [2]. Various factors such as the plant size, the ratio of plant material to water, the duration of extraction and the temperature during extraction, are involved in the quantity and quality of the essential oil extracted from the plant in the distillation method [3]. During the distillation process, the plant material is constantly in contact with water, and therefore processes such as hydrolysis, polymerization, and dissolution of compounds in water can affect the quantity and quality of extracted essential oil [4]. The change in plant size changes the contact surface of plant materials with water, which can affect the rate of absorption and desorption of water by plant tissues and the release of effective compounds from the plant [3]. The meability of plant tissues as well as the type and position of the secretory structures of plants are different from each other, and this causes the amount and speed of the release of effective compounds during the distillation process to be different depending on the plant species [5]. Therefore, it is necessary to optimize the extraction conditions to achieve the highest quantity and quality of effective compounds for any plant species. Many studies have been conducted in this field, and the results of all of them confirm the influence of extraction conditions on the quantity and quality of extracted active ingredients [3, 4, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15]. According to the importance of the mentioned topic, in this research, the effect of plant material to water ratio and also plant particle size on the amount and composition of *Satureja sahendica* Bornm. essential oil was investigated. *S. sahendica* is a perennial plant species from the Lamiaceae family and endemic to Iran, which is distributed in the northwest and west of Iran [16]. The essential oil content of *S. sahendica* is about 1-3% and the most important components of its essential oil are thymol, *p*-cymene and *γ*-terpinene [17, 18]. *S. sahendica* has antimicrobial and antioxidant properties [19] and like other savory species, it is used in the treatment of infectious diseases, muscle pain, indigestion, diarrhea and blood lipids [20, 21]. Also, this plant is used as a spice in the food industry [16].

2. Materials and methods

2.1. Experimental treatments

In this research, the effect of plant particle size in three mesh sizes: 35 (diameter 0.5 mm), 18 (diameter 1 mm) and 12 (diameter 1.6 mm), as well as the ratio of plant material to water (w/v) in three levels: 1:5 (10 g of plant material in 50 ml of water), 1:10 (10 g of plant material in 100 ml of water) and 1:15 (10 g of plant material in 150 ml of water) were investigated on the essential oil content and composition of *S. sahendica*.

2.2. Plant sample preparation

S. sahendica used in this research was collected in June 2021 from its natural habitat located in Boukan city with latitude 36°47'59" N, longitude 46°06'39" E, and altitude 1371 m. Plant samples were dried at room temperature for one week. Then, the dried plant materials were crushed by a grinder and passed through sieves with the mentioned mesh sizes to prepare for essential oil extraction.

2.3. Essential oil extraction

The essential oil was extracted based on the water distillation method using a Clevenger apparatus. For this purpose, about 10 g of plant sample was weighed and poured into the balloon of the device, and then water (50, 100 and 150 ml in the ratios of 1:5, 1:10 and 1:15 plant material to water) was added to it. The essential oil extraction process continued for 2.5 hours and then the volume of isolated essential oil was measured and finally, its yield (% v/w) was calculated. The extracted essential oils were poured into glass vials and stored at 4 °C. It should be noted that anhydrous sodium sulfate was used to remove water from essential oils.

2.4. Essential oil analysis

GC/MS and GC/FID were used for quantitative and qualitative analysis of essential oils. The specifications and working conditions of the mentioned devices were as follows:

GC/MS: Agilent 7890B/5977A GC/MSD (HP5 column with 30 m length, 0.25 mm inner diameter, and 0.25 μm film thickness) was used to identify essential oil components. The temperature of the injection chamber was set at 280 °C. The initial temperature of the column was set at 50 °C for 1 min and then raised to 260 °C at a rate of 10 °C/min. The ionization source temperature and ionization energy were 230 °C and 70 eV, respectively. The spectral scanning range was 50–550 m/z. Helium was used as a carrier gas at a pressure of 34 psi with a flow rate of 1 ml/min.

GC/FID: GC analysis was performed using an Agilent 7890B GC equipped with a flame ionization detector (FID). The column specifications as well as the thermal program of the column were similar to GC/MS. The temperature of the injection chamber and detector were 280 and 290 °C, respectively. Similar to GC/MS, helium with a flow rate of 1 ml/min was used as a carrier gas.

The essential oil compounds were identified by comparing their calculated retention indices with the NIST and Wily databases. The quantity of compounds was obtained by calculating the area under the peak obtained for each compound in the GC/FID chromatogram.

3. Results and discussion

3.1. Essential oil content

In the ratios of 1:5 and 1:10 plant material to water, no significant difference was observed between the essential oil content of plant material with mesh sizes of 12 and 18, but with the reduction of plant particle size (mesh size of 35), the yield of essential oil increased significantly (1.55%) (Fig 1). In the ratio of 1:15 plant material to water, the highest essential oil content (2.15%) was obtained from the plant materials with the largest size (mesh size of 12), and between the other two mesh sizes, no significant difference was observed in terms of essential oil content.

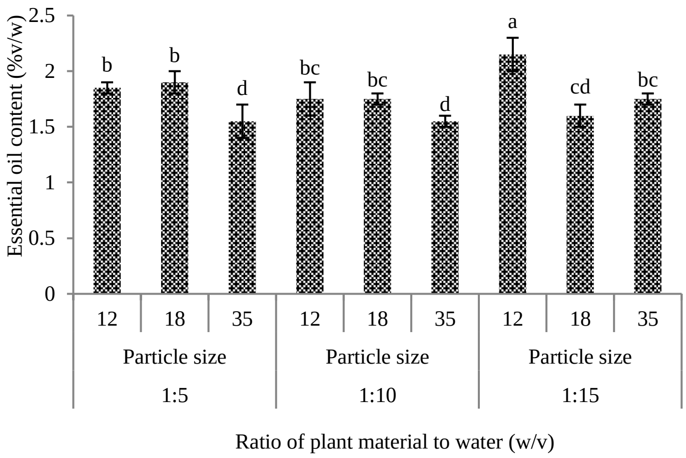

Fig 1 Essential oil content of *Satureja sahendica* Bornm. affected by plant particle size and ratio of plant material to water

In previous studies, the essential oil content of *S. sahendica* was reported as 1.45% [22], 1.09-2.02 [18], 0.95-2.2% [16] and 0.42% [23]. These differences in essential oil content can be due to differences in habitat conditions, farm management, harvesting time, drying method, extraction method and extraction time. In different plant species, essential oils are stored in different secretory structures, so the speed of essential oil release during the distillation process is not the same in all plants and even different tissues belonging to the same plant species. Therefore, in order to extract the highest amount of essential oil, the extraction conditions should be optimized for each plant species as well as each plant tissue. In some plant organs such as leaves and petals, secretory structures are often superficial, which are easily ruptured during the distillation process and their essential oils come out, and therefore they do not need to be crushed too much. But organs such as roots, stems, rhizomes, and fruits have a more rigid texture and their secretory structures are often internal, and as a result, extracting their essential oils is difficult, so they must be crushed into smaller pieces [5, 24]. A possible explanation for more essential oil yield extracted from larger plant materials is that larger plant materials are deposited at the bottom of the container due to their greater weight and therefore are closer to the heat source and their secretory structures are affected by more heat. As a result, they rupture faster and easier and their essential oil comes out. But plant materials with smaller sizes, due to their lower weight, are suspended on the surface of the water and have a greater distance from the heat source, so rupturing their secretory structures requires more time and energy [6]. Another reason that has been reported for the decrease in the essential oil yield along with the decrease in plant particle size is that smaller plant particles have a great tendency to absorb and retain plant compounds, including essential oils, and as a result, the release of these compounds from the plant is reduced [9]. The result of another research has shown that when the size of the plant particles is reduced too much, they stick together and become a mass, so it is difficult to extract the essential oil from them, and finally, the efficiency of the extracted essential oil decreases [10]. Some reports stated that the efficiency of essential oil extraction increases with reducing plant particle size, possibly due to increasing contact surface of plant materials with water, which results in more water absorption by plant material, and finally increase in rupturing of secretory structures and release of essential oil [3, 4, 7, 11, 15]. Some studies have reported the highest efficiency of essential oil extraction with medium-sized plant particles (compared to healthy or powdered samples) [9, 10]. It seems that this contradiction in the results is due to the difference in the plant species and their secretory structure. The ratio of water to plant material during the distillation process should be such that all plant materials are immersed in water so that the essential oil extraction is done well. Also, the volume of water should be enough to easily rupture the cell membranes and extract the essential oil [15]. In low ratios of water to plant material, the plant material is half burned and the extraction efficiency decreases [4]. Another reason for the decrease in the efficiency of essential oil extraction with the increase in the ratio of plant material to water is that for a given weight of plant material, there is less volume of water to penetrate into plant tissues and extract compounds [25]. In the ratio of 1:5 plant material to water, there is 5 ml of water to extract essential oil from 1 g of plant material, but in the ratio of 1:15, there is 15 ml of water for 1 g of plant material, and therefore it is reasonable to extract more essential oil in the ratio of 1:15. On the other hand, it has been reported that in high ratios of water to plant material, the extraction efficiency decreases due to the increase in the dissolution of effective ingredients in water and their hydrolysis [4, 26]. It seems that the optimal ratio of water to plant material to achieve the best results depends on the type of plant material and its effective compounds.

3.2. Essential oil components

The number, type and amount of identified compounds in *S. sahendica* essential oil were different in the various treatments. The lowest number of identified compounds (5), which accounted for 91.48% of the total essential oil, belonged to the mesh size of 35 and the ratio of 1:10 plant material to water. The highest number of identified compounds (22) was found for mesh size of 18 in ratios of 1:10 and 1:15 plant material to water, which represented 97.64 and 96.46% of the total essential oil, respectively. The dominant essential oil components in ratios of 1:5 and 1:10 plant material to water were thymol and *p*-cymene, while in the ratio of 1:15 plant material to water, the dominant compounds were different depending on plant particle size. As in a mesh size of 12, thymol and *α*-terpinene; in a mesh size of 18, thymol and *p*-cymene; and in a mesh size of 35, thymol and carvacrol were identified as the dominant components of essential oil. Thymol was identified as the dominant component of essential oil in all treatments, and its highest amount (81.79%) was obtained from samples with a mesh size of 35 and in the ratio of 1:10 plant material to water. The thymol content decreased slightly with a decrease in the mesh size of plant samples from 12 to 18, and then increased with a further decrease in the mesh size from 18 to 35. In the same mesh size but with different ratios of plant material to water, the changes in thymol content were different, so that in mesh size of 12, with a decrease in the ratio of plant material to water from 1:5 to 1:10, thymol decreased, but with a greater decrease from 1:10 to 1:15, thymol increased. In a mesh size of 18, with a decrease in the ratio of plant material to water, thymol decreased, and in a mesh size of 35, it first increased and then decreased (Table 1).

In previous studies, thymol, *p*-cymene and *γ*-terpinene have been reported as the dominant components of *S. sahendica* essential oil [16, 18, 22]. Thymol, as the dominant component of *S. sahendica* essential oil, is an oxygenated monoterpene, and its high amount indicates a better quality of essential oil. The thymol content can be different depending on genetic and pre- and post-harvesting conditions, so that its amount has been reported between 34.7-45.3% [16], 37.5-46.7% [18], and 30.6-41.3% [22]. In the present study, the thymol content ranged from 52.31-81.79%, which indicates superior genetic and more favorable habitat conditions of the studied population.

Oxygenated monoterpenes were the main class of essential oil compounds in all treatments, and their highest amount belonged to the smallest plant materials (mesh size of 35). On the other hand, the smallest plant materials had the lowest amount of monoterpene hydrocarbons. Also, the lowest total amount of monoterpene compounds (monoterpene hydrocarbons and oxygenated monoterpenes) was detected in the smallest plant materials. The amount of monoterpenes was not significantly affected by the ratio of plant material to water. In the ratio of 1:5 and 1:15 plant material to water, the highest amount of sesquiterpenes was obtained from the smallest plant materials, while in the ratio of 1:10, the highest amount of the mentioned compounds was identified in the medium-sized samples (Table 1).

The higher amount of monoterpenes in larger plant samples can have different reasons. The contact surface of plant materials increases with a decrease in their size, and therefore the release of volatile compounds becomes easier. As a result, during crushing and preparation of samples for essential oil extraction, the evaporation of monoterpene compounds with low molecular weight and high volatility [27] increases with the decrease in the plant particle size, while sesquiterpenes do not evaporate easily, because these compounds are heavier with lower volatility compared to monoterpenes. Also, there is a possibility of more oxidation and hydrolysis of monoterpenes in finer plant material due to more contact surfaces.

The effect of plant particle size and the ratio of water to plant material on the quantity and quality of active ingredients of medicinal plants have been proven in previous studies. In the research conducted on the *Curcuma longa* L., increasing the mesh size increased the amount of extracted curcumin [3]. The extraction rate of sesquiterpenes curcumenone, curcumenol, curdione, curzerenone and furanodienone in *Curcuma wenyujin* has increased with the increase in the ratio of solvent to plant material [6]. A decrease in the amount of anethole extracted from fennel has been reported with an increase in the mesh size [11]. An increase in the extraction rate of phenolic compounds has been observed in *Calendula officinalis* L. along with a decrease in the plant particle size and an increase in the ratio of solvent to plant material [28]. The amount of limonene extracted from *Pimpinella affinis* Ledeb. by water distillation method has shown an increase with increasing mesh size [29].

The reduction or increase in the extraction yield of a specific phytochemical compound from a plant due to the change in the plant particle size and also the ratio of solvent to plant material depends on the chemical nature of the compound. Low or high molecular weight, volatility or non-volatility, low or high boiling point, as well as polarity or non-polarity of medicinal compounds affect their extraction rate.

Table 1 Essential oil components (%) of *Satureja sahendica* Bornm. affected by plant particle size and ratio of plant material to water

Components	R.T. (min)	Ratio of plant material to water (w/v)								
		1:5			1:10			1:15		
		Plant particle size (mesh)			Plant particle size (mesh)			Plant particle size (mesh)		
		12	18	35	12	18	35	12	18	35
α-Thujene	5.95	tr	tr	tr	tr	tr	tr	0.48	tr	tr
α-Pinene	6.12	0.25	0.21	tr	0.31	0.35	tr	tr	0.38	tr
Camphene	6.25	0.65	0.53	0.23	0.68	0.59	tr	tr	0.64	tr
β-Pinene	6.53	0.12	tr	tr	0.14	0.12	tr	tr	0.12	tr
β -Myrcene	7.10	0.15	tr	tr	tr	0.14	tr	tr	0.16	tr
α-Phellandrene	7.45	0.42	0.37	tr	0.57	0.58	tr	tr	0.66	tr
α-Terpinene	7.96	0.15	0.18	tr	0.22	0.24	tr	9.96	0.27	tr
p-Cymene	8.22	21.44	21.0	9.58	28.08	27.21	4.73	tr	30.58	0.98
γ-Terpinene	8.87	0.15	0.23	tr	0.51	0.96	tr	tr	0.87	tr
cis-Sabinene hydrate	9.07	tr	tr	tr	0.17	0.23	tr	tr	0.23	tr
Linalool	9.79	2.63	2.17	1.71	2.19	2.18	2.18	2.26	2.16	2.43
Borneol	11.1	0.38	0.34	0.29	0.32	0.33	tr	tr	0.31	0.32
Terpineol	11.3	1.05	0.92	1.12	0.81	0.78	1.59	0.4	0.73	1.75
p-Cymen-8-ol	11.6	0.73	0.65	0.84	0.62	0.67	tr	0.44	0.35	1.35
Thymol	13.7	61.16	59.16	69.63	53.49	53.22	81.79	75.1	52.31	71.63
Carvacrol	14.0	2.19	5.50	3.99	4.54	4.29	tr	2.01	2.43	7.22
Thymyl acetate	14.8	0.33	0.30	0.31	0.26	0.32	tr	0.37	0.28	0.40
trans-Caryophyllene	16.0	0.45	0.59	1.23	0.50	0.57	1.19	0.41	0.51	0.76
p-Cymen-7-ol	16.3	tr	tr	0.23	0.14	0.14	tr	tr	0.10	tr
Ledene	17.5	tr	tr	0.2	tr	0.11	tr	tr	tr	tr

(+)Spathulenol	18.8	1.14	3.32	4.08	2.75	4.13	tr	3.55	2.77	5.14
Caryophyllene oxide	19.2	3.13	tr	0.2	0.13	0.13	tr	tr	0.13	tr
(-)Spathulenol	20.0	tr	tr	tr	tr	tr	tr	0.27	0.11	tr
cis-Z-*α*-Bisabolene epoxide	20.3	0.33	0.36	0.45	0.31	0.35	tr	0.55	0.36	0.63
Monoterpene hydrocarbons		23.33	22.52	9.81	30.68	30.42	4.73	10.44	33.91	0.98
Oxygenated monoterpenes		68.47	69.04	78.12	62.37	61.93	85.56	80.58	58.67	85.1
Total monoterpens		91.8	91.56	87.93	93.05	92.35	90.29	91.02	92.58	86.08
Sesquiterpene hydrocarbons		0.45	0.59	1.43	0.5	0.68	1.19	0.41	0.51	0.76
Oxygenated sesquiterpenes		4.6	3.68	4.73	3.19	4.61	tr	4.37	3.37	5.77
Total sesquiterpenes		5.05	4.27	6.16	3.69	5.29	1.19	4.78	3.88	6.53
Total identified compounds		96.85	95.83	94.09	96.74	97.64	91.48	95.8	96.46	92.61

R.T.: Retention time

tr: Values less than 0.1%

4. Conclusion

In general, the results of this research showed that the decrease in the plant particle size caused a decrease in the extracted essential oil of *S. sahendica*. The optimal ratio of plant material to water in order to obtain more essential oil was different depending on the plant particle size, so that in very small (mesh size of 35) and very large (mesh size of 12) plant samples, the ratio of 1:15 was the most suitable and in medium-sized plant samples, the ratio of 1:5 gave better results. The number, type and amount of essential oil components were strongly influenced by the applied treatments, and determining the most appropriate treatment can be different depending on the target compound. If thymol is the target compound, the mesh size of 35 and the ratio of 1:10 plant material to water was the best treatment.

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بهینه‌سازی شرایط استخراج اسانس مرزه سهندی (*Satureja sahendica* Bornm.)

جلال خورشیدی[۱و۲]*، سید هیمن امان‌اللهی[۳]، پخشان بایزیدی‌آذر[۳]

۱- استادیار، گروه علوم و مهندسی باغبانی، دانشکده کشاورزی، دانشگاه کردستان، سنندج، ایران

۲- مرکز پژوهشی اصلاح و توسعه گیاهان دارویی، دانشکده کشاورزی، دانشگاه کردستان، سنندج، ایران

۳- دانشجوی کارشناسی ارشد، گروه علوم و مهندسی باغبانی، دانشکده کشاورزی، دانشگاه کردستان، ایران

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* مسئول مکاتبات:

j.khorshidi@uok.ac.ir

چکیده

میزان و کیفیت اسانس استخراج شده از گیاه به‌شدت تحت تأثیر شرایط استخراج قرار دارد و لذا بهینه‌سازی شرایط استخراج می‌تواند ما را در دست‌یابی به بیش‌ترین و با کیفیت‌ترین اسانس کمک نماید. مرزه سهندی (*Satureja sahendica* Bornm.) از گیاهان دارویی اندمیک ایران است که به دلیل میزان بالای اسانس و نیز محتوای تیمول بالا در اسانس، از اهمیت بالایی برخوردار است. در این پژوهش، تأثیر اندازه قطعات گیاهی (سایز مش‌های ۱۲، ۱۸ و ۳۵) و نسبت ماده گیاهی به آب (۱ به ۵، ۱ به ۱۰ و ۱ به ۱۵) بر میزان و ترکیبات تشکیل‌دهنده اسانس گیاه مذکور ارزیابی گردید. استخراج اسانس به روش تقطیر با آب با کلونجر و آنالیز اسانس‌ها با دستگاه‌های GC/MS و GC/FID انجام گرفت. نتایج نشان داد که با کاهش اندازه قطعات گیاهی، میزان اسانس به‌طور معنی‌داری کاهش یافت. مطلوب‌ترین نسبت ماده گیاهی به آب برای حصول اسانس بیش‌تر، بسته به اندازه قطعات گیاهی متفاوت بود. بیش‌ترین میزان اسانس (۲/۱۵ درصد حجمی/وزنی) از مواد گیاهی با سایز مش ۱۲ و نسبت ۱ به ۱۵ مواد گیاهی به آب به‌دست آمد. تعداد، انواع و میزان ترکیبات تشکیل‌دهنده اسانس به‌شدت تحت تأثیر تیمارهای اعمال‌شده قرار گرفتند. بیش‌ترین میزان تیمول (۸۱/۷۹ درصد) به‌عنوان مهم‌ترین ترکیب اسانس مرزه سهندی، از نمونه‌های با سایز مش ۳۵ و نسبت ۱ به ۱۰ ماده گیاهی به آب، به‌دست آمد. انتخاب مناسب‌ترین شرایط استخراج، بسته به هدف و ترکیب شیمیایی مورد نظر اسانس، می‌تواند متفاوت باشد.