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Improving the Efficiency and Antioxidant Activity of Essential Oil Extraction from *Abies sachalinensis* by Underwater Shockwave Pretreatment for the Construction of Low-Energy and Sustainable Essential Oil Extraction System

Hideaki Kawai [1,2], Eisuke Kuraya [3,*], Akiko Touyama [3], Osamu Higa [3], Kazuki Tokeshi [1], Yoshie Tsujikawa [4], Kazuyuki Hokamoto [4] and Shigeru Itoh [5]

[1] Graduate School of Science and Technology, Kumamoto University, 2-39-1 Kurokami, Chuo-ku, Kumamoto 860-8555, Japan
[2] ASAHI GIKEN Inc., 1-17-19 Tokura, Toyonaka, Osaka 561-0845, Japan
[3] Science and Technology Division, National Institute of Technology, Okinawa College, 905 Henoko, Nago 905-2192, Japan
[4] Institute of Industrial Nanomaterials, Kumamoto University, 2-39-1 Kurokami, Chuo-ku, Kumamoto 860-8555, Japan
[5] National Institute of Technology, Okinawa College, 905 Henoko, Nago 905-2192, Japan
* Correspondence: kuraya@okinawa-ct.ac.jp; Tel.: +81-980-55-4043

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Abstract: Essential oils (EOs) from *Abies sachalinensis* (Sakhalin fir), a conifer species found in Sakhalin Island and Hokkaido in Japan, effectively remove nitrogen dioxide and possess antifungal activity. EOs also exert a relaxing effect and enhance air quality. Underwater shock waves generate instantaneous high pressure that ruptures cell walls, enhancing the performance of steam distillation and oil extraction. In this study, we aimed to increase the yield and quality of *A. sachalinensis* extracts using shockwaves. Leaves and branches were subjected to shockwave pretreatment or left untreated before EO extraction by steam distillation. EO yield of untreated dried leaves was 2.4 g/kg of dry leaf weight (DW). Upon application of a 3.0 kV, 3.6 kJ shockwave, the yield increased with the number of shockwave cycles. After ten cycles, yield increased 13.6-fold. Pretreatment with shockwaves for 10 cycles resulted in approximately 6- and 13-fold reductions in total energy consumption relative to fresh and dried leaves, respectively. Antioxidant activity increased more than 30-fold in shockwave-pretreated leaves than in untreated dried leaves after 10 cycles. This novel process can significantly reduce the energy used for EO extraction in steam distillation, thereby contributing to the development of a sustainable, low-energy EO production system.

Keywords: *Abies sachalinensis* essential oil; underwater shockwave pretreatment; essential oil yield; antioxidant activity

1. Introduction

Abies sachalinensis (Sakhalin fir) is a coniferous tree of the Pinaceae family native to Sakhalin Island, the southern Kuril Islands (Russia), and Hokkaido (Japan). Previous phytochemical studies of the *Abies* spp. have identified terpenoids, flavonoids, lignans, and other chemical components [1,2]. Essential oils (EOs) are essentially plant extracts made by steaming different plant parts (flowers, bark, leaves, and fruits) and capturing fragrance-producing compounds. EOs are generally complex mixtures of several hundred compounds. The chemical composition of *A. sachalinensis* EOs has been investigated [3–5], and biological and physiological activities, such as antifungal activity [6], anxiolytic-like properties [7], and antitumor initiating effects [8], have been reported. Moreover, the EO of *A. sachalinensis* has been used to produce deodorants and skin refreshers [4].

There are various pollutants in the environment that we live in. The diseases caused by such pollutants constitute a health concern worldwide; hence, there is an urgent need to develop suitable methods to remove such pollutants. Recent studies suggest that using highly volatile EOs could help reduce atmospheric emissions. In particular, *A. sachalinensis* EOs are reportedly effective in removing nitrogen dioxide [9]. γ-Terpinene, myrcene, and β-phellandrene are known as activity-removing compounds for nitrogen dioxide. Furthermore, regarding the removal mechanism, these substances generate particulate matter with a particle size of 1000 nm or more, detoxifying nitrogen dioxide. Moreover, EOs provide a relaxing effect, and their use is expected to improve overall air quality [1,9]. These functionalities depend on the antioxidant activity of EOs, and efficient extraction of antioxidants in plant materials is important for biological and physiological activities and detoxification of air pollutants.

Owing to their aroma, EOs are primarily used in the food industry as flavors; they are also used as antioxidants, antiradics, and functional antibacterial agents [10]. Although some EOs are extracted from citrus fruits by pressing, steam or hot water distillation are typically used for extraction. Therefore, the extraction of EOs requires a considerable amount of energy, including steam generation and cooling of the generated steam and volatile aromatic substances. Steam distillation coupled with microwaves is an additional option [11,12]; however, this method has disadvantages, such as changes in EO composition due to localized heating of a part of the plant material; moreover, large-scale distillation equipment is cost prohibitive.

Underwater shockwaves generate instantaneous high pressure; when applied to plant material, shockwaves penetrate the whole cell. Generally, plants have air spaces in their tissues [13], such as between and inside cells, with numerous interfaces with different acoustic impedances. When a shockwave penetrates an interface with different impedance, a reflected expansion wave is generated at the intracellular air–space interface, and the cell is peeled off by tensile force owing to spalling, resulting in cellular destruction [14]. Cell disruption caused by air–space interface in the tissue is rapidly disrupted not only by pressure waves exceeding the speed of sound due to shockwaves but also by ultrasonic standing waves. The disruption rate increases parabolically with increasing gas-liquid ratio [15]. In addition, as the impedance of internal plant tissues is uneven, the shockwave repeatedly reflects and propagates, with high pressure of the shockwave acting on low-impedance bubbles and high-impedance plant cell walls, thereby damaging the structural integrity of the cell and generating numerous cracks along cell walls [16]. These cracks function as permeation pathways in extraction processes such as steam distillation and oil extraction [17,18]. This application can increase the extraction yield of EO by subsequent steam distillation. Our recent studies have revealed that the instant high pressure of underwater shockwaves permeates the whole plant cell and selectively destroys the cell wall of plants. When applied to compressive pretreatment of foods such as yuzu (*Citrus junos*) [19–21] and carrots (*Daucus carota* L.), the shockwave process effectively extracts antioxidants such as flavonoids and carotenoids, respectively [22].

Novel extraction processes—such as ultrasound, microwaves, and turbohydrodistillation, which efficiently extract plant EOs without compromising quality and quantity—have been studied [23]. These technologies involve various heating methods and the stimulation of plant materials during extraction but not direct treatment of plant materials. Notably, the technology proposed in this study can be used as a pretreatment not only for traditional steam distillation but also for other extraction techniques currently under development. Notably, the shockwave treatment process exhibits high reliability and excellent performance. Shockwave treatment of *Alpinia zerumbet* leaves on a pilot scale has been shown to improve the yield of EOs and the antioxidant capacity of the aqueous extract obtained by steam distillation [24]. Our previous studies confirm that when *A. zerumbet* leaves are pretreated with shockwaves, crushing results in multiple cracks in the cell wall, which acts as a permeation pathway for water vapor during steam distillation. Furthermore, the antioxidant capacity of the obtained EOs and water extract was improved, suggesting the possibility

of exhibiting physiological activity. The proposed technique has demonstrated excellent performance in improving the extraction of volatile constituents from aromatic plants when used prior to the steam distillation process [25]. The energy required to generate instant high pressure through the shockwave in water was as little as 3.6 kJ/cycle, suggesting that low energy can considerably improve extraction efficiency. Furthermore, the antioxidant ability of the obtained EOs and aqueous extract was enhanced, suggesting that they may exhibit physiological activity. The application of an underwater shockwave pretreatment technology to *A. sachalinensis* leaves might allow the generation of a pathway for water vapor in the tissue by spalling destruction and severing of cell structure by low energy crushing fracture, allowing for more effective extraction of EOs via steam distillation. In addition, the implementation of underwater shockwave treatment as a pretreatment step has been shown to be effective for extracting functional constituents from biomass. In particular, it is expected to significantly improve the antioxidant capacity of EOs, as various volatile components unevenly distributed in the tissues can be efficiently extracted.

In this study, we introduced a novel application of this pretreatment process to improve the efficiency of low-energy extraction of EO from the leaves and branches of *A. sachalinensis*. Moreover, this process has the potential to be applied to a wide range of extractive processes, including those used in herbal medicines. Dynamic control of instantaneous high pressure based on underwater shockwaves could pave the way for new fields of industrial technology, creating a low-energy, sustainable EO production system.

2. Materials and Methods

2.1. Plant Materials

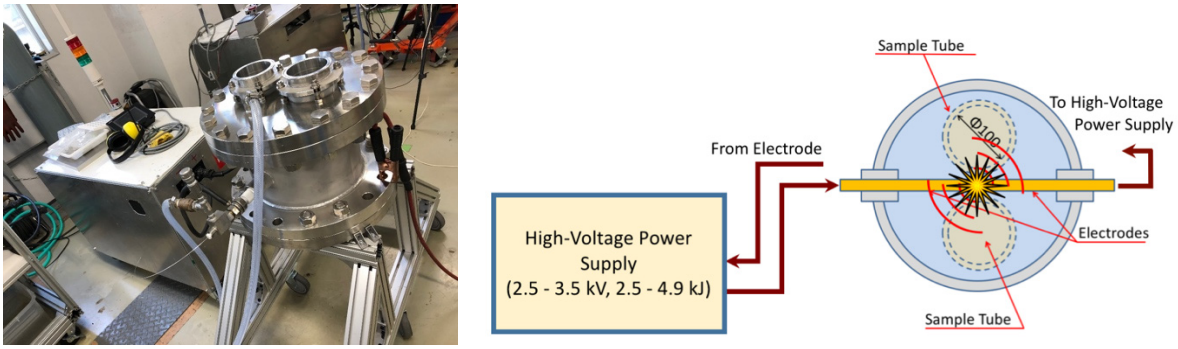
A. sachalinensis leaves and branches were harvested in July 2017 and supplied by Hikobayu LLC, Niseko, Hokkaido. Fresh samples were immediately steam-distilled or pretreated with underwater shockwaves and steam-distilled to obtain an EO. The dried samples were used after oven-drying the leaves and branches of *A. sachalinensis* (40–45 °C) to a moisture content of 10% or lower.

2.2. Chemicals

Analytical grade 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 6-hydroxy-2,5,7,8-tetra methylchroman-2-carboxylic acid (Trolox) were used as standards for the DPPH assay and were obtained from Sigma-Aldrich (St. Louis, MO, USA). Folin–Ciocalteu reagent, gallic acid (GA), *n*-alkanes, and ethanol as analytical-grade reagents, and sodium carbonate, chloric acid, *n*-hexane, tris(hydroxymethyl)aminomethane as special-grade reagents were obtained from Nacalai Tesque (Kyoto, Japan). All solutions were prepared with distilled and deionized water.

2.3. Underwater Shockwave Pretreatment of A. sachalinensis Leaves and Branches

Figure 1 shows the schematic of the pilot scale treatment equipment developed and used in this study for the underwater shockwave (SW) pretreatment of *A. sachalinensis* leaves. The equipment includes a pressure vessel (305.7 mm i.d., 300 mm height; SUS304), high-voltage power supply unit, and high-voltage controller. The high-voltage power supply generates 2.5 to 3.5 kV in an 800 μF capacitor; the underwater shockwaves are generated inside the vessel by a high current electrical discharge on an aluminum wire (1.2 mm diameter; 8 mm length). The leaves and branches were placed in a silicone tube (100 mm i.d., 110 mm o.d., 400 mm length; SR1554, Tigers Polymer Co., Ltd., Osaka, Japan), completely separated from the water and shockwave generation source, and subjected to various cycles of an instantaneous high-pressure load at 3.0 kV and 3.6 kJ.



(**a**) (**b**)

Figure 1. Schematic of the experimental setup: (**a**) overview of high-voltage power supply and vessel and (**b**) top view of the vessel.

2.4. Energy Evaluation of EO Extraction by Steam Distillation of Leaves and Branches of A. sachalinensis

Energy from steam distillation of leaves and branches of *A. sachalinensis* was evaluated based on Figure S1 and calculated using Equations (1)–(3).

$$Q_{EP} = \frac{Consumption\ of\ SW\ energy\ \left(\text{MJ}\cdot\text{kg}^{-1}\text{DW leaf}\right)}{W_{EO}} \quad (1)$$

$$Qi \cong Q_{ESD} = \frac{4.19 \times 75.0 \times W_W + 75.0 \times H_{cpm} \times W_{pm} + 2257 \times W_{SV} + 2257 \times W_{VC} + 4.19 \times 75.0 \times W_{VC}}{W_{EO}} \quad (2)$$

$$Q_{TE}\left(\text{MJ}\cdot\text{kg}^{-1}\ \text{EO}\right) = Q_{EP} + Q_{ESD} \quad (3)$$

where

Q_{EP}: energy consumed for pretreatment (MJ/kg EO)
Qi: heat input (MJ/kg EO)
Q_{ESD}: energy consumed for steam distillation (MJ/kg EO)
Q_{TE}: total energy (MJ/kg EO)
4.19: sensible heat (kJ/kg °C)
2257: latent heat (kJ/kg)
Wsv: steam generation volume (kg)
75.0: the temperature difference during heating or cooling (25.0 °C → 100 °C (ΔT = 75.0 °C))
Hcpm: Heat capacity of plant materials (kJ/kg °C)
Wpm: Weight of plant materials (kg)
Ww: water weight (kg)
Wvc: volume of steam to cool (kg)
W_{EO}: essential oil weight (kg)

The energy loss due to heat dissipation during distillation was not considered while calculating the total energy.

In this study, a distillation apparatus (Large Type; Tokyo Seisakushyo Ltd., Tokyo, Japan), shown in Figure S1, was used to evaluate the energy of essential oil extraction via steam distillation of *A. sachalinensis* leaves and branches. The vessel was made of stainless steel (240 mm i.d., 235 mm height; SUS304) and had a volume of approximately 10 L. A stainless steel separator inside the vessel completely separated the plant material from the water used to generate steam. Before starting distillation, 2 L of water was placed in the vessel, and the lower part of the vessel was heated by a heater to generate steam for distillation. The cooler was made of glass, and water at 4 °C was poured through it to liquefy the vapor, which was cooled to 25 °C to obtain the essential oil and distilled water.

To assess the effect on dried leaves, approximately 1 kg of dried leaves (approximately 1.8 kg FW) were placed in a distillation apparatus and steam-distilled for 1.5 h. Fresh leaves and dried leaves treated with shockwaves were steam-distilled in the same manner. Steam distillation was performed to extract EO and approximately 0.5 L of water. The extracted EOs were weighed, and the EO yield (WEO) was subsequently calculated in terms of DW (kg). EO samples were stored at 4 °C until further testing and analysis.

2.5. Analysis of Volatile Components of EOs

The EOs were analyzed using a gas chromatograph coupled to a mass spectrometer (GC/MS; QP-2010 Plus; Shimadzu Co., Kyoto, Japan) equipped with an autoinjector AOC-20i (Shimadzu). Quantitative determinations of EO components were performed using peak area measurements. GC/MS analyses were carried out with a DB-WAX column of 60 m length, 0.32 mm ID, and 0.5 µm thickness (Agilent Technologies, Inc., Santa Clara, CA, USA). The column temperature was programmed to increase from 40 °C (3 min hold) to 165 °C at 5 °C/min and then increased at 10 °C/min to 220 °C (3 min hold). The injector and detector temperatures were set at 250 °C. The range of masses was scanned from 30 to 600 amu. Control of the GC/MS system and the data peak processing were performed using Shimadzu's GC/MS solution software, version 4.3 (Shimadzu Co., Kyoto, Japan). Volatile constituents were identified by comparing mass fragmentation patterns with MS libraries (NIST05 and FFNSC Library ver. 1.2; Shimadzu Co., Kyoto, Japan) and considering linear retention index. The linear retention indices were determined for all components using a homologous series of *n*-alkanes (C8–C24) injected under the same chromatographic conditions as the samples.

2.6. DPPH Radical Scavenging Assay

The antioxidant abilities of the EOs were assessed through the DPPH analysis. The scavenging activity was estimated according to the Blois method from 96-well microplate with some modifications [26]. The EO was diluted to 30 mg/mL using ethanol. Subsequently, each well of the 96-well microplate was supplemented with an aliquot of this solution (20 µL), ethanol (80 µL), and ethanolic DPPH (126.8 µM and 100 µL). The mixture was vigorously shaken and allowed to stand in a dark room at 37 °C for 30 min. For Trolox, a standard solution (20–200 µM) was prepared and subjected to the DPPH assay in the same manner. Furthermore, a calibration curve was prepared. The absorbance was subsequently measured using a Varioskan Flash Multimode Reader (Thermo Fisher Scientific, Oy, Finland) at 520 nm. The scavenging effect was expressed as Trolox equivalents/mg oil (µmol TE/mg EO). The results are expressed as mean test values in triplicate analyses for each sample.

2.7. Folin–Ciocalteu Assay of EOs

The antioxidant capacity of compounds with phenolic hydroxyl groups in the EOs was determined using the Folin–Ciocalteu reagent with the GA as a standard [27]. In brief, 10 µL of Folin–Ciocalteu reagent was added to 20 µL sample in a 96-well microplate. After mixing, 40 µL of 10% sodium carbonate was added, and the mixture was allowed to stand for 30 min, stirring intermittently. After adding 130 µL of distilled and deionized water, the absorption of 750 nm was measured with a Varioskan Flash Multimode Reader (Thermo Fisher Scientific Oy, Finland). Total phenolic content was expressed as GA equivalents (GAE) in mg GAE/kg EO.

3. Results

3.1. Effect of Underwater Shockwave Pretreatment on Essential Oil Extraction

The processing equipment for the pretreatment of *A. sachalinensis* leaves and branches using underwater shockwaves was built in-house. In this study, the effects of underwater shockwaves on both dried and fresh materials were evaluated. To assess the effect on fresh leaves and branches, 2 kg of fresh *A. sachalinensis* leaves and branches were finely cut,

placed into a silicone tube that was completely separated from water and the source of shockwave generation, and subjected to an instant high-pressure load for 1 to 10 cycles at 3.0 kV and 3.6 kJ. To assess the effect on dried leaves and branches, 2 kg of leaves and branches (approximately 1.4 kg DW) was finely cut and oven-dried at 40–45 °C to a moisture content of 10% or lower. Subsequently, the dried leaves were subjected to an instant high-pressure load for various cycles at 3.0 kV and 3.6 kJ in the same manner as for the fresh leaves. Figure 2 shows the images of dried leaves subjected to shockwaves for various numbers of cycles. For fresh leaves, after being subjected to shockwaves, the cell walls were destroyed when a momentary high pressure was applied. The leaves and branches turned into finer powder as the number of cycles increased.

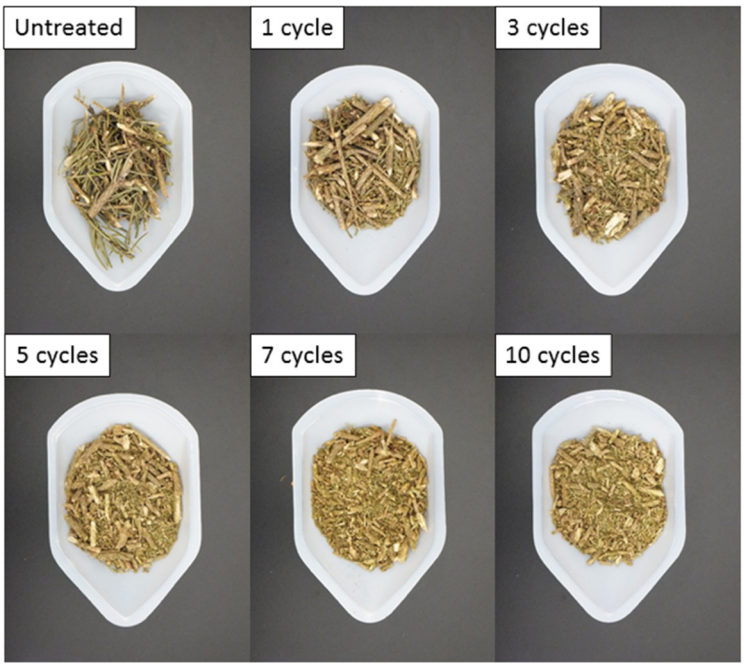


Figure 2. Images of the effects of underwater shockwave pretreatment at 3.0 kV and 3.6 kJ on dry leaves and branches for various numbers of cycles.

The untreated and shockwave-treated *A. sachalinensis* leaves and branches were steam-distilled, and the EOs were extracted. Table 1 shows the EO yield of *A. sachalinensis* by steam distillation with SW pretreatment; energies of SW pretreatment, pretreatment, and steam distillation; and total energy. The EO yields for unprocessed and shockwave-treated fresh leaves (fresh leaves + SW) were 5.1 and 16.2 g/kg leaf dry weight (DW), respectively. Results indicated that for fresh leaves, EO yield increased approximately 3.2 times after the pretreatment of underwater shockwave.

Table 1. Essential oil yield of *A. sachalinensis* by steam distillation with SW pretreatment; energies of SW pretreatment, pretreatment, steam distillation; and total energy.

	Essential Oil Yield g/kg DW Leaf		Energy of SW Pretreatment kJ/kg DW Leaf	Energy of Pretreatment Q_{EP} MJ/kg EO *1	Energy of Steam Distillation Q_{ESD} MJ/kg EO	Total Energy Q_{TE} MJ/kg EO *3
	Fresh	Dried				
Control	5.1	2.4	-	-	596.6/1267.9	596.6/1267.9
SW1 *2	-	16.7	11.8	0.71	182.2	182.9
SW3	-	19.7	40.3	2.05	154.4	156.5
SW5	16.2	24.1	69.8	2.90	187.7/126.3	187.7/129.2
SW7	-	28.7	97.4	3.39	106.0	109.4
SW10	-	32.7	134.6	4.12	93.0	97.2

*1: The plant material is finely cut but does not contain the energy of the cutting process; *2: The number indicates the number of additional cycles of the shockwave at 3.0 kV and 3.6 kJ; *3: Energy loss due to heat loss during distillation is not considered when calculating total energy.

In general, drying the leaves prior to steam distillation causes slight cracks in the cell wall, and steam tends to enter the cell, increasing the EO yield with gas phase extraction [24]. However, the EO yield from dried leaves was 2.4 g/kg DW, which was approximately half the weight of untreated fresh leaves. It is presumed that the leaves and branches of *A. sachalinensis* became woody and hardened because of the drying process. Furthermore, the hydrophobicity of the wax component in *A. sachalinensis* made it difficult for water vapor to pass through, reducing the yield of EOs. In contrast, the yield of EOs obtained from dried leaves via shockwave pretreatment increased with an increase in the treatment cycles. The EO yields for 5 (SW5) and 10 cycles (SW10) were 24.1 and 32.7 g/kg DW, respectively. The EO yields of dried leaves + SW5 improved 1.5-fold and 10-fold for fresh leaves + SW5 and untreated dried leaves, respectively. Shockwave pretreatment creates multiple cracks in the plant tissue, splitting or crushing the cellular structure and fibers [16,19,24], allowing for more efficient steam extraction. In the case of dried leaves, the EO yield of dried leaves + SW10 increased to 13.6 times that of untreated dried leaves. By increasing the number of cycles, the effect was significantly increased, and at 10 cycles, the leaves of *A. sachalinensis* became powdered (Figure 2), improving the passage of water vapor into the cell. Therefore, EOs were efficiently extracted, significantly increasing the extraction yield.

The increase in EO yield with SW pretreatment also significantly reduced the total energy required to extract the EOs. The total energy required to extract EO from just finely cut leaves by steam distillation was 596.6 and 1267.9 MJ/kg EO for fresh and dried leaves, respectively. In contrast, SW pretreatment increased the EO yield and decreased the total energy to 97.2 MJ/kg EO for SW10. The SW10 pretreatment resulted in approximately 6- and 13-fold reductions in total energy consumption compared to fresh and dried leaves, respectively, indicating that not only an increase in EO yield but also a significant reduction in energy was required for EO extraction. Various methods for efficiently extracting EOs without compromising quality or quantity have been demonstrated [11,12,28]. Microwave- or ultrasound-assisted steam distillation could reduce the extraction time to approximately one third that of conventional steam distillation and could significantly reduce energy consumption [23]. The shockwave treatment technology proposed in this study is only a pretreatment technology and does not compete with these technologies. Further energy savings can be expected by applying underwater shockwave treatment to plant materials as a pretreatment for microwave and ultrasonic extraction technologies.

3.2. Effect of Underwater Shockwave Pretreatment on Volatile Contents

Quantitative determination of volatile constituents was carried out using a peak area analysis of the GC/MS spectra. The EO components are presented in Table 2. The major volatile constituents in the EOs obtained from fresh leaves were α-pinene, camphene, β-pinene, myrcene, limonene, β-phellandrene, bornyl acetate, and borneol. These volatile components evidently differed for fresh and dried leaves. Moreover, not only the yield of the EOs decreased by drying but also the ratio of the main components remarkably decreased, except for bornyl acetate and borneol, yielding a large sensory change. In contrast, when dried leaves were treated with a shockwave, the relative concentration of the main volatile compounds decreased by increasing drying. As the shockwave load cycle increased, many components were processed. Unlike other components, the relative concentration of borneol decreased as the shockwave treatment cycle increased. Through shockwave pretreatment, the extraction yield of the main components increased as the load cycle increased. However, the extraction amount of borneol did not depend on the treatment cycle, and the relative concentration decreased owing to the increase in EO yield. In addition to α-terpinyl acetate and juniper camphor, other volatile compounds exhibited similar trends.

Table 2. Major volatile compounds identified in *A. sachalinensis* essential oils.

Compounds	RI* DB-WAX	Relative Concentration (%)						
		Untreated		SW Pretreatment				
		Fresh	Dried	SW1	SW3	SW5	SW7	SW10
Santene	990	0.81	0.07	1.07	1.00	0.95	0.93	0.96
Tricyclene	1012	0.64	0.04	1.19	1.30	1.32	1.58	1.59
α-Pinene	1027	7.73	0.66	13.25	14.21	15.35	15.07	16.15
α-Thujene	1031	0.02	-	0.06	0.06	0.07	0.05	0.06
α-Fenchene	1063	0.01	-	0.03	0.03	0.03	0.03	0.03
Camphene	1072	9.71	1.22	12.57	13.16	12.80	15.12	14.69
β-Pinene	1115	5.92	0.85	7.25	7.36	8.51	6.35	7.49
Sabinene	1128	0.01	tr	0.05	0.04	0.06	0.04	0.05
3-Carene	1157	0.01	0.12	0.90	0.86	1.13	0.49	0.79
Myrcene	1171	2.36	0.62	1.78	1.87	1.93	2.00	1.90
α-Phellandrene	1173	0.08	0.08	0.23	0.23	0.23	0.20	0.22
α-Terpinene	1188	0.07	0.06	0.12	0.12	0.12	0.10	0.11
Limonene	1208	5.02	2.76	5.28	5.12	4.96	4.96	4.97
β-Phellandrene	1218	10.56	5.80	16.51	16.20	18.24	13.33	15.23
γ-Terpinene	1256	0.12	0.13	0.17	0.17	0.11	0.15	0.17
p-Cymene	1281	0.06	0.06	0.06	0.06	0.33	0.05	0.05
Terpinolene	1295	0.70	0.73	0.94	0.91	0.60	0.90	0.91
Fenchyl acetate	1484	1.19	2.37	1.42	1.39	0.87	1.41	1.36
Camphor	1537	1.94	1.60	0.53	0.43	0.41	0.46	0.38
Linalool	1559	0.03	3.31	0.84	0.77	0.58	0.76	0.64
Linalyl acetate	1568	0.35	1.31	0.47	0.46	0.33	0.46	0.38
Bornyl acetate	1599	27.65	38.28	22.87	22.12	13.49	23.86	22.09
Isobornyl acetate	1605	0.23	0.29	0.14	0.14	0.40	0.14	0.13
Caryophyllene	1617	0.40	0.64	1.06	1.10	0.69	1.27	1.18
Terpinen-4-ol	1621	0.14	0.48	0.11	0.11	0.08	0.09	0.08
(*E*)-β-Farnesene	1676	-	0.21	0.10	0.10	0.07	0.10	0.09
Humulene	1690	0.14	0.24	0.43	0.46	0.27	0.54	0.49
α-Terpinyl acetate	1715	1.53	1.23	0.42	0.42	0.34	0.37	0.33
Borneol	1722	19.45	30.44	7.19	7.22	3.93	6.46	5.02
β-Himachalene	1731	-	0.12	0.13	0.13	0.17	0.11	0.12
δ-Cadinene	1736	-	0.13	0.22	0.22	0.33	0.20	0.20
β-Bisabolene	1742	-	0.52	0.52	0.52	0.35	0.51	0.49
α-Farnesene	1760	-	0.11	0.08	0.08	0.06	0.11	0.10
Nerolidol	2053	tr	0.24	0.15	0.16	0.27	0.15	0.14
Juniper camphor	2264	1.06	1.47	0.52	0.51	0.33	0.50	0.49
Total (%)		97.93	96.21	98.66	99.01	89.69	98.87	99.08

RI*: retention index relative to *n*-alkanes on a DB-WAX column. tr < 0.01%, -: not detected.

For dried leaves, both major volatile constituents and the EO yield changed. Peak percentages of α-pinene, camphene, β-pinene, and β-phellandrene were higher for the shockwave-treated leaves than untreated leaves. The relative concentrations of major volatile components such as myrcene, limonene, and bornyl acetate were almost unchanged by shockwave treatment. However, because of the significant increase in EO yield, these components extracted from the unit mass of leaves increased significantly.

Previous histochemical analysis indicated that the EO components of plant leaves are unevenly distributed in the leaves and cells of the abaxial epidermis [29]. Especially for dried leaves, the numerous cracks created by shockwave treatment act as permeation pathways, which increase the ratio of EOs extraction in the steam distillation process. These results indicate that in a fresh leaf, the structure of the cells is destroyed by treatment with shockwaves, but the effect on the composition of EOs depends on the amount of water contained in the leaf.

The EO of *A. sachalinensis* is proven to be an active elimination agent, similar to γ-terpinene, myrcene, and β-phellandrene, which effectively removes nitrogen dioxide. EOs have a relaxing effect; the use of EOs should improve overall air quality. Table 3 shows the yields of these volatile components, that effectively remove the nitrogen dioxide identified in the EOs of *A. sachalinensis*. The yield of these components obtained from dried leaves by shockwave pretreatment increased with an increase in treatment cycles. The β-phellandrene yields of SW5 and SW10 were 4.40 and 4.98 g/kg DW, respectively. The active removal agent yields of dried leaves + SW10 were 35.8-fold greater than those of untreated dried leaves. These results suggest that shockwave treatment not only increases EO yields but also effectively removes dangerous components, such as nitrogen dioxide, which cause serious damage to the respiratory system.

Table 3. Yield of volatile components that effectively removes nitrogen dioxide. Major volatile compounds identified in *A. sachalinensis* essential oils.

	Yield g/kg DW Leaf						
	Untreated		SW Pretreatment				
	Fresh	Dried	SW1 *1	SW3	SW5	SW7	SW10
β-Phellandrene	0.54	0.14	2.76	3.20	4.40	3.83	4.98
γ-Terpinene	0.006	0.003	0.028	0.033	0.027	0.043	0.056
Myrcene	0.12	0.015	0.30	0.37	0.47	0.57	0.62

*1: The number indicates the number of additional cycles of the shockwave at 3.0 kV and 3.6 kJ.

3.3. Evaluation of the Antioxidant Activity of EOs

In this study, the DPPH and Folin–Ciocalteu analyses were used to assess the antioxidant capacity of the EOs. DPPH is a compound that possesses a proton free radical. Its proton radical scavenging action is a well-known mechanism for measuring antioxidant ability, and it shows maximal absorption at 520 nm. When DPPH encounters proton radicals, the purple color quickly fades. Consequently, this assay was used to identify the entrapment of stable DPPH radical species by antioxidants [26,30].

The Folin–Ciocalteu assay, which uses a phenolic reagent based on the reducing power of phenolic hydroxyl groups, is one of the most typical analytical methods for measuring antioxidant activity [27]. The Folin–Ciocalteu assay is mainly used to analyze polyphenols showing physiological activity contained in foods, but it is extremely important for showing the antioxidant activity of compounds with phenolic hydroxyl groups in the EOs. Many EOs contain volatile components with phenolic hydroxyl groups, such as thymol, eugenol, and carvacrol, which show high antioxidant activity [10,31–33]. These compounds show not only high antioxidant activity but also good antibacterial properties in foods [34]. They are used to assess the antioxidant capacity of compounds by comparing their reductive power with the reductive power of GA.

Figure 3a shows the scavenging activity of the DPPH free radicals of the EOs obtained by steam distillation of the dried leaves of *A. sachalinensis*. The dried leaves were shockwave pretreated, and the scavenging activity of the resulting EO was significantly increased compared to that of untreated samples. Increasing the shockwave load cycle also increased the scavenging activity with untreated, SW3, and SW7 scavenging activities of 489.0, 518.1, and 718.3 μmol TE/mg EO, respectively. Scavenging activity increased with increasing load cycle but decreased with SW10 to 522.3 μmol TE/mg EO. Figure 3b shows the amount of antioxidants in the EO obtained via steam distillation of dried *A. sachalinensis* leaves. The amount of antioxidants was estimated from the yield of EOs and the scavenging activity of free radicals. The amount of antioxidants extracted from 1 kg of dried leaves increased significantly with an increasing load cycle. The amounts of untreated, SW3, and SW7 antioxidants were 1.2, 10.2, and 20.6 mol TE/kg DW leaf, respectively. The effect of extracting antioxidants from the dried leaves of *A. sachalinensis* was maximal in SW7, and the amount extracted was almost saturated even after further treatment. On the other hand, the effect of the underwater shockwave on the EO extraction rate was highest in SW10; therefore, the relative concentration of antioxidants in the EO decreased, and, as a result, the scavenging activity of SW10 decreased with respect to SW7. These results revealed that the EO yield is highly dependent on the shockwave pretreatment load cycle. Furthermore, it is suggested that more antioxidants are extracted from the leaf tissue of *A. sachalinensis* than terpenoids, which are the main components of EOs and show almost no antioxidant activity.

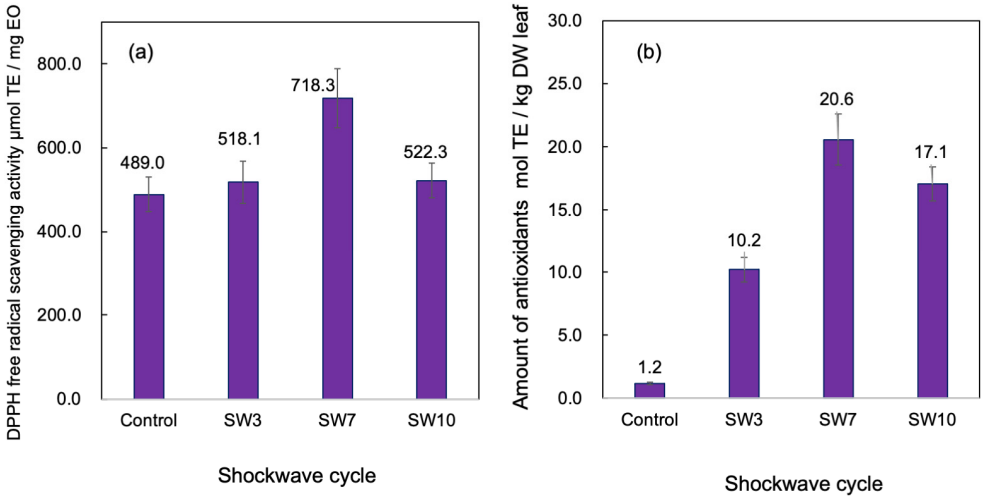


Figure 3. (**a**) DPPH free radical scavenging activity of essential oils and (**b**) the amount of antioxidants in essential oils obtained by steam distillation from *A. sachalinensis* dried leaves. Bars represent the mean ± standard deviation of three independent experiments. The number of SW indicates the number of additional cycles of the shockwave at 3.0 kV and 3.6 kJ.

Figure 4 shows (a) the antioxidant activity of EOs and (b) the amount of antioxidants with phenolic hydroxyl groups in EOs, obtained by steam distillation from *A. sachalinensis* dried leaves. The dried leaves were shockwave-pretreated, and the antioxidant capacity of the resulting EO was significantly increased compared to that of untreated leaves. Increasing the shockwave load cycle also increased the antioxidant activity, with untreated, SW3, and SW7 antioxidant activities of 0.37, 0.44, and 0.99 mg GAE/g EO, respectively. The amount of antioxidants with phenolic hydroxyl groups extracted from 1 kg of dried leaves also increased significantly as the load cycle increased. Extractions of untreated, SW3, and SW7 antioxidants yielded 0.89, 8.76, and 28.34 mg GAE/kg DW leaves, respectively. SW7 antioxidant extracts were 31.8 times higher than untreated leaves.

Antioxidants with high DPPH free radical scavenging activity or with phenolic hydroxyl groups remove nitrogen dioxide from the environment while exhibiting high antibacterial and antifungal properties. Therefore, these results indicate that underwater shockwave pretreatment not only increases the yield of *A. sachalinensis* EO but also increases the functionality of the EOs.

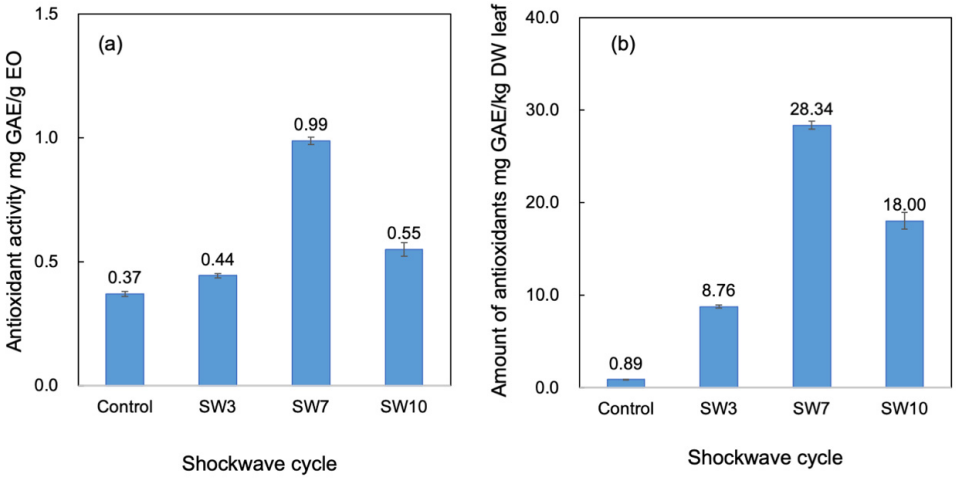


Figure 4. (**a**) Antioxidant activity of essential oils and (**b**) the amount of antioxidants with phenolic hydroxyl groups in essential oils obtained by steam distillation from *A. sachalinensis* dried leaves. Bars represent the mean ± standard deviation of three independent experiments. The number of SW indicates the number of additional cycles of the shockwave at 3.0 kV and 3.6 kJ.

4. Conclusions

This study shows that applying underwater shockwave treatment to the leaves of *A. sachalinensis* as a pretreatment step can effectively extract EOs through subsequent steam distillation. In addition, we have demonstrated the high applicability and reliability of underwater shockwave pretreatment in significantly improving the antioxidant activity of EOs and the yield of EO extracts from the leaves and branches of *A. sachalinensis*. Our results confirm that through the shockwave pretreatment of *A. sachalinensis* leaves and branches, spalling destruction forms multiple cracks in the cell walls, which act as permeation pathways for water vapor during steam distillation. The proposed technique exhibited excellent performance in extracting volatile constituents of aromatic plants when it was used before the steam distillation process. Although evaluated in combination with conventional steam distillation in this study, underwater shockwave treatment is an innovative pretreatment that can be combined with other extraction techniques because it directly treats the plant material before distillation. Moreover, this novel process can significantly reduce the energy used for EO extraction in steam distillation, suggesting that it can contribute to the development of a low-energy, sustainable EO production system. This process could be widely applied to the extraction process of medicinal and other plants, and the dynamic control of high instantaneous pressures based on underwater shockwaves could pave the way for new industrial applications.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/pr10122534/s1, Figure S1: Evaluation of the energy of essential oil extraction by steam distillation of leaves and branches of *Abies sachalinensis*.

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