Organic Chemistry Research

Article

Organic Chemistry Research
www.orgchemres.org

Cite this: *Org. Chem. Res.* **2024**, *Vol. 10*, 44-49.
DOI: 10.22036/org.chem.2024.468205.1346

Application of new techniques for extraction of the volatile components and essential oils

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Received: July 20, 2024; Accepted: September 12, 2024

Abstract: The conventional methods for the essential oil extraction (hydro-distillation and steam distillation) have some disadvantages. Losses of some volatile constitute, low extraction efficiency, degradation of unsaturated or ester compounds through thermal or hydrolytic effects and toxic solvent residue in the extract may be encountered using these extraction methods. The development of new extraction techniques for the food and herbal drugs industries has received a lot of attention lately due to the environmental restrictions and the need for minimizing the energy costs. In order to reduce the extraction time, and the operation costs and possibly improve the oil yield and the quality of the essential oil, new approaches such as microwave-assisted extraction, supercritical fluid extraction, and ultrasound-assisted extraction have also been sought. Results of our study indicated that the changes in yield of the volatile oil during extraction depended on extraction method and temperature, type constituent and the biological characteristics of the plants. Extraction methods had significant effects on aroma quality of the dried samples in Iranian herbs. In conclusion, extraction of the essential oil from some Iranian medicinal and aromatic plants with microwave-assisted steam hydro-diffusion was better in terms of extraction time, phenolic and aroma fractions, and product quality.

Keywords: Medicinal and aromatic plants, Chemical composition, Essential oil yield, Novel techniques

1. Introduction

Herbs are a rich genetic resource and one of the most valuable assets of each country. Iran is among the 8 most important countries in the world with diverse medicinal plant flora.[9] The genus *Satureja* L. (Savory) belongs to the Lamiaceae family and consists of 200 species of herbs and shrubs, often aromatic, widely distributed in the Asian and Mediterranean regions.[6] The essential oil of *S.bachtiarica*, isolated from the plant's aerial parts, possesses antibacterial, antifungal, and antioxidant properties.[18] It includes various phenolic compounds, monoterpenes, and sesquiterpenes. The main compounds found in *S.bachtiarica* essential oil are thymol, carvacrol, γ-terpinene and ρ-cymene. This oil can be used as a food preservative in the food industry[3] and for hygienic and cosmetic purposes such as deodorants, toothpastes and mouthwashes.[5]

The extraction methods of essential oils from medicinal plants are divided into two models: conventional methods, which include hydrodistillation, steam distillation, steam and water distillation, as well as extraction with organic solvents; and innovative methods, which include supercritical fluid extraction, microwave-assisted extraction, and ultrasound-assisted extraction.[15]

However, the problems associated with conventional extraction methods include loss of volatile compounds, low extraction efficiency, degradation of esteric and unsaturated compounds due to thermal and hydrolytic effects. Additionally, because essential oil compounds are very sensitive in the conventional methods, toxic solvent residue, the longtime of the extraction and the direct heating maybe encountered chemical changes and polymerization in the essential oils.[10,11,21]

The new methods of essential oil extraction are considered to provide not only high quality and quantity of essential oil, but also to be organic solvent-free, fast, with low energy and water consumption.[16,20] These extraction methods are effective in determining the efficiency, types, and contents of chemical compounds in the essential oils as well as their properties. This has led to the benefits of modern extraction techniques and drawbacks of conventional methods. Over the years, some

traditional processes and other thermal extraction techniques have been replaced with procedures based on microwave extraction. Some recent studies have successfully utilized a microwave oven for essential oil extraction from herbs.[10,17,13,19,20] However, based on the author's searches, no work has been published on the microwave-assisted extraction of *Savory* species. In this study, we compared the antioxidant activity (using DPPH procedures) and total phenolic compounds of *Satureja bachtiarica* Bunge. essential oils obtained from different extraction methods in order to determine the most advantageous method for extraction with high protective antioxidant capacity.

2. Materials and methods

Plant material

The fresh aerial parts (up to approximately 5 cm, 100 g) of *Satureja bachtiarica* Bunge. were gathered from Chaharmahal va Bakhtiari province in the southwestern part of Iran, at an elevation of about 220 meters above sea level in June 2021. The plant was confirmed by voucher specimens (no.1999) that have been placed at the Research Institute of Forests and Rangeland Herbarium in Shahrekoard, Iran. The fresh aerial parts of Bakhtiari savory were dried in a dark room at a temperature of 30 ± 5 °C for six days. An Endecott food processor was used to produce tiny powder and a 16 mesh sieve was used to remove large pieces of debris**.**

Chemicals

Homologous series of C_5-C_{24} n-alkanes, β-Carotene, DPPH (Sigma-Aldrich, Steineheim, Germany) and Anhydrous sodium sulphate, Folin-ciocalteu, Gallic acid, BHT, Sodium carbonate, Linoleic acid, Tween 40, Ascorbic acid, Diethyl ether, Methanol, Ethanol, Chloroform HPLC grade (Merck Co. Darmstadt, Germany)

Conventional methods

The coventional methods consisted of hydrodistillation and traditional steam and water distillation.

Hydrodistillation (HD_{BP} and HD_{RIFR}). Hydrodistillation method was performed by two Clevenger-types apparatus: British pharmacopia (HD_{BP}) and Research Institute of Forests and Raglans of Iran (HD_{RIFR}) (Figure 1), 100 g of plant with 1 L of water for 4 h was extracted at least triplicate.

The essential oils obtained from all of the different extraction methods were dried by anhydrous Na_2so_4 and transferred to sealed dark vials and stored at 4 ºC until analysis.

Steam and water distillation (SWD). In this procedure the special innovative flask (designed by Memarzadeh and Ghasemi Pirbalouti patent no.89031 in Iran) was used. The vapor produced by 1 L water from lower part of flask passed through the 100 g essential oil rich plant material before being condensed into a receiving Clevenger-type apparatus (British pharmacopia approach) for 4 h and at least triplicate.

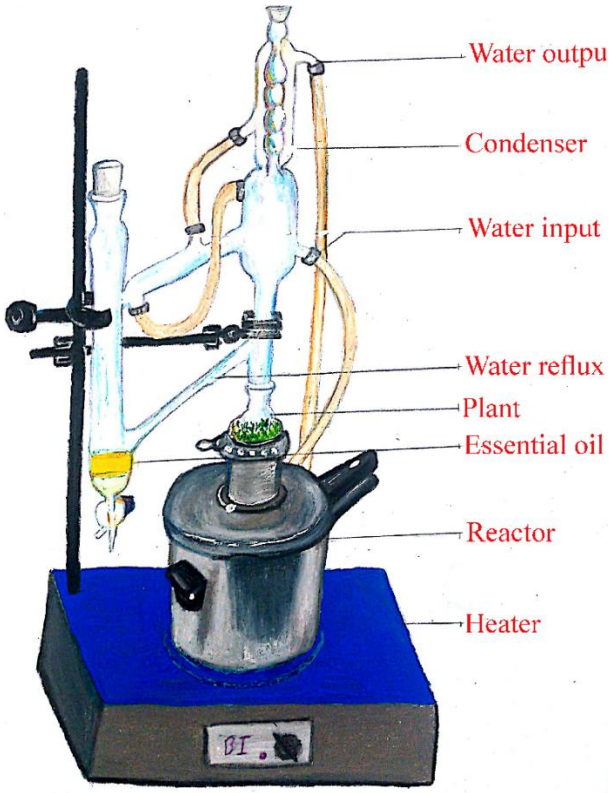

Figure 1. Hydrodistillation of Research Institute of Forests and Raglans of Iran metod (HD_{RIFR}).

Innovative methods (Apparatus and Procedure)

The innovative methods consist of steam distillation (SD_{innove}), Microwave-assisted hydro-diffusion (MHD)and Microwave-assisted steam hydro-diffusion (MSHD).

Innovative steam distillation (SD_{innove}). In an innovative steam distillation technique (SD_{innove}) that was designed by Memarzadeh and Ghasemi Pirbalouti (Figure 2) the vapor was produced by the simple steam generator and then steam was transferred from the tube that was embedded in the lower part of 1 L special flask and passed through the 100 g essential oil rich plant material before being condensed into a receiving Clevenger-type apparatus (British pharmacopia approach) for 4 h.

Microwave-assisted steam hydro-diffusion ($MSHD_{400\ and\ 800W}$).

The domestic microwave oven used as an energy source was a modified model MG-4012WM/00 from LG, Korea, and two methods MSHD and MHD were employed. In this process, 100 g of Bakhtiari savory that had been macerated in 1 L of distilled water for 30 minutes was placed on the lattice surface in the middle of a special flask containing another liter of deionized water. The set-up was then placed within the microwave oven cavity, with a condenser positioned on top (outside the oven) to collect the extracted essential oil. The vapor produced by the microwave oven passed through the essential oil-rich plant material before being condensed into a receiving Clevenger type apparatus (Figure 3). The

microwave oven operated at two power levels, 400W and 800W, for a period of 30 minutes, which was sufficient to extract all essential oil from the samples. During this time, collected essential oils were decanted from the condensate at 10-minute intervals.

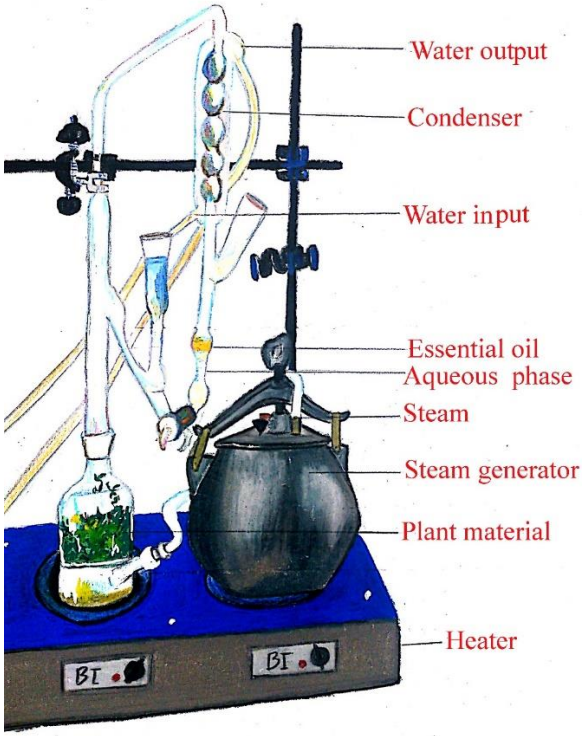

Figure 2. Innovative steam distillation method ($SD_{innovative}$).

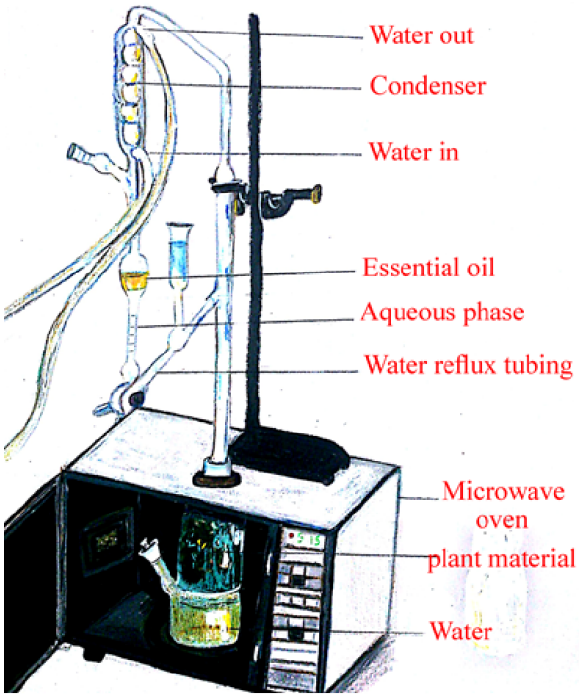

Figure 3. Microwave- assisted steam hydro-diffusion method (MSHD).

Microwave-assisted hydro-diffusion ($MHD_{400 \text{ and } 800 \text{ W}}$)
This method were done base on part 2.4.2 with the difference that the bottom of the special flask was empty.

Determination of essential oil yield
Essential oil content was determined using the Europian Pharmacopea on basis of dry matter carried out in triplicates. Essential oil yield (%) was measured using the following formula.[36]

$$\text{EO yield}(\%) = \frac{\text{mass of EO obtained(g)}}{\text{mass of dry matter(g)}} \times 100$$

Determination of total phenolic contents
The total phenolic content (TPC) in each extract was determined using the Folin-Ciocalteu procedure[21] with minor modifications. In brief, 500 µL of a 100 µg/mL solution of essential oil in methanol was mixed with 5 mL of Folin-Ciocalteu reagent (diluted 1:10 with distilled water) and shaken slowly for 5 minutes. Then, 3 mL of a 2% sodium carbonate solution was added to the test tube and shaken well for another minute. The mixture was left at room temperature for one hour until it turned blue in color. Subsequently, the absorbance of the mixture was measured at 760 nm using an ultraviolet-visible (UV-Vis) spectrophotometer (Varian-Cary100 conc). A blank sample consisting of water and reagents served as the reference. Gallic acid equivalents (GAE) were used as the reference standard, and TPC was expressed as mg of GAE per gram of each extract containing essential oil. Butylated hydroxytoluene (BHT) and ascorbic acid were used as positive reference standards. Each test was performed at least three times, and the results were reported as mean ± standard deviation (SD) from triplicates.[14]

Antioxidant activity
DPPH Radical scavenging Assay. Brifely, 100 µL of the sample of each essential oil was blended at chosen concentrations (500, 250, 125, 62.5 µg/mL) with 3.9 mL from 25 mg/L methanol DPPH solution. The mixture was shaken vigorously and allowed to stand at dark room temperature for 30 min. The absorbance of the samples was measured at 517 nm using a spectrophotometer UV-Vis (Varian-Cary100 conc). The methanol used as at the blank and a control (A_B Control) including methanol and DPPH solution was also measured. BHT and Ascorbic acid were used as the positive refrence standards. Each test was performed at least three times.The inhibition percentage of the samples was calculated accordingto the following formula:

$$\%\text{Inhibitation} = (1 - A_A/A_B) \times 100$$

A_A and A_B are the absorbance values of the DPPH radical in the presence of the essential oil samples and the control, respectively.[2]

The inhibition percentage was plotted versus the essential oil samples concentration and 50% of the inhibitory concentration (IC_{50}) of the DPPH value was determined by linear regression analysis.
percentages were reported as mean ± SD of triplicate.

Statistic analysis
All the tests were carried out in three replications; in order to analyze the data, SPSS software, version 20, was used. The means comparision was carried out using One way ANOVA and Duncan´s multiple range test ($p \leq 0.05$).

3. Results and discussion

Effect of extraction methods on the essential oil yield
The results indicated that different extraction methods had a significant effect on the essential oil yield of *Satureja bachtiarica* Bunge ($p \leq 0.05$). The HD_{BP} and $MSHD_{800w}$ methods showed the highest essential oil yield (v/w on dry weight basis), while the lowest essential oil yield was obtained by the HD_{RIFR} and MHD_{400w} methods (Table 1). Specifically, HD_{BP} showed a yield of 1.4 ± 0.1 mL EO/100 g dry plant in a 4-hour extraction time, and $MSHD_{800w}$ showed a yield of 1.37 ± 0.6 mL EO/100 g dry plant in just 30 minutes extraction time. These methods achieved maximal yields, with $MSHD_{800w}$ being faster than HD_{BP} in terms of extraction time due to its efficient heat transfer speed compared to traditional hydrodistillation.

Table 1. Effect of extraction methods on the essential oil yield

E. method	Yield ± SD
$HD_{(BP)}$	1.400 ± 0.010 **a**
$SWD_{(BP)}$	1.270 ± 0.076 **c**
$SD_{(innove)}$	1.283 ± 0.029 **bc**
$HD_{(RIRF)}$	1.060 ± 0.040 **e**
MHD_{400w}	1.033 ± 0.058 **e**
MHD_{800w}	1.167 ± 0.058 **d**
$MSHD_{400w}$	1.370 ± 0.060 **ba**
$MSHD_{800w}$	1.167 ± 0.058 **d**
ANOVA	**$p \leq 0.05$**

Note: Significant different at 5% level probability with Duncan test have been indicated with different letters ($p \leq 0.05$).

An approved reference method for quantifying essential oils, microwave-assisted hydrodistillation (MSHD) rapidly delivers energy to the extractant and plant matrix.[22] These findings are consistent with a study by[10] on *Thymus vulgaris* L., which demonstrated that microwave-assisted hydrodistillation decomposed leaf glands in only 30 minutes, while traditional hydrodistillation took 75 minutes to achieve similar results.
In summary, these results confirm that while other microwave-assisted methods may not have a positive effect on maximizing yield, they do offer time-saving benefits compared to traditional techniques.

Relationship between total phenolic contents and antioxidant activities of *Saturejabachtiarica* Bunge essential oil under different extraction methods
Total phenolic contents of essential oils. The Total Phenolic Content (TPC) is a key indicator widely used to demonstrate the overall antioxidant activity in samples.[21] Significant differences ($p \leq 0.5$) were detected in the TPC values of Bakhtiari savory essential oils under different extraction methods, with results reported in mg (GAE: Gallic Acid Equivalent) per g of essential oil (Table 2). The highest TPC value was found using $MSHD_{800w}$ (268 ± 6.34 mg GAE/g EO), while the lowest TPC value was recorded for SD_{innove} (205.91 ± 2.5 mg GAE/g EO). Phenolic compounds are known to scavenge reactive oxygen intermediates without provoking further oxidative reactions.[2] BHT and Carvacrol were used as positive controls, with TPC values of 327.72 ± 2.30 mg GAE/g and 259.04 ± 0.57 mg GAE/g, respectively. The results of this study are consistent with literature on Satureja Hortensis.[11] Additionally, environmental factors such as origin and elevation can lead to changes in TPC values; for instance, the TPC value equal to 185.5 mg GAE/g EO on *Satureja Coneifolia* Ten[16] differed from the findings of this study.

Table 2. Total phenolic contents (TPC) and antioxidant activity of essential oil under different extraction method

E. method	DPPH	TPC
	IC50 (mg/ml)	**(mg GA/g)**
HD_{BP}	3.44 ± 0.76 **ed**	249.52 ± 3.64 **c**
SWD_{BP}	2.74 ± 0.57 **d**	258.37 ± 9.13 **c**
SD_{innove}	3.25 ± 0.69 **d**	205.91 ± 2.55 **f**
HD_{RIFR}	3.27 ± 0.20 **d**	255.12 ± 5.10 **c**
MHD_{400w}	4.34 ± 0.18 **f**	229.35 ± 5.27 **d**
MHD_{800w}	3.16 ± 0.37 **d**	219.14 ± 1.60 **e**
$MSHD_{400w}$	4.03 ± 0.48 **fe**	226.67 ± 2.99 **de**
$MSHD_{800w}$	1.95 ± 0.13 **b**	268.22 ± 6.34 **b**
BHT	0.13 ± 0.00 **a**	327.72 ± 2.30 **a**
Carvacrol	2.73 ± 0.08 **d**	259.04 ± 0.57 **c**
ANOVA	**$p \leq 0.05$**	**$p \leq 0.05$**

Note: Significant different at 5% level probability with Duncan test have been indicated with different letters ($p \leq 0.05$).

Antioxidant activity. DPPH scavenging activity: Antioxidant activity is commonly assessed using the DPPH scavenging activity test.[4] In this study, different extraction methods were compared to determine the ability of their essential oils to scavenge the DPPH free radical, with BHT used as a positive control. The IC_{50} values ranged from 1.95 ± 0.13 to 4.34 ± 0.18 mg/mL.
Significant differences ($p \leq 0.05$) in the IC_{50} values of Bakhtiari savory essential oils under different extraction methods were observed. The essential oil of $MSHD_{800w}$ exhibited the highest antioxidant activity with the lowest IC_{50} value (1.95 ± 0.13 mg/mL), while the essential oil of SD_{innove} showed the weakest antioxidant activity with the highest IC_{50} value (4.34 ± 0.18 mg/mL) (Table 2). Antioxidant activity has an inverse relationship with IC_{50} and a direct relationship with

total phenolic content. These results are consistent with findings from two previous studies.[8,12]

4. Conclusions

Extraction of secondary metabolites from medicinal plants is essential and significant. Essential oils, which include polyphenols, terpenoids, alkaloids, and phenylpropanoids, are among the most valuable secondary metabolites. In this study, the extraction time, yield, total phenolic content, and antioxidant activity of essential oils obtained from 8 different extraction methods were compared in two models: conventional (HD_{BP}, HD_{RIFR}, SWD_{BP}) and innovative methods (SD_{innove}, MHD_{400w}, MHD_{800w}, $MSHD_{400w}$,$MSHD_{800w}$).

The reduction in extraction time (40 minutes compared to 4 hours for conventional methods), high oil yield (1.2 ml/ 100 g dry plant), natural odor (similar to the original savory), high antioxidant activity and total phenol content led to $MSHD_{800w}$ being identified as the best extraction method for obtaining *Satureja bachtiarica* Bunge essential oil.

In conclusion, “microwave-assisted extraction" was observed as a green technology due to its lower extraction time, higher essential oil yield, good quality of the obtained essential oil. It also proved to be a promising tool for extracting essential oils from medicinal plants and aromatic herbs for use in pharmaceuticals industry food industry cosmetics hygiene products and aromatherapy.

Declaration of Interests

The authors declare that there exists no conflict of interest.

Author Contributions

Sayedeh Mansoureh Memarzadeh: Investigation, Data curation, Formal analysis, Writing - original draft, validation.
Abdollah Ghasemi Pirbalouti: Supervision, Conceptualization, Writing - review & editing.
Ali Gholami: Project administration, Methodology.
Farita Nourizadeh: Methodology.

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Acknowledgements

The authors are grateful to University of Kashan for supporting this work.

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