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Article

COMPARISON OF DIFFERENT EXTRACTION METHODS OF ESSENTIAL OILS FROM *LAVANDULA ANGUSTIFOLIA* AND DETECTION OF TARGET COMPOUNDS WITH UPLC-MS/MS

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ABSTRACT

Three extraction methods were used comparatively to extract linalool and linalyl acetate from lavender flowers, namely hydrodistillation, Soxhlet extraction (in ethanol) and accelerated solvent extraction (ASE) (with water or methanol, 80ºC, 1500 psi). The best results were obtained with ASE (methanol). The fresh flowers gave higher yields of linalool and linalyl acetate. The possibility of using UPLC-MS-MS for rapid and precise identification (based on spectra of fragments of target compounds) of essential oils was successfully demonstrated. UPLC-MS analysis of the isolated oils revealed that linalool and linalyl acetate are the major components of all the samples.

Keywords: *Lavandula angustifolia*, hydrodistillation, Soxhlet extraction, accelerated solvent extraction, essential oil, UPLC-MS-MS, linalool, linalyl acetate.

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1. INTRODUCTION

Essential oils are volatile aromatic compounds found in a wide range of terrestrial plants, e.g. flowers, leafs, roots, stems and seeds[1]. They are a combination of highly hydrophobic organic compounds that can be extracted by various techniques[2] among them, the most used being hydrodistillation[3], Soxhlet extraction[4], microwave extraction[5], cold pressing[6] and supercritical fluid extraction[7]. One of the most published technique for the extraction of the essential oil from plants is hydrodistillation[3]. On the other hand, hydrodistillation is a very time-consuming method, during which highly volatile components as well as water-soluble components can get lost[8]. Usual extraction techniques of essential oils and the other chemicals that are present in the plant materials include techniques that use solvents at atmospheric pressure or at high pressure and high temperature [9-11]. The methods that use solvents for extraction can be very selective, as the solvent properties are selected to extract only a few compounds that are more soluble in the chosen solvent. The drawback of these approach is the fact that together with the volatile components of the essential oils will be extracted other compounds, equality soluble in that solvent. Furthermore, the temperature and the pressure can influence not only the extraction yields of the target compound but also can contribute to the degradation of these molecules. This can be avoided by the technique called accelerated solvent extraction (ASE), in which the extraction period can be controlled and the deleterious effects of high temperature and high pressure can be minimized[12].

Some of lavender essential oil constituents present beneficial biological activities[13] such as: the treatment of anxiety [14] and sleep disorders [15], or antimicrobial[16] and antifungal[17] effects or natural preservatives in cosmetic products [18].

The genus *Lavandula* from *Labiatae* family, comprise three species utilized for their production of essential oils: *L. angustifolia* Miller contains essential oil of the highest quality, *L. latifolia* Medicus has the lowest yields, and *L. hybrida* (*L. angustifolia* × *L. latifolia*) has the highest yields, but not the highest quality oil[19].

Essential oils of *L. angustifolia* Miller is a colorless to pale yellow liquid, with a floral fragrance. Over 300 compounds have been found in species of *Lavandula*. The two main compounds in *Lavandula* are linalool and linalyl acetate[20].

The chemical composition of *L. angustifolia* oil have been the subject of several publications[21-23]. Most of the data concerning the investigations of the chemical composition of essential oils of *Lavandula* species were obtain by GC and GC-MS analysis[21-23]. In the present paper the chemical composition of the essential oils of *L. angustifolia* cultivated in Romania was analyzed by using liquid chromatography coupled to mass spectrometry (UPLC-MS).

The aim of this study was to compare different sample preparation methods for their suitability for the subsequent UPLC/MS-MS determination of major compounds of lavender essential oils. Three sample preparation procedures for the extraction of essential oils were compared, namely, hydrodistillation which is the most usual method for the isolation of

essential oils, Soxhlet extraction, and accelerated solvent extraction. The possibility of using UPLC-MS was also tested, as traditionally these compounds are analyzed by gas-chromatography techniques.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

Methanol (Sigma, #34966) and ethanol (Sigma, #32221) were purchased from Amex SRL, Bucharest, Romania.

2.2. Plant material

Freshly picked flowers of *Lavandula angustifolia* cultivated in Timiş (Romania) in 2015 and 2016 were purchased from the local market. The fresh material was dried at constant temperature (23ºC), in a dark place[24].

2.3. Preparation of lavender flower samples.

Known amounts of fresh or dried lavender flowers (usually around 1 g) were manually grinded in a mortar. To achieve reproducible extraction yields the samples were passed through a sieve with mesh sizes between 20 and 30 (particle diameters ranging over 0.60-0.85 mm). The dried samples were kept within sealed bag in the cold and dry place until they were used[25].

2.4. Hydrodistillation

The hydrodistillatin extraction was carried for 150 min with 200 mL water and 30 g of dried or fresh lavender flowers, using a Clevenger-type apparatus[3,5]. The oil phase was separated through a separatory funnel.

2.5. Soxhlet extraction

Four experiments of traditional Soxhlet extraction[16] were carried for 300 min with 45 mL of ethanol and (a) 2 g of dried lavender flowers or (b) 2 g of fresh lavender flowers, and with 45 mL of water and (c) 2 g of dried lavender flowers or (d) 2 g of fresh lavender flowers.

The obtained extracts were centrifuged at 5000 rpm for 5 min and then the oil was separated through a separatory funnel.

2.6. Accelerated solvent extraction

Two type of solvents were used for ASE[4] methanol and water. Around 1 g of fresh flowers was loaded into extraction cell of an ASE200 extraction system (Dionex Corporation, USA). In both cases, the volume of solvent used for extraction was 16 mL. The extraction was carried out at 1500 PSI and 80°C for 10 minutes. After extraction, the extracts were mixed with mobile phase (1:1), filtered on 0.2 μm syringe filters and injected in the chromatographic system.

2.7. UPLC-MS analysis

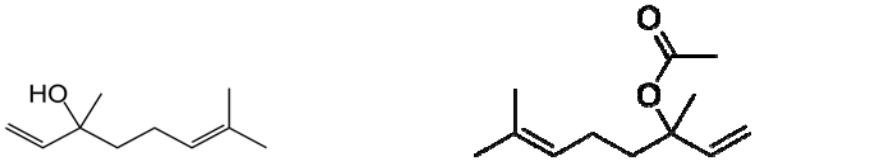
The chromatographic analysis was carried out on a Waters Acquity UPLC-MS system (Binary Solvent Manager, Xevo TQD MS-detector equipped with an electrospray ionization interface)[26] with a UPLC BEH C18, 1.7 μm (2.1×100 mm) column, using a gradient elution procedure. Mobile phase A consisted in 0.02% formic acid in 5% methanol and mobile phase B was 0.02% formic acid in methanol. The gradient profile was: 0 – 0.2 min, 30% A and 70% B; 0.2 – 1 min, linearly increase untill 100% B; 1 – 2.9 min, hold 100% B; 2.9 – 3.0 min, linearly decrease untill 70% B (initial condition). The column temperature was set at 30°C. The analyses were run at a flow rate of 0.3 mL/min, and the sample volume injected was 10 μL.

The electrospary ionization (ESI) parameters for Xevo TQD MS detector were fixed as follows: capillary voltage at 3.0 kV, source temperature at 150°C, desolvation temperature at 400°C, and desolvation gas at 500 L/h. Nitrogen was used as the desolvation gas, and argon was employed as the collision gas.

3. RESULTS AND DISCUSSIONS

In the absence of appropriate standards, the optimization of the extraction procedure has to rely on qualitative results. From the literature regarding the composition of lavender oil results that linalool and linalyl acetate are the major compounds of essential oils extracted from this plant[16]. In fact, in many papers dealing with the yield of extraction of essential oils from lavender, these two compounds are considered as marker molecules[18,27]. The chromatographic analytical method, based on detection with a triple-quadrupole was optimized to separate and to detect the compounds with m/z of 155 ($M+1^+$, for linalool) and

197 ($M+1^+$, for linalyl acetate) and to search the daughter fragments of these parent ions. The chemical structures of the target analytes are presented in Figure 1.



Linalool (MW 154.25 g/mol) Linalyl acetate (MW 196.29 g/mol)

Figure 1. The chemical structures of the two major compound from lavender oil

Based only on the information about the target compounds (molecular mass, log P values) we have undertaken a separation procedure and a detection method to allow the identification of linalool and linalyl acetate in the absence of standards. For setting up a MS-MS detection method, in some preliminary results were search the best conditions of detection and fragmentation to find the ion daughters of positive charged parent ions, i.e. $M+1^+$ = 155 m/z for linalool and $M+1^+$ = 197 m/z for linalyl acetate, respectively. Instead of real standards, a commercial lavender oil was used. The oil was diluted with weak mobile phase (10% MeOH, containing also 0.02% formic acid, to promote positive ionization) and the signal was recorded at various values of parameters of interface and triple-quadrupole: capillary voltage 2 – 4 kV, source temperature at 150°C, desolvation temperature at 200 - 500°C, desolvation gas velocity at 200 - 800 L/h, cone voltage 10 – 70 V, collision energy 0 – 70 V. The results have shown that the best values of the parameters of the interface and triple-quadrupole for detection of the parent ions and one major fragment are: capillary voltage 3.0 kV, source temperature at 150°C, desolvation temperature at 400°C, desolvation gas velocity at 500 L/h, cone voltage 30 V, collision energy 20 V.

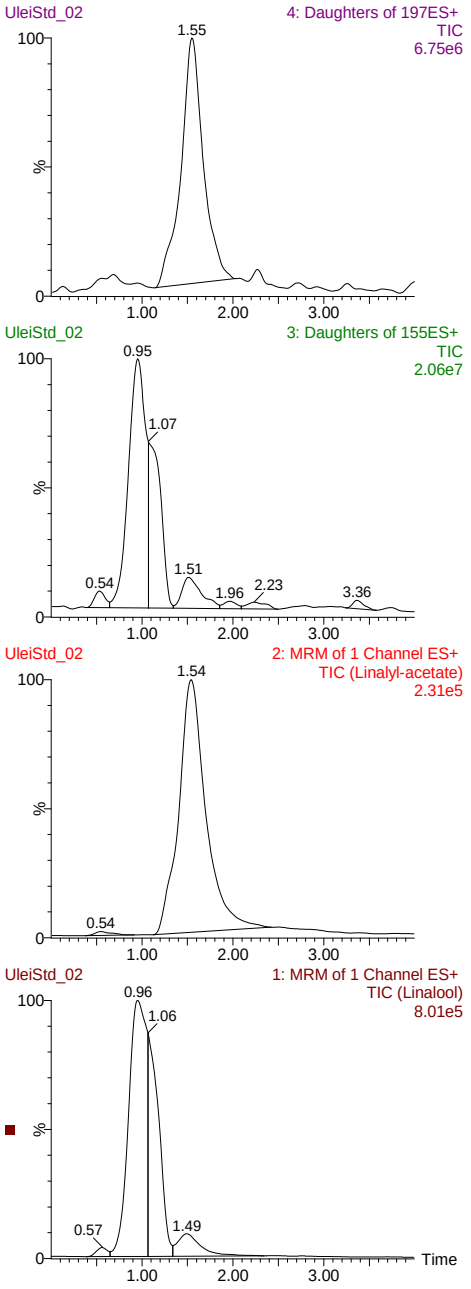


Figure 2.

The chromatographic separation of linalyl acetate and linalool from a sample of commercial lavender oil.

The first upper panel present the chromatogram of linalyl acetate, when the MS-MS method was settled to search the daughter fragments of ion with m/z 197, corresponding to linalyl acetate. Similarly, the second panel present the chromatogram of linalool, were the detector was programed to search the daughter fragments of ion with m/z 155.

The lower panels present the chromatograms obtained when the detector was programed in multiple reactions monitoring mode, searching only for the fragment with m/z 137 of the parent ion 197, for linalyl acetate and with m/z 137 of the parent ion 155, for linalool, respectively.

The next step was the optimization of the chromatographic separation of the target compounds, again, first from the commercial lavender oil product. After searching several elution gradient formulations, the optimal separation was achieved when the gradient profile was: 0 – 0.2 min, 30% A and 70% B; 0.2 – 1 min, linearly increase untill 100% B; 1 – 2.9

min, hold 100% B; 2.9 – 3.0 min, linearly decrease untill 70% B (initial condition). Figure 2 presents the separation of linalool and linalyl acetate from commercial lavender oil.

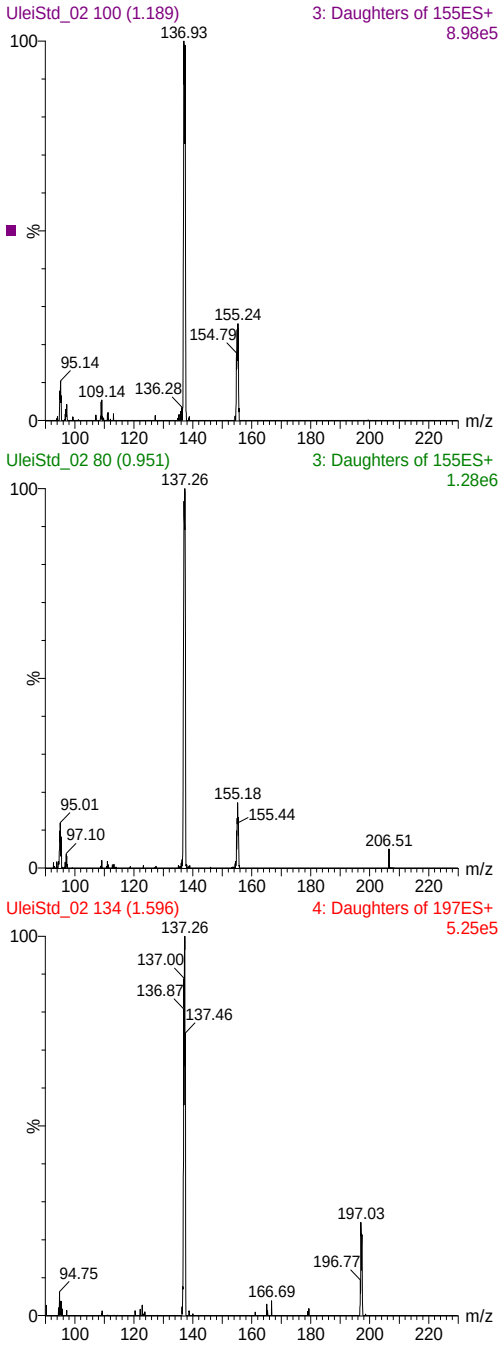


Figure 3.

The spectra of the daughter fragments of compounds separated in Figure 2..

The lower panel presents the ion fragments when the detector was settled to search for daughter of ion with m/z 197.

The first two panels present the ion fragments of parent ion with m/z 155 and these spectra are a confirmation of the fact that the compounds that coelute at 0.95 and 1.07 min have the same mass and produce the same daughter when subjected to the same collision energy.

The evidence that the eluted compounds from Figure 2 are linalool and linalyl acetate is presented in Figure 2 where the spectra of daughter fragments of parent ion with m/z 155 corresponding to linalool and of parent ion with m/z 197 corresponding to linaly acetate, respectively are presented.

In Figure 2 the second and the fourth panels present the chromatograms of linalool, and because the peaks present a shoulder, it is an indication that the peaks are not pure, i.e. there are two compounds that co-elute at close retention times. In Figure 3 the first two panels represent the spectra recorded at 0.98 and 1.18 minutes, respectively, i.e. of the first part and the one of the major peak from Figure 2 the second panel. As both these spectra are very similar, we may conclude that the two-peaks that co-elute in from Figure 2 belong to the same compound. It is well known that linalool has a stereogeneric center at C3 and for this, there are two stereoisomer: the R-isomer is known as licareol and the S-isomer as coriandrol.

To extract the essential oils from lavender in this study were used three methods: hydrodistillation, Soxhlet extraction and ASE. Figure 4 compares the peak areas of the linalool and linalyl acetate from essential oils obtained by Soxhlet extraction and ASE with the commercial product, used as imitation standard.

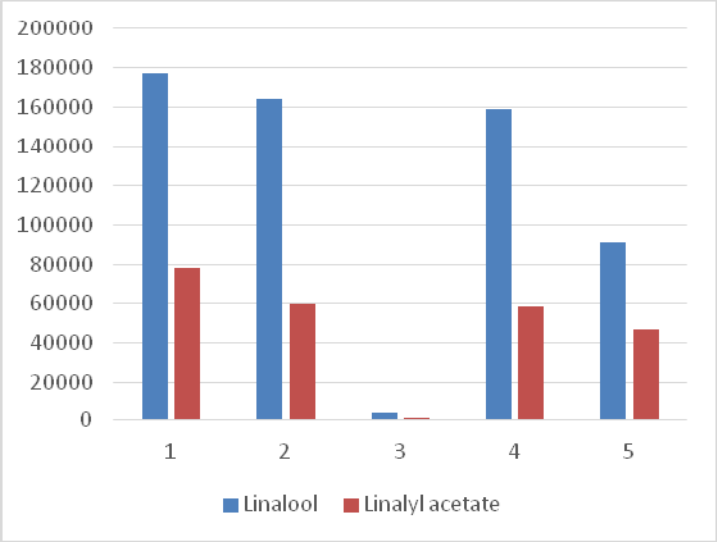


Figure 4. Comparison of yields of extraction, expressed as areas of the separated peaks, of different extraction methods: 1 = commercial lavender oil, used for comparison; 2 = extraction from dried flowers with methanol using ASE method; 3 = extraction from dried flowers with water using ASE method; 4 = Soxhlet extraction from fresh flowers with ethanol; 5 = Soxhlet extraction from dried flowers with ethanol.

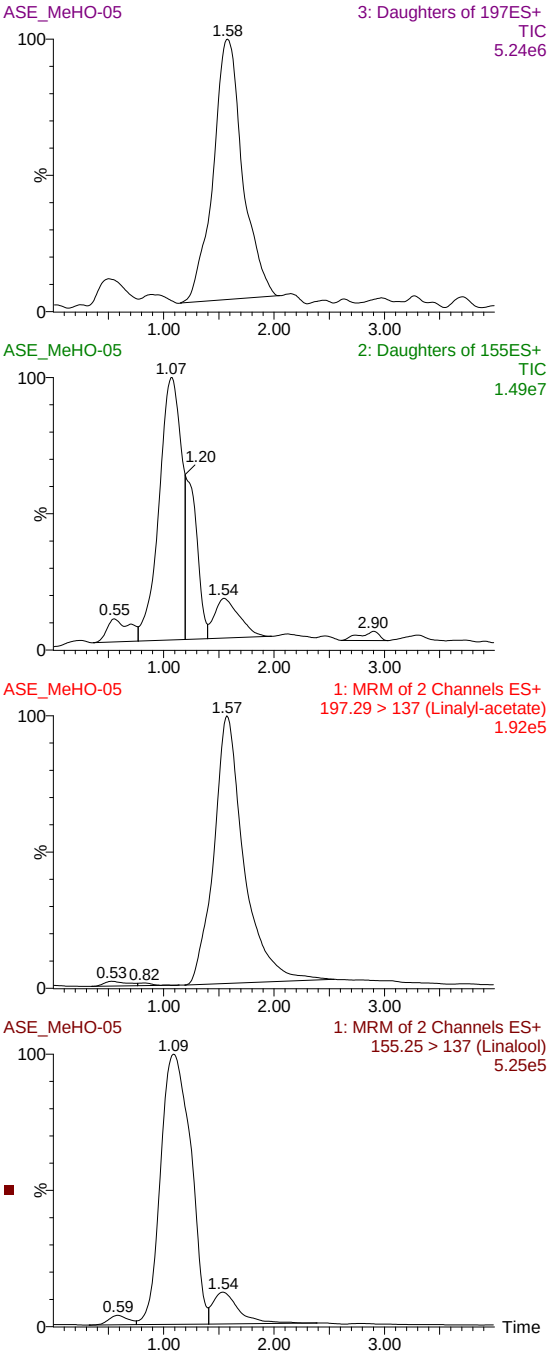


Figure 5.

The chromatograms of the ASE extract realized with methanol. For details of realization of the extraction see the text. For details of MS-MS method see Figure 2.

In Figure 5 there is presented an example of the chromatograms of the essential oil obtained using the ASE technique applied to dried flowers of lavender. Comparing the chromatograms with those of the commercial oil (Figure 2) one may see that the ASE extract contain both target compounds in high amounts.

The results from Figure 4 confirm that the best extraction method for the essential oils (linalool and linalyl acetate) is accelerated solvent extraction, when the extraction solvent is methanol. When water was used as extraction solvent, the extraction yield was very low (column 3 in Figure 4). The fresh flowers provide higher amount of essential oil than the dried flowers, as it can be seen by comparing the columns 4 and 5 from the same figure. The extraction methods should be further optimized as now the extraction yields for the target compounds are lower that the commercial product.

This is the first publication of use of an UPLC-MS-MS method for the separation of linalool and linalyl acetate from essential oils of lavender. The method can identify these compounds by their MS-MS spectra even in the absence of calibration standards. Using a gradient elution profile, the target compounds were separated in less than 3 minutes, with a resolution of 1.72.

4. CONCLUSIONS

Results presented in the present study showed a great variation in the yield, of essential oils obtained by different solvents and between fresh and dried flowers of *Lavandula*. The data obtained indicate that the content of main compounds (linalool and linalyl acetate) was similar with the content of the commercial product.

In the case of lavender, the amount of extracted compounds seems to be higher in fresh flowers, comparing with the dried material.

The optimized UPLC-MS-MS method can identify by MS spectra the linalool and linalyl acetate and can separate them in less than 3 minutes.

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REFERENCES

1. Ali B.; Al-Wabel N.A.; Shams S.; Ahamad A.; Khan S.A.; Anwar F. Essential oils used in aromatherapy: A systemic review. *Asian Pacific Journal of Tropical Biomedicine* **2015**, *5*, 601-611.
2. Asbahani A.E.; Miladi K.; Badri W.; Sala M.; Addi E.H.A.; Casabianca H.; Mousadik A.E.; Hartmann D.; Jilale A.; Renaud F.N.R.; Elaissari A. Essential oils: From extraction to encapsulation. *International Journal of Pharmaceutics* **2015**, *483*, 220-243.
3. Golmakani M.-T.; Rezaei K. Comparison of microwave-assisted hydrodistillation withthe traditional hydrodistillation method in the extractionof essential oils from Thymus vulgaris L. *Food Chemistry* **2008**, *109*, 925-930.

4. Rodríguez-Solana R.; Salgado J.M.; Domínguez J.M.; Cortés-Diéguez S. Characterization of fennel extracts and quantification of estragole: Optimization and comparison of accelerated solvent extraction and Soxhlet techniques. *Industrial Crops and Products* **2014**, *52*, 528-536.
5. Filly A.; Fabiano-Tixier A.S.; Louis C.; Fernandez X.; Chemat F. Water as a green solvent combined with different techniques for extraction of essential oil from lavender flowers. *Comptes Rendus Chimie* **2016**, *19*, 707-717.
6. Ferhat M.A.; Meklati B.Y.; Chemat F. Comparison of different isolation methods of essential oil from Citrus fruits: cold pressing, hydrodistillation and microwave 'dry' distillation. *Flavour and Fragrance Journal* **2007**, *22*, 494-504.
7. Pereira P.; Cebola M.-J.; Oliveira M.C.; Bernardo-Gil M.G. Supercritical fluid extraction vs conventional extraction of myrtle leaves and berries: Comparison of antioxidant activity and identification of bioactive compounds. *The Journal of Supercritical Fluids* **2016**, *113*, 1-9.
8. González-Rivera J.; Duce C.; Falconieri D.; Ferrari C.; Ghezzi L.; Piras A.; Tine M.R. Coaxial microwave assisted hydrodistillation of essential oils from five different herbs (lavender, rosemary, sage, fennel seeds and clove buds): Chemical composition and thermal analysis. *Innovative Food Science & Emerging Technologies* **2016**, *33*, 308-318.
9. Luque de Castro M.D.; Jiménez-Carmona M.M.; Fernández-Pérez V. Towards more rational techniques for the isolation of valuable essential oils from plants. *TrAC Trends in Analytical Chemistry* **1999**, *18*, 708-716.
10. Länger R.; Mechtler C.; Jurenitsch J. Composition of the Essential Oils of Commercial Samples of Salvia officinalis L. and S. fruticosa Miller: A Comparison of Oils Obtained by Extraction and Steam Distillation. *Phytochemical Analysis* **1996**, *7*, 289-293.
11. Rodrigues M.R.; Krause L.C.; Caramao E.B.; dos Santos J.G.; Dariva C.; Vladimir de Oliveira J. Chemical composition and extraction yield of the extract of Origanum vulgare obtained from sub- and supercritical CO2. *J Agric Food Chem* **2004**, *52*, 3042-3047.
12. Richter J.; Schellenberg I. Comparison of different extraction methods for the determination of essential oils and related compounds from aromatic plants and optimization of solid-phase microextraction/gas chromatography. *Anal Bioanal Chem* **2007**, *387*, 2207-2217.
13. Bakkali F.; Averbeck S.; Averbeck D.; Idaomar M. Biological effects of essential oils – A review. *Food and Chemical Toxicology* **2008**, *46*, 446-475.
14. Franco L.; Blanck T.J.J.; Dugan K.; Kline R.; Shanmugam G.; Galotti A.; von Bergen Granell A.; Wajda M. Both lavender fleur oil and unscented oil aromatherapy reduce preoperative anxiety in breast surgery patients: a randomized trial. *Journal of Clinical Anesthesia* **2016**, *33*, 243-249.
15. Fismer K.L.; Pilkington K. Lavender and sleep: A systematic review of the evidence. *European Journal of Integrative Medicine* **2012**, *4*, e436-e447.
16. Hanamanthagouda M.S.; Kakkalameli S.B.; Naik P.M.; Nagella P.; Seetharamareddy H.R.; Murthy H.N. Essential oils of Lavandula bipinnata and their antimicrobial activities. *Food Chemistry* **2010**, *118*, 836-839.
17. Soylu E.M.; Kurt Ş.; Soylu S. In vitro and in vivo antifungal activities of the essential oils of various plants against tomato grey mould disease agent Botrytis cinerea. *International Journal of Food Microbiology* **2010**, *143*, 183-189.
18. Lesage-Meessen L.; Bou M.; Sigoillot J.C.; Faulds C.B.; Lomascolo A. Essential oils and distilled straws of lavender and lavandin: a review of current use and potential application in white biotechnology. *Applied microbiology and biotechnology* **2015**, *99*, 3375-3385.
19. Muñoz-Bertomeu J.; Arrillaga I.; Segura J. Essential oil variation within and among natural populations of Lavandula latifolia and its relation to their ecological areas. *Biochemical Systematics and Ecology* **2007**, *35*, 479-488.

20. Sahraoui N.; Vian M.A.; Bornard I.; Boutekedjiret C.; Chemat F. Improved microwave steam distillation apparatus for isolation of essential oils: Comparison with conventional steam distillation. *Journal of Chromatography A* **2008**, *1210*, 229-233.
21. Singh P.A., H.; Rawat, M.S.M.; Joshi (nee Pant), G.; Jangwan, J.S. GC-MS Analysis of Essential Oil from Lavandula angustifolia Cultivated in Garhwal Himalaya. *The Natural Products Journal* **2015**, *5*, 268-272.
22. Shellie R.; Mondello L.; Marriott P.; Dugo G. Characterisation of lavender essential oils by using gas chromatography–mass spectrometry with correlation of linear retention indices and comparison with comprehensive two-dimensional gas chromatography. *Journal of Chromatography A* **2002**, *970*, 225-234.
23. Zagorcheva T.; Stanev S.; Rusanov K.; Atanassov I. Comparative GC/MS analysis of lavender (Lavandula angustifolia Mill.) inflorescence and essential oil volatiles. *Agricultural Science & Technology (1313-8820)* **2013**, *5*,
24. Zheljazkov V.D.; Astatkie T.; Hristov A.N. Lavender and hyssop productivity, oil content, and bioactivity as a function of harvest time and drying. *Industrial Crops and Products* **2012**, *36*, 222-228.
25. Wang H.; Liu Y.; Wei S.; Yan Z. Comparative seasonal variation and chemical composition of essential oils from the leaves and stems of Schefflera heptaphylla using microwave-assisted and conventional hydrodistillation. *Industrial Crops and Products* **2012**, *36*, 229-237.
26. Bratan A.; Mincea M.; Lupa I.-R.; Pirtea M.-G.; Ostafe V. Quantification of Nicotine and Cotinine in Teenager's Urine. *Studia Universitatis Babes-Bolyai Chemia* **2014**, *LIX*, 47-61.
27. Prusinowska R.; Śmigielski K.B. Composition, biological properties and therapeutic effects of lavender (Lavandula angustifolia L.). A review. *Herba Polonica* **2014**, *60*, 56-66.