Trop J Nat Prod Res, April 2023; 7(4):2713-2717 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Effect of Isolation Techniques on the Quantity, Quality, and Antimicrobial Activity of *Lavandula dentata* Essential Oils

Sarah B.E. Jilali[1]*, Ihamdane Rachid[2], Beniaich Ghada[3], Moubchir Tarik[4], Rezouki Sanae[5], Khadmaoui Abderrazzak[1]

[1]*Laboratory of Biology and Health, Ibn Tofil University, kenitra, Morocco*
[2]*Department of Chemistry, Organic chemistry, Catalysis and Environment Laboratory, University Ibn Tofail, kenitra, Morocco*
[3]*Department of Biology, Laboratory of Engineering, Electrochemistry, Modeling and Environment (LIEME), Faculty of Sciences Dhar EL Mahraz, USMBA, Fez, Morocco.*
[4]*Polyvalent Laboratory in Research and Development, Poly-disciplinary Faculty, Sultan Moulay Slimane University, Beni Mellal, Morocco.*
[5]*Laboratory of Plant, Animal and Agro-industry Productions, Faculty of Sciences, University of Ibn Tofail, Kenitra, Morocco*

ARTICLE INFO

Article history:
Received 15 January 2023
Revised 04 April 2023
Accepted 05 April 2023
Published online 01 May 2023

ABSTRACT

The present study evaluated the effect of extraction equipment and technique on the yield, content of main compounds and antibacterial activity of the essential oil of Lavandula dentata (EO) collected in the region of Sefrou, Morocco. The chemical composition study was performed using GC-MS methods for various essential oils extracted by Clevenger (HD) and microwave-assisted Clevinger hydrodistillation (MAHD). The result showed that the use of the (MAHD) technique provides a yield of 1.93% compared to the (HD) method, which provides only 1.65%. Regarding its chemical composition, seven chemicals were considered as the major components of L. *dentata* EO, the most important of them are: Linalool (HD: 34.84% and MAHD: 38.74%) succeeded by Linalyl aetate (HD: 27.28% and MAHD: 28.28%) and the Ketone: Camphor (HD: 8.85% and MAHD: 10.85%). The antibacterial activity of *L. dentata* EOs revealed that they have a significant antibacterial power against Gram-positive bacteria in comparison with Gram-negative bacteria and that this activity is very important for oils extracted by the MAHD method. These results strongly encourage the scientific efforts to develop the qualitative and quantitative value of aromatic and medicinal plants and their uses in various fields.

Keywords: essential oil, *Lavandula dentata*, antibacterial activity, extraction methods.

Introduction

Essential oils of aromatic and medicinal plants are a volatile mixture of chemical molecules with an oily viscosity that is often produced by plants. They are produced by all plant components and can be stored in a variety of sites, including secretory and epidermal cells, as well as glandular trichomes.[1,2] These essential oils are an important source of bioactive molecules that have chemical and biological properties.[3,4] Essential oils and their components are mainly used as food flavors; they have proven their therapeutic effectiveness through their antifungal activities as the essential oil of *Mentha pulegium*,[5] insecticides as the essential oil of *Origanum compactum* and *Dittrichia viscosa*,[6,7] Antioxidant and antibacterial activity, such as the essential oil of *Anvillea Radiata*.[8]
For the extraction of essential oils, hydrodistillation is the most commonly used technique at present.[9] However, studies have reported that this method can act negatively by causing the loss of material and energy.[9] Currently, research has been directed towards the optimization of extraction parameters in order to improve the yield and quality of essential oils and consequently reduce the cost and environmental pollution (such as CO_2 emissions), which encourages researchers to develop green alternative techniques based on cost-effective and sustainable criteria.

*Corresponding author. E mail: sarahbneljilali@gmail.com
Tel: +212 678 13 73 48

Citation: Jilali SBE, Rachid I, Ghada B, Tarik M, Sanae R, Abderrazzak K. Effect of Isolation Techniques on the Quantity, Quality, and Antimicrobial Activity of *Lavandula dentata* Essential Oils. Trop J Nat Prod Res. 2023; 7(4):2713-2717 http://www.doi.org/10.26538/tjnpr/v7i4.7

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

The valorization of aromatic and medicinal plants is done in two different ways; the first one consists of an exploitation as dry plants, the second one focuses on essential oils and other aromatic extracts extraction. Essential oils have been isolated from aromatic and medicinal plants by hydro-distillation, steam distillation, and organic solvent extraction for decades. However, these traditional methods are somewhat costly, especially since they are extremely energy and solvent intensive.[9,10]
In order to reduce the time and cost of extraction and at the same time improve the quality of the essential oils, new extraction techniques have been developed such as microwave-assisted extraction. The understanding and optimization of this extraction process are important for the research and development of the aromatic and medicinal plant sector in Morocco.
Lavandula dentata is an aromatic plant with a wide spectrum of biological activities, including antidepressant, anti-inflammatory, antioxidant, antibacterial and antifungal properties.[11-13] However, there is insufficient research and information on the activities of Moroccan varieties, especially against antibiotic-resistant pathogenic microorganisms. Several researches have revealed that the extraction method, duration, and conditions affect the quantity and quality of essential oils extracted from aromatic and medicinal plants. Therefore, the objective of the present study was to evaluate the effects of the extraction technique on the yield, composition, and antibacterial activity of *L. dentata* essential oils from the Sefrou region (Northern Morocco).

Materials and Methods

Plants material
The present study was carried out using the leaves of *L. dentata*; The whole plant was collected in June 2021, during peak flowering, in the region of Sefrou, Middle Atlas of Northern Morocco. A botanical

 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

identification of *L. dentata* was made by a botanist and assigned the reference D/O12/10372 before being placed in a herbarium. The leaves were then dried in the shade for 7 days in a dry, ventilated area at 25°C.

Extraction of essential oils

The extraction of essential oils was performed by two different methods, Clevenger hydrodistillation (HD) and microwave-assisted Clevinger hydrodistillation (MAHD).

Hydro-distillation by Clevenger (HD): This extraction method was performed using a Clevenger apparatus. The distillation was performed by boiling 200 g of the wild plant for 5 hours with 1.2 L of distilled water in a 2 L flask topped with a 60 cm long graduated column connected to a cooler. During the distillation process, the flask is maintained at 80°C. The oil-laden vapors pass through a cooler, which condenses and falls into a burette. The distillate includes hydrolate and essential oils. The essential oils were extracted with a micropipette and stored in an Eppendorf tube at 4°C, protected from light.

Microwave Assisted Clevenger Hydro-distillation (MAHD): Microwave hydrodistillation was carried out using the microwave oven with a maximum delivered power of 600 W, in which 200 gr of leaves were heated for 180 min with 1.2 liters of water added. This period was sufficient to extract all the essential oils from the sample. Each extraction was performed at least three times.

Chromatographic analysis (GC-MS): GC-MS was used to determine the composition of the essential oils (GC-MS). A GC system with a flame ionization detector and an HP-5MS capillary column was used for the analysis. Temperature-programmed GC was used setting temperatures at 35°C to 250°C and a gradient of 5°C/min. Retention indices were determined by GC with two fused silica capillary columns (30 m 0.25 mm). The operating temperature was set at 35°C and then increased to 250°C at a rate of 5°C/minute, with the lower and higher temperatures held for 3 and 10 minutes, respectively. The flow rate of the carrier gas (helium) was 1.0 mL/min. In fractionated mode, one sample of 1.0 L was injected (fractionation ratio, 1: 100). The essential oil constituents were identified by comparing their mass spectra with those of the NIST and Adams library.

Essential oil yield

The essential oil yields were expressed in g per 100 g of dry vegetable material and were calculated using the following equation[3]:

$$R_{EO}(\%) = \frac{m_{EO}}{m_{md}} \times 100$$

With: R_{EO} the essential oil yield in (%), m_{EO}: the mass of the essential oil in g and m_{md}: the mass of the dry plant matter.

Antibacterial activity

The essential oils obtained are tested for their antibacterial activity by two methods.

Disc diffusion method: The agar diffusion technique is used to investigate antibacterial activity in the disc diffusion method[14]. *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli* are used as test microbes. TSA Medium is dispensed with a 100 L volume of bacterial culture (5.10+5 CFU/ml).

Sterile filter paper discs (6 mm) are soaked with 15ul essential oil and placed on the top of the TSA medium, which has already been inoculated with the test bacteria. Finally, the boxes are incubated at 37 °C for 24 hours. The positive and negative controls are respectively chloramphenicol (50 mg/ml) and DMSO. The presence of a clear zone around the discs after incubation demonstrates the inhibitory action.

Micro-dilution method: The micro-dilution technique with resazurin is used to determine the minimum inhibitory concentration (MIC) of essential oils. [15] 100 µl of the essential oil solution (2.5 mg / ml of 90% DMSO) is pipetted into the first row of 96-well plates, followed by 100 µl of tryptic soy broth in all other wells of the micro-dilution plates. A sequence of dilutions is started by transferring 100 µl of test material from the first row to the next row's wells in the same column, so that each well contains 100 µl of test material at serially decreasing concentrations. Then 10 µl of bacterial suspension (10+8 CFU/ml) is added to each well. All positive color changes from purple to pink or colorless were recorded. The MIC value was determined by taking the lowest concentration at which the color change occurred.[16]

Statistical analysis

Statistical analysis of the results was performed using SPSS for Windows® (version 21.0). The data were subjected to one-way analysis of variance (ANOVA) to determine the difference between the extreme values of the group. Fisher's Least Significant Difference (LSD) test was used to distinguish between significant and non-significant means.

Results and Discussion

The monitoring results of essential oil extraction kinetics of L. *dentata*, by the two extraction methods (Figure 1) showed that; the extraction n kinetics by HD and MAHD are almost the same rate, also Figure 1 shows that the yield of lavender essential oils varied with the distillation time. In fact, Clevenger distillation yields 1.65 g of essential oil per 100 g of dry plant. While microwave distillation gives a yield of 1.93 g of essential oil per 100 g of dry plant.

Similar results of essential oil yield variation with time were observed for some plant materials extracted by hydrodistillation or by microwave, such as *Origanum compactum*[9] *Nigella sativa L.*.[17]*sandalwood.* [18]

The chemical composition results of *Rosmarinus officinalis* essential oils extracted by the two methods of extraction are grouped in Table 1. These results identified 9 compounds for the HD method and 10 compounds for the MAHD method which represent a total of 90.38% in the HD method and 95.04% in the MAHD method. The results show that the chemical composition of the essential oils obtained by the two methods is similar for both MAHD and HD methods with a slight quantitative difference in some components. The majority component is Linalool, with a slightly better rate in the MAHD than in HD, which is respectively 38.55% and 35.62%. However, the proportions of Linalyl acetate 25.23% in HD and 28.55% in MAHD) and 1,8-Cineole (5.17% in HD and 2.22% in MAHD)

Using the two methods Clevenger Hydrodistillation (HD), and Clevenger Microwave-Assisted Hydrodistillation (MAHD), the chemical composition of the essential oils of *L. dentata* presents the majority compounds and the other minority compounds, in order to highlight the effect of the extraction techniques on the chemical composition. Figure 2 shows the majority compounds of the essential oils extracted by the two methods.

It is noted that the nature of the components is the same for the three types of essential oils, as well as being characterized by the low percentage of monoterpenes; Among the compounds that are abundant we cite the monoterpenols which are represented by a domination of linalool (35.62% to 38.55%). Terpene esters are present in a similar percentage to monoterpenols, with linalyl acetate as the most abundant, followed by camphor. Finally, the oils studied are rich in monoterpenols and terpene esters but poor in terpene oxide and sesquiterpenes.

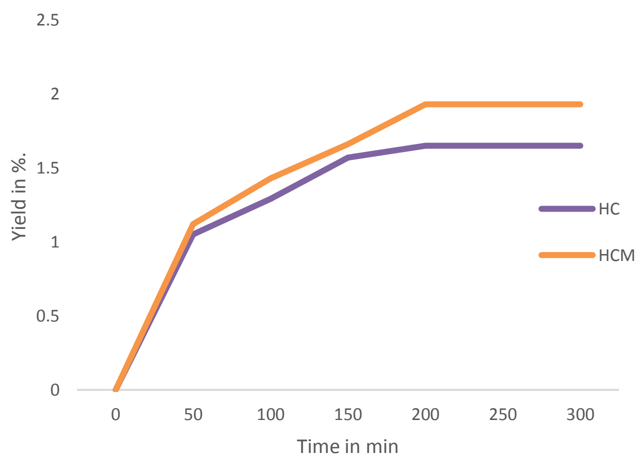

Figure 1: The yield of lavender essential oils by the two methods studied

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 1: Majority compounds of the studied essential oils extracted by the two types of distillation (HD and MAHD).

RI	Compounds	Formula	HD(%)	MAHD (%)
948	α-Pinene	$C_{10}H_{16}$	-	0.1
1059	1,8-Cineole	$C_{10}H_{18}O$	5.17	2.22
1082	Linalool	$C_{10}H_{18}O$	35.62	38.55
1121	Camphor	$C_{10}H_{16}O$	10.88	12.01
1138	Borneol	$C_{10}H_{18}O$	3.42	2.88
1137	Terpinen-4-ol	$C_{10}H_{18}O$	5.22	5.98
1143	α-Terpineol	$C_{10}H_{18}O$	0.9	1.1
1272	Linalyl acetate	$C_{12}H_{20}O_2$	25.23	28.55
1270	Lavandulyl acetate	$C_{12}H_{20}O_2$	3.4	2.9
1440	β-Farnesene	$C_{15}H_{24}$	0.54	0.75
	Total		90.38	95.04

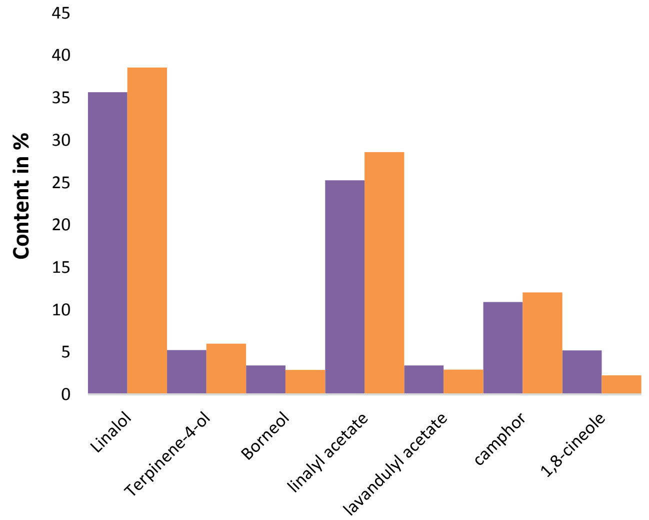

Figure 2: Comparison of the chemical composition of the essential oils of *Lavandula dentata* from the two methods studied

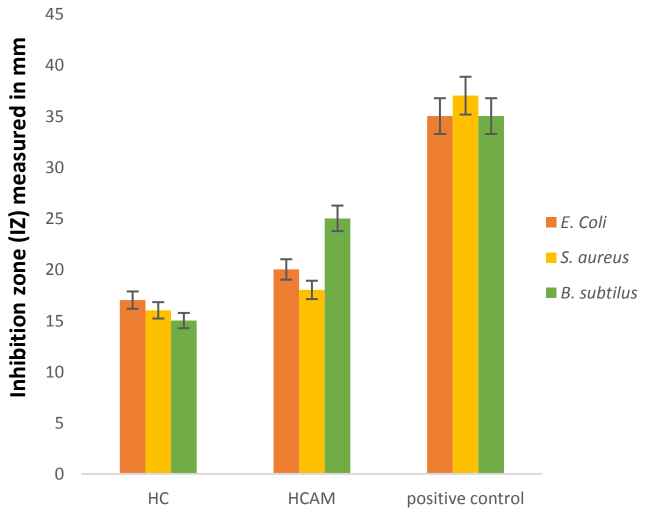

Figure 3: Antibacterial activity of HD and MAHD essential oil against pathogenic bacteria (inhibition zone (IZ) measured in mm)

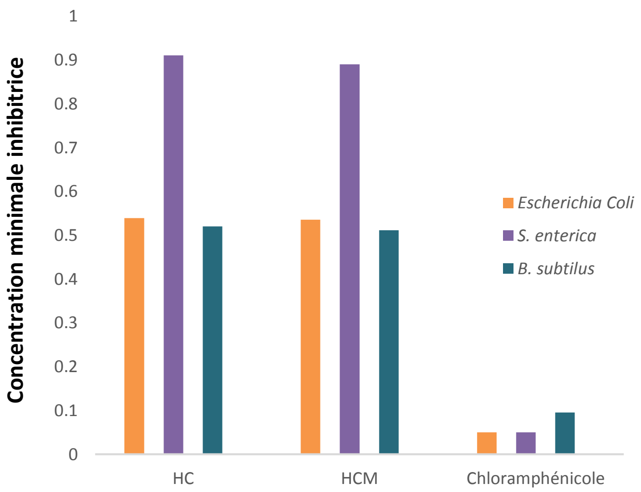

Figure 4: MIC of Lavender essential oil extracted by HD and HCAM against pathogenic bacteria

It should be noted that the small fluctuations in the contents of the compounds mentioned in Table 1 differ according to the extraction method. Indeed, comparing the results obtained from the two types of extraction of essential oil of *Lavandula vera*, we note that the one extracted by the HD simple method is lower in linalool (35.68%), and camphor (10.88%) compared to the MAHD method without neglecting the moderate presence of other components.

Essential oils were characterized by their high content of Linalool. This compound was also found in essential oils of *L. dentata* coming from other biotopes, but in variable proportions .[19,20] According to our results, compared to some previous studies on the phytochemical composition of *L. dentata*, we can conclude that the differences in the phytochemical composition of the essential oil are most likely attributable to environmental variables such as plant age, vegetative cycle, or even genetic factors.[3,21] The drying procedure also had a substantial impact on the amount of essential oil as well as the phytochemical.[3,22]

Figure 3 and 4 depicts the antibacterial activity of HD and MAHD essential oils against pathogenic bacteria. The essential oil has varying degrees of antibacterial activity. The results of the disc diffusion test show that the essential oil tested has significant antibacterial activity against Gram-positive bacteria but not against Gram-negative bacteria.

MAHD extracted oils show a higher antibacterial effect than HD extracted oils. This can be explained by the high content of some compounds in MAHD extracted oils such as linalool.

Many dangerous bacteria can be inhibited from growing by the presence of VOs in therapeutic plants. No one can dispute the contribution of VOs to the discovery of possible new microbicidal therapies in the field

of infectious disorders due to microbial resistance. Among The important substances used today to create effective antibacterial agents include 2-isopropyl-5-methyl phenol, linalool, eugenic acid, menthols and caryophyllene.[23,24]
The essential oils showed inhibition zones against all microorganisms tested. The data obtained from the disc diffusion method indicated that *S. aureus* was the most sensitive microorganism with the largest zones of inhibition (15-25 mm) and *Escherichia coli* showed the smallest zones of inhibition (17- 20mm). It is relatively difficult to attribute an essential oil's antimicrobial effect to one or a few active ingredients. In addition to the main components, secondary components also play a crucial role due to synergistic, although antagonistic and additive effects.[25]

Conclusion

The extraction methods results revealed the presence of significant fingerprints on the quantity, quality and antibacterial activity of the studied lavender essential oils. The yield obtained by MAHD was higher than that obtained by HD, GC-MS also shows that most of the compounds of the oils obtained by MAHD, such as linalool, linalyl acetate and camphor, that have a high percentage in comparison to those obtained by HD; this qualitative and quantitative variation directly influences the antibacterial activity. This shows that optimizing oil extraction by modifying several parameters can be an economical and sustainable way to valorize aromatic and medicinal plants.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential Oils- A review. Food Chem. Toxicol.2008; 46: 446–475.
2. Dhifi W, Bellili S, Jazi S, Bahloul N, Mnif W. Essential Oils' Chemical Characterization and Investigation of Some Biological Activities: A Critical Review. *Medicines.* 2016; 3*:* 25.
3. Pophof B, Stange G, Abrell L. Volatile Organic Compounds as Signals in a Plant—Herbivore System: Electrophysiological Responses in Olfactory Sensilla of the Moth *Cactoblastis cactorum*. Chem. Senses. 2005; 30: 51–68.
4. Rezouki S, Allali A, Bouchra L, Eloutassi N, Fadli M. The impact of the harvesting period and drying conditions on the essential oil yield of *Rosmarinus officinalis*, *Thymus satureioides* and *Origanum compactum* from the taza-taounate region. Asian J. Agric. Biol. 2021; 202004251.
5. Allali A, Rezouki S, Fadli A, El Moussaoui A, Bourhia M, Salamatullah AM, Alzahrani A, Alyahya HK, Albadr NA, Agour A, Eloutassi N. Chemical Characterization and Antioxidant, Antimicrobial, and Insecticidal Properties of Essential Oil *from Mentha pulegium* L. Evid.-Based Complement. Altern. Med. 2021; 1108133
6. Allali A, El Abdali Y, Rezouki S, Abdelfattah M, Bourhia M., Salamatullah AM, Alzahrani A, Alyahya HK, Albadr NA, Nafidi HA, Ouahmane L, Fadli M. Chemical composition and antifungal, insecticidal and repellent activity of essential oils from *Origanum compactum benth.* used in the Mediterranean diet. Front. Plant Sci. 2022; 13. doi: 10.3389/fpls.2022;798259
7. Mssillou I, Agour A, Allali A, Saghrouchni H, Bourhia M, El Moussaoui A, Salamatullah AM, Alzahrani A, Aboul-Soud MAM, Giesy JP. Antioxidant, Antimicrobial, and Insecticidal Properties of a Chemically Characterized Essential Oil from the Leaves of *Dittrichia viscosa* L. Molecules. 2022; 27: 2282.
8. Beniaich G, Hafsa O, Maliki I, Bin Jardan YA, El Moussaoui A, Chebaibi M, Agour A, Zouirech O, Nafidi HA, Khallouki F, Taleb M. GC-MS Characterization, In Vitro Antioxidant, Antimicrobial, and in Silico NADPH Oxidase Inhibition Studies of *Anvillea Radiata* Essential Oils. Horticulturae.2022; 8: 886.
9. Rezouki S, Allali A, Eloutassi N, Fadli M. Biotechnological valorization of aromatic plants in Morocco: impact of extraction methods on the yield and chemical composition of *Origanum compactum* Benth. of Taounate (Northern Morocco). P. Cell. Biotech. Mol. Biol. 2020; 21:61-62
10. Gharby S, Oubannin S, Ait Bouzid H, Bijla L, Ibourki M, Gagour J, Koubachi J, Sakar E.H, Majourhat K, Lee L.H, Harhar H, Bouyahya A. An Overview on the Use of Extracts from Medicinal and Aromatic Plants to Improve Nutritional Value and Oxidative Stability of Vegetable Oils. *Foods.* 2022; *11*:3258
11. Zuzarte M, Vale-Silva L, Gonçalves MJ. Antifungal activity of phenolic-rich *Lavandula multifida* L. Essential oil. Euro. J. of Clin. Microb. & Infect. Disea. 2012; 31 (7): 1359–1366.
12. El Abdali, Y, Agour A, Allali A, Bourhia M, El Moussaoui A, Eloutassi N, Salamatullah AM, Alzahrani A, Ouahmane L, Aboul-Soud MAM, Giesy JP, Bouia A. *Lavandula dentata* L.: Phytochemical Analysis, Antioxidant, Antifungal and Insecticidal Activities of Its Essential Oil. Plants.2022; 11(3) :311.
13. El Abdali Y, Beniaich Gh, Mahraz MA, El Moussaoui A, Bin Jardan YA, Akhazzane M, Chebaibi M, Nafidi HA, Eloutassi N, Bourhia M, Bouia A. Antibacterial, Antioxidant, and in silico NADPH Oxidase Inhibition Studies of Essential Oils of *Lavandula dentata* against Foodborne Pathogens. Evid-Based Compl. and Altern. Med. 2023; 12:9766002.
14. Kelen M, Tepe B. Chemical composition, antioxidant and antimicrobial properties of the essential oils of three *Salvia* species from Turkish flora Bioresource. Technology. 2008; 99: 4096-4104.
15. Sarker SD, Nahar L, Kumarasamy Y. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. Methods. 2007; 42: 321-324.
16. Hussain A, Wahab S, Zarin I, Hussain MDS. Antibacterial activity of the leaves of *coccinia indica* (w. and a) wof india. Adv. in boil. Resea. 2010; 4:(5):241-248.
17. Benkaci-Ali F, Baaliouamer A, Meklati BY. Kinetic Study of Microwave Extraction of Essential Oil of *Nigella sativa* L. Seeds. Chromatographia. 2006; 3–4:227–31.
18. Kusuma H, Mahfud M. Comparison of kinetic models of oil extraction from sandalwood by microwave-assisted hydrodistillation. Inter. Food Resea. J. 2017; 24:1697–702.
19. Tsuro M, Inoue M, Kameoka H. Variation in essential oil components in regenerated lavender (*Lavandula vera* DC) plants, Sci. Hortic. 2001; 88: 309–317.
20. Kirimer N, Mokhtarzadeh S, Demirci BF, Goger KM, Khawar F. Phytochemical profiling of volatile components of *Lavandula angustifolia* Miller propagated under in vitro conditions Ind. Crop. Prod. 2017; 96:120-125,
21. Barra A. Factors Affecting Chemical Variability of Essential Oils: A Review of Recent Developments. *Nat. Prod. Commun.* 2009; 4 (8): 1147-54.
22. Caputo L, Amato G, de Bartolomeis P. *et al.* Impact of drying methods on the yield and chemistry of *Origanum vulgare* L. essential oil. Sci Rep. 2022; 12:3845
23. Akthar M.S, Degaga B, Azam T. Antimicrobial activity of essential oils extracted from medicinal plants against the

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

pathogenic microorganisms: a review. Biol. Sci. Pharm. Res. 2014; 2:1-7
24. Osuntokun, OT, Ogunleye AJ. Prospects of essential oils in drug discovery. Adv cytol pathol. 2017; 2:1-3.
25. Bassolé IHN, Juliani HR. Essential oils in combination and their antimicrobial properties. Molecules. 2012; 17:3989–4006.