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ESSENTIAL OIL EXTRACTION FROM *LAVANDULA ANGUSTIFOLIA, JUNIPERUS COMMUNIS*, AND *HELICHRYSUM ITALICUM* FROM MALESIA E MADHE AND EVALUATION OF THEIR ANTIMICROBIAL AND ANTIFUNGAL ACTIVITY

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Abstract

The importance of this study was to evaluate the effect of the variable climate and factors such as soil and geographic location on essential oil yield quality and to prove the effectiveness of these essential oils in destroying several bacterial and fungal pathogens from human infections. Antibiotic-resistant bacteria have become nowadays a major concern worldwide so the usage of essential oils such as *Lavandula angustifolia*, *Juniperus communis*, and *Helichrysum italicum* could serve as an alternative way of combating resistant bacteria in different patients. The purpose of this study was to extract and evaluate the antimicrobial and antifungal properties of *Lavandula angustifolia, Juniperus communis,* and *Helichrysum italicum*.

Lavandula angustifolia, Juniperus communis, and *Helichrysum italicum* were collected from different locations in Malesia e Madhe District in Albania. The extraction of the essential oils from these plants was done by using a distillation process at the ecotoxicological laboratory at the Faculty of Natural Sciences, University of Shkodra "Luigj Gurakuqi". The essential oils were evaluated for antimicrobial and antifungal activity as follows: antimicrobial activity was done by disc diffusion test in Müller-Hinton Agar against *Staphylococcus aureus, Salmonella typhimurium,* and *Escherichia coli*, while against antifungal activity for *Candida albicans* on Sabourand agar. Microorganisms used in our study are samples which are taken from patients diagnosed with an infection from the throat, infection from the skin, infection from urine, etc. from the Center for Microbiological Diagnostication "Wolfdieter Sixl" at the above-mentioned faculty.

Essential oil yields of dried leaves and fruits ranged from *Lavandula angustifolia* 5.1 - 6.3%, *Juniperus communis* 0.7 - 1.1%, and *Helichrysum italicum* < 1%. The study demonstrates that these essential oils from *Lavandula, Juniperus, and Helichrysum* not only have bactericidal and fungicidal effects against microorganisms such as *Staphylococcus aureus, Salmonella typhimurium, Escherichia coli,* and *Candida albicans*, but they inhibit the bacteria and fungus growth comparable with antibiotics and antifungals like ampicillin, amoxicillin, ciprofloxacin, negram, nitrofurantoin, econazole, nystatin, ketoconazole, miconazole, clotrimazole, etc.

The inhibitory activity of the examined essential oil proved to be comparable to the antimicrobial and antifungal activity drugs used.

***Key words**: Lavandula angustifolia, Juniperus communis, Helichrysum italicum, Essential oil, Antibacterial, Antifungal activity.*

1. Introduction

Antibiotic-resistant bacteria have become nowadays a major concern worldwide, so the usage of essential oils from plants, such as *Lavandula angustifolia, Juniperus communis,* and *Helichrysum italicum* could serve as an alternative way of combating resistant bacteria in different patients. In Albania are present about 51% of the Balkan plant species and 33% of the European plant species [1]. Albania is rich in the natural genetic diversity of medicinal and aromatic plants, representing one of the European countries with the richest flora. This diversity is attributed to favorable climate conditions, ranging from subtropical to coastal to continental inland. Its geographical position in the Mediterranean region and the Balkan Peninsula results in many different types of natural landscapes [2].

Lavender oil is active against many species of bacteria, including those resistances to antibiotics such as methicillin resistance Staphylococcus aureus and vancomycin resistance enterococcus [3, 4]. Lavender is widely used for the essential oils derived from it. Lavender oils have many health benefits and biological properties [5]. Volatile compounds of plant extracts, particularly essential oils are known as secondary plant metabolites which have been used primarily in aromatherapy, cosmetics, and medicinal purposes [6].

Various essential oils of different plants such as thyme, oregano, mint, cinnamon, cumin, salvia, clove, and eucalyptus have been observed to possess strong antimicrobial properties [7]. Commercially used essential oil is obtained from the flowering tops by steam distillation [8]. The genus *Helichrysum* (Miller) belongs to the Asteraceae family and includes more than a thousand taxa that have a high occurrence in the Mediterranean areas of Europe [9]. *Helichrysum italicum* (Roth.) G. Don fil., due to its various beneficial health effects, represents an important plant in the traditional medicine of Mediterranean countries [10]. It grows widely in natural, dry, and sandy-rocky areas of Mediterranean regions, and is adapted to survive in environments that lack water [11].

The main factors influencing the composition of plant extracts and essential oils are the environmental characteristics of growing sites (ecology, climate, and geographical location), the developmental stage of the plant, the texture, and acidity of soils, and the plant's genotype or subspecies [12]. *Juniperus communis* L. is a widely spread scrub throughout the territory of Albania. Berries export plays an important role in the economic aspect of the population of Albania. From the statistics taken in last year's results, Albania is one of the countries with the biggest exports in the Balkan area for *Juniperus communis* L. [13]. The yield of the three essential oils studied depends on main climatic conditions such as temperature, daylight, and rainfall in the region of Malesia e Madhe, Shkoder.

This study aimed to evaluate the effect of the variable climate and soil on three essential oils yield quality from *Lavandula angustifolia, Juniperus communis, Helichrysum italicum* and to evaluate the "*in vitro*" antimicrobial effect of these three essential oils in destroying several bacterial and fungal pathogens in human infections.

2. Materials and Methods

2.1 Sampling of medicinal plants

During our study, the medicinal plants were collected in Malesia e Madhe, Region or more specifically: *Lavandula angustifolia* was taken from Gruemira point, *Helichrysum italicum* was taken from Koplik, and *Juniperus communis* was taken from Rrjolli (Figure 1). They were harvested by local farmers.

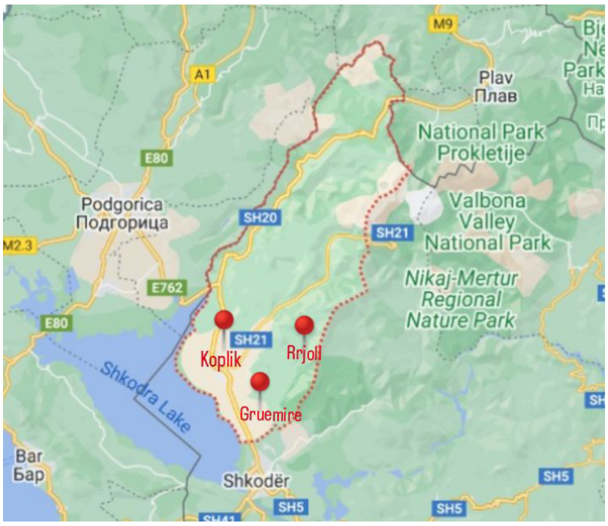

Figure 1. Sampling points of medical plants *Lavandula angustifolia, Juniperus communis* and *Helichrysum italicum*

2.2 Essential oil isolation

The essential oils of *Lavandula angustifolia, Juniperus communis, and Helichrysum italicum* were isolated from the dried and crushed samples which were received from different farmers in Malësia e Madhe, Albania. The true lavender *(Lavandula angustifolia)* and Italian strawflower (*Helichrysum italicum)* were harvested in May and September, while *the* dried fruits of *the* common juniper (*Juniperus communis)* were collected only in September 2023.

Dried materials from flowers and berries were analyzed in September and November 2023. The determination of essential oils from dried plant materials was done by using the hydrodistillation method (ISO-6571-2008) [14]. Approximately 100 grams of dried material were weighed from each sample and transferred in the 1,000 mL round bottom flask, where for about 2 hours a quantity of 500 mL distilled water was added. The essential oils of *Lavandula angustifolia, Helichrysum italicum,* and *Juniperus communis* were separated by decantation and then dried on anhydrous sodium sulfate, the volume of essential oil was measured, and the yield was calculated.

Essential oils were stored at 4 ^{0}C until measuring antimicrobial activity. The extraction of the essential oils from these plants was done by using a hydrodistillation process at the Ecotoxicological Laboratory in the Faculty of Natural Sciences, University of Shkodra "Luigj Gurakuqi" (Figure 2).

Figure 2. Dried material preparation and cooking

2.3 Disc diffusion assay with essential oils

The "*in vitro*" antimicrobial activity of essential oils from *Lavandula angustifolia, Juniperus communis,* and *Helichrysum italicum* were tested by disc diffusion assay in Müller-Hinton Agar against *Staphylococcus aureus, Salmonella typhimurium,* and *Escherichia coli*, while disc diffusion assay against antifungal activity for *Candida albicans* was tested on Sabouraud agar. Microorganisms used in our study were samples taken from patients diagnosed with an infection from the throat, infection from the skin, infection from urine, etc., from the Center for Microbiological Diagnostication "Wolfdieter Sixl" at the Faculty of Natural Sciences, University of Shkodra "Luigj Gurakuqi".

Three essential oils were tested "*in vitro*" for their antibacterial and antifungal properties by disc diffusion. All three essential oils were obtained from hydrodistillation. The disc diffusion method was conducted to the method previously described by (Bauer *et al.*, [15]). Bacterial cultures from the throat, skin, urine, etc. were firstly inoculated in selective media such as blood agar, endo agar, and desoxycholate citrate agar at 37 ^{0}C for 18 - 24 hours, while fungal cultures were inoculated in Sabourand Dextrose Agar CAF 50 at 37 ^{0}C for 3 - 5 days. A sterile bacteriological loop was used to transfer the bacterial and fungal cultures from blood agar, endo agar, etc. on the surface of Müller-Hinton agar.

The assay by disc diffusion was conducted for 3 parallel samples. Antimicrobial and antifungal activity of the essential oils was tested for 50 µL and 100 µL essential oils from *Lavandula angustifolia, Juniperus communis,* and *Helichrysum italicum*. The discs of three essential oils were aseptically applied to the surface of the Müller-Hinton agar which were incubated at 37 ^{0}C for 18 - 24 hours. The antimicrobial and antifungal activity was evaluated by measuring the diameter of the zone of inhibition against the test microorganisms. A measurement of the diameter of the zone of inhibition was evaluated in millimeters: (< 10mm) - no antimicrobial activity; (10 - 15 mm) - weak antimicrobial activity; (16 - 20 mm) - moderate antimicrobial activity; and (> 20 mm) - strong antimicrobial activity [17]. Ampicilline, amoxilline, ciprofloxacine, negrame, nitrofurantoine, econazole, nistatine, ketoconazole, myconazole, clotrimazole were used as references.

3. Results and Discussion

Essential oil yields of dried leaves and fruits ranged such as *Juniperus communis* 0.7 - 1.1%, *Lavandula angustifolia* 5.1 - 6.3%, and *Helichrysum italicum* < 1% (Figure 3).

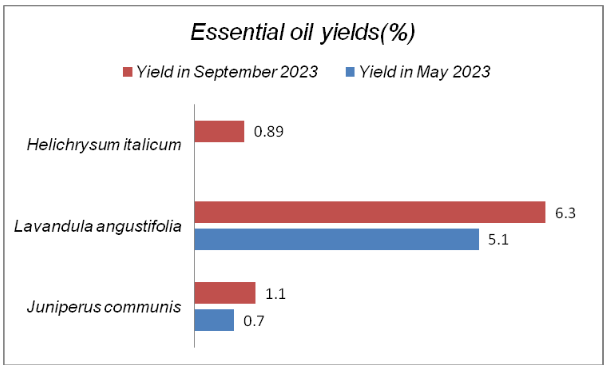

Figure 3. The percentage of the essential oil yields in percentage

The highest quantity of oil yield was measured during the period of September because of favorable climatic conditions from May to September 2023 (Figure 4), [16].

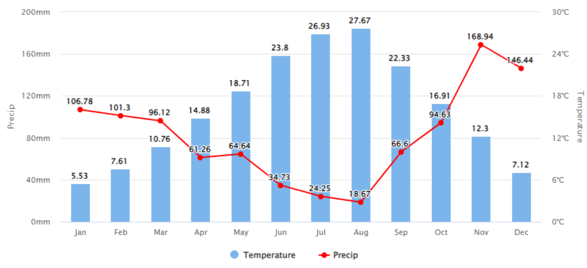

Figure 4. The chart shows the mean monthly temperature and precipitation of Koplik in recent years

The antibacterial activity of the essential oils was tested in 50 µL and 100 µL of essential oils from *Lavandula angustifolia, Juniperus communis,* and *Helichrysum italicum*. Essential oils of *Juniperus communis* and *Lavandula angustifolia* gave a comparable moderate sensitive zone of inhibition from 7 to 18 mm with the antibiotics and antimycotics used in our daily routines, such as ampicillin, amoxicillin, ciprofloxacin, negram, nitrofurantoin, econazole, nystatin, ketoconazole, miconazole, and clotrimazole.

Helichrysum italicum essential oil doesn't have any effect on *Escherichia coli* in both concentrations - 50 μL and 100 μL (Table 1).

The inhibition zone of *Helichrysum italicum* for *Staphylococcus aureus* in concentration 50 μL had an inhibition zone lower than 4 mm (no antimicrobial activity) and in 100 μL concentration had an inhibition zone which varied from 7 mm to 12 mm which is weak antimicrobial activity. *Helichrysum italicum* essential oil for *Salmonella typhimurium* in concentration from 50 μL had an inhibition zone lower than 4 mm (no antimicrobial activity) and in 100 μL concentration had an inhibition zone which varied from 8 mm to 13 mm (weak antimicrobial activity). The inhibition zone of *Helichrysum italicum* for *Candida albicans* in concentration 50 μL had an inhibition zone lower than 3 mm (no antifungal activity), while in concentration from 100 μL had an inhibition zone which varied from 7 - 12 mm (weak antimicrobial activity) (Table 1).

Lavandula angustifolia in concentrations of 50 μL and 100 μL for *Escherichia coli* gives an inhibition zone that varies from 12 mm to 18 mm (moderate antimicrobial activity) (Table 2).

Lavandula angustifolia in concentrations of 50 μL and 100 μL for *Staphylococcus aureus* gave an inhibition zone that varied from 10 mm to 16 mm (weak to moderate antimicrobial activity). The inhibition zone of *Lavandula angustifolia* in concentrations of 50 μL and

Table 1. Antimicrobial and antifungal effects of *Helichrysum italicum*

Microbial strain	Volume (μL)	Inhibition (mm)		
		Sample 1	Sample 2	Sample 3
Escherichia coli	50	-	-	-
	100	-	-	-
Staphylococcus aureus	50	-	-	-
	100	7	11	12
Salmonella typhimurium	50	-	-	-
	100	8	10	13
Candida albicans	50	-	-	-
	100	7	11	12

Table 2. Antimicrobial and antifungal effects of *Lavandula angustifolia*

Microbial strain	Volume (μL)	Inhibition (mm)		
		Sample 1	Sample 2	Sample 3
Escherichia coli	50	15	12	13
	100	17	14	18
Staphylococcus aureus	50	14	12	10
	100	16	15	14
Salmonella typhimurium	50	11	12	15
	100	15	16	17
Candida albicans	50	10	11	12
	100	13	14	16

Table 3. Antimicrobial and antifungal effects of *Juniperus communis*

Microbial strain	Volume (μL)	Inhibition (mm)		
		Sample 1	Sample 2	Sample 3
Escherichia coli	50	7	11	14
	100	10	15	18
Staphylococcus aureus	50	11	13	9
	100	15	15	12
Salmonella typhimurium	50	9	12	11
	100	14	15	16
Candida albicans	50	12	10	11
	100	14	13	17

100 μL for *Salmonella typhimurium* gave an inhibition zone that varied from 11 mm to 17 mm (weak to moderate antimicrobial activity). The inhibition zone for *Candida albicans* from *Lavandula angustifolia* in concentrations of 50 μL and 100 μL gave an inhibition zone that varied from 10 mm to 16 mm (weak to moderate antifungal activity) (Table 2).

Juniperus communis essential oil in concentrations of 50 μL and 100 μL gives an inhibition zone for *Staphylococcus aureus* from 9 mm to 13 mm (weak antimicrobial activity) (Table 3).

Juniperus communis in both concentrations gave an inhibition zone for *Escherichia coli* which varied from 7mm to 18 mm (weak to moderate antimicrobial activity) while for *Salmonella typhimurium* inhibition zones varied from 9 mm to 16 mm (no antimicrobial activity-moderate antimicrobial activity). *Juniperus communis* essential oil for *Candida albicans* gave an inhibition zone from 10 mm to 17 mm (no antimicrobial to moderate antimicrobial activity) (Table 3).

Lavandula angustifolia showed moderate antimicrobial and antifungal activity followed by Juniperus communis, while Helichrysum italicum didn't possess any significant antibacterial or antifungal effect on our bacterial and fungal samples. The results of our study are comparable to those of other researchers for the same medicinal plants, who highlighted that the essential oils of *Lavandula angustifolia,* and *Juniperus communis* were moderately active against *Escherichia coli, Staphylococcus aureus, Salmonella typhimurium* while essential oil of *Helichrysum italicum* had no antimicrobial effect against *Escherichia coli* and showed weak antimicrobial effect against *Staphylococcus aureus, Salmonella typhimurium,* and *Candida albicans* [17, 18].

The higher the essential oil concentration the higher the inhibition zone. The concentration of 100 μL of essential oils gives an inhibition zone until 18 mm. Antibacterial effects that illustrate the inhibition zone of antibiotics in concentrations of 50 μL for ampicillin, amoxicillin, ciprofloxacin, negram, and nitrofurantoin against *Staphylococcus aureus, Escherichia coli,* and *Salmonella typhimurium* are given in Tables 4, 5, and 6 which are comparable to the results taken from the essential oil (Tables 1, 2, and 3). Ciprofloxacin showed moderate antimicrobial activity in *Escherichia coli* infections with an inhibition zone from 18 mm for sample 1, to 20 mm for sample 3, followed by amoxicillin with an inhibition from 16 mm moderately sensitive in sample 2 (Table 4).

Negram, nitrofurantoin, and ampicillin had an inhibition zone that varies from 5 mm to 15 mm inhibition zone which is weak antimicrobial activity.

Table 5 shows the antimicrobial activity of antibiotics against *Staphylococcus aureus.* Antibiotics such as ampicillin, amoxicillin, ciprofloxacin, negram, and nitrofurantoin were used in three parallel samples, and they showed an inhibition zone that varied from 6 mm to 16 mm (weak to moderate antimicrobial activity).

Ciprofloxacin, negram, and amoxicillin in samples 2 and 3 showed moderate antimicrobial activity from 14 mm to 16 mm.

Table 6 shows the antimicrobial activity of antibiotics against *Salmonella typhimurium.* Ampicillin and amoxicillin had weak antimicrobial activity against *Salmonella typhimurium*, while ciprofloxacin and negram showed moderate antimicrobial activity in sample 1 with an inhibition zone 15 mm to 16 mm. Ampicillin, ciprofloxacin, and negram gave an

Table 4. Antimicrobial effects of antibiotics in *Escherichia coli*

Escherichia coli	**Volume (μL)**	**Sample 1**	**Sample 2**	**Sample 3**
Ampicillin	50	15 mm	7 mm	10 mm
Amoxicillin	50	0 mm	16 mm	6 mm
Ciprofloxacin	50	18 mm	0 mm	20 mm
Negram	50	15 mm	0 mm	0 mm
Nitrofurantoin	50	6 mm	7 mm	5 mm

Table 5. Antimicrobial effects of antibiotics in *Staphylococcus aureus*

Staphylococcus aureus	**Volume (μL)**	**Sample 1**	**Sample 2**	**Sample 3**
Ampicillin	50	14 mm	6 mm	7 mm
Amoxicillin	50	15 mm	7 mm	5 mm
Ciprofloxacin	50	14 mm	15 mm	10 mm
Negram	50	15 mm	16 mm	0 mm
Nitrofurantoin	50	15 mm	0 mm	7 mm

Table 6. Antimicrobial effects of antibiotics in *Salmonella typhimurium*

Salmonella typhimurium	Volume (µL)	Sample 1	Sample 2	Sample 3
Ampicillin	50	0 mm	20 mm	7 mm
Amoxicillin	50	0 mm	0 mm	5 mm
Ciprofloxacin	50	16 mm	16 mm	17 mm
Negram	50	15 mm	20 mm	8 mm

Table 7. Antifungal effects of antimycotics in *Candida albicans*

Candida albicans	Volume (µL)	Sample 1	Sample 2	Sample 3
Econazole	50	4 mm	15 mm	13 mm
Nystatin	50	15 mm	5 mm	15 mm
Ketoconazole	50	6 mm	4 mm	8 mm
Miconazole	50	16 mm	4 mm	10 mm
Clotrimazole	50	15 mm	16 mm	13 mm

inhibition zone for *Salmonella typhimurium* which varied from 16 mm to 20 mm (moderate to strong antimicrobial activity) for sample 2. For sample 3 only ciprofloxacin showed an inhibition zone from 17 mm (moderate antimicrobial activity) for *Salmonella typhimurium* while ampicillin, amoxicillin, and negram showed an inhibition zone of 5 mm to 8 mm (no antimicrobial activity).

Table 7 gives the inhibition zones in mm which represent the antifungal effects of antimycotics such as econazole, nystatin, ketoconazole, miconazole, and clotrimazole against *Candida albicans*.

All antimycotics in three parallel samples had an inhibition zone that varied from 4 mm to 16 mm (no antifungal to moderate antifungal activity). None of the antifungals used in our study in the three parallel samples showed a strong antifungal effect.

4. Conclusions

- The results represented in this study showed the impact of climate on the essential oil yields in a year study - 2023. The most significant differences are found in EO yield between two different periods of sampling which are linked with climate factors, such as rainfall, air temperature, humidity, and sunny days. The highest percentage of oil was in September 2023 for three medicinal plants studied.
- Essential oil yields of dried leaves and fruits ranged such as *Juniperus communis* 0.7 - 1.1%, *Lavandula angustifolia* 5.1 - 6.3%, and *Helichrysum italicum* < 1%.
- The study demonstrates that these essential oils from *Lavandula angustifolia, Juniperus communis,* and *Helichrysum italicum* present moderate bactericidal and fungicidal effects against microorganisms such as *Staphylococcus aureus, Salmonella typhimurium, Escherichia coli,* and *Candida albicans*. Lavandula angustifolia showed moderate antimicrobial and antifungal activity followed by Juniperus communis, while Helichrysum italicum didn't have any significant antibacterial and antifungal effects in our bacterial and fungal samples.
- The inhibitory activity of the examined essential oil from *Lavandula angustifolia, Juniperus communis,* and *Helichrysum italicum* proved to be comparable to the antimicrobial and antifungal activity drugs used in our daily routine.
- *Lavandula angustifolia* and *Juniperus communis* could serve as an alternative way in combination with antibiotics to combat resistant bacteria in different patients with infections from bacterial and fungal problems.

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5. References

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