ACTIVE PRINCIPLES IN BASIL ESSENTIAL OIL – *Ocimum basilicum* L. COTTON LININGS WITH ANTIBACTERIAL PROPERTIES

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*Ocimum basilicu*m L. is an aromatic plant in the family *Lamiaceae* with bioactive properties used since ancient times in traditional medicine. The active ingredients of basil essential oil can be used in perfumes, pharmaceuticals, medicine, cosmetics or spices. In this study, the essential oil of basil was obtained by hydrodistillation in the Clevenger continuous extractor. It was characterized by GC-MS and 53 constituent compounds were identified. The majority compounds were highlighted: linalool, 64,569%, p-allyl anisol, 5,163%, Eucalyptol, 3,745%, α-Cadinene, 3,510%. Kovats indices were calculated and FT-IR analysis was performed to confirm the specific constituent compounds. The essential oil of basil was microbiologically analyzed against *Escherichia coli* (ATCC 10536) Gram-negative bacteria and against *Staphylococcus aureus* (ATCC 6538) Gram-positive bacteria by diffusometric working method. Antibacterial activity was determined by measuring the diameter of the inhibition zone around the samples. Samples of filter paper and cotton fabric were used to simulate shoe lining and bandages. Basil essential oil has resistance against the tested strains, observing the increase of the inhibition zone with the increase of the amount of essential oil used in treatments (20 μL, 30 μL, 50 μL). The results showed that *Ocimum basilicum* L. may be a good candidate as a plant-derived antibacterial agent for medical footwear, wound dressings and other medical applications.

Keywords: *Ocimum basilicum* L., bioactive compounds, antibacterial activity

INTRODUCTION

Natural products obtained from medicinal and aromatic plants have been widely consumed for centuries due to their therapeutic properties and aromaticity (Alsaraf *et al*., 2020). The need for medicinal and aromatic plants is in a growing demand for the active component principles that can be used in perfumes, pharmaceuticals, medicine, cosmetics or spices (Tursun, 2022). Basil (*Ocimum basilicum* L.) is considered one of the common aromatic plants with pharmacological properties such as antioxidant, chemo-preventive, anti-inflammatory, antimicrobial, immunomodulatory activity (Shalaby *et al*., 2020; Tursun, 2022, El-Nekeety *et al*., 2021), sedative and digestive due to the presence of specific compounds in the essential oil. It is also used as a toxin eliminator, to treat cough, colds, in the treatment of insomnia and constipation (Shalaby *et al*., 2020). Basil has effects on dermal pathology and in wound healing, including acne, eczema, boils, psoriasis and rashes (Tursun, 2022).

This work aims to highlight the composition of basil essential oil and the use of active principles in obtaining cotton linings with antibacterial properties.

EXPERIMENTAL

Materials

Cotton fabric has been used for impregnation with different concentrations of basil essential oil to simulate the lining of shoes or bandages. In the microbiological analyses, the filter paper was also used. Other chemical reagents were of analytical grade.

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Extraction of Volatile Oil

The essential oil of basil was extracted from 100g of the shredded dried plant, in a balloon with a round bottom of 2L, in the ratio of 1:10 m / V (plant: distilled water), by continuous hydrodistillation in the Clevenger extractor, for 240 min. (Ilić *et al*., 2022). Approximately 0.7 mL of essential oil was obtained which was dried on sodium anhydrous sulphate to remove traces of water (Ilić *et al*., 2022).

ATR-FTIR Spectroscopy

ATR-FTIR analysis was performed with a FT-IR/ATR spectrometer-Jasco 4200 operating in the range of 4000 to 600cm^{-1}, with a spectral resolution of 0.5 cm^{-1}.

GC-MS Analysis

An Agilent 6890 N gas chromatograph was used to identify the compounds in the volatile oil. An Agilent 5958 C mass spectrometer was used as a detector to obtain the molecular weights of the compounds separated in the chromatograph. The Kovats retention indices have been calculated to confirm the identification of the constituent compounds in the essential oil of basil. For the calculation of kovats retention indices was used the standard mixture of alkanes - C8-C20 and the calculation formula (1):

$$K = 100 \bullet \left[n + \frac{(\log t_{Rx} - \log t_{Rn})}{(\log t_{Rn+1} - \log t_{Rn})} \right] \quad (1)$$

T_{Rx} = retention time of the compound to be analyzed;
T_{Rn} = the retention time of the alkane with n C atoms in the molecule of which the bit is placed to the left of the peak to be analyzed;
T_{Rn+1} = retention time of the alkane with n+1 carbon atoms placed to the right of the peak to be analyzed from the chromatogram.

Microbiological Analysis

The samples were tested on ATCC strains from the ICPI biotechnology laboratory collection, namely, on *Escherichia coli* (ATCC 10536) Gram-negative bacteria and *Staphylococcus aureus* (ATCC 6538) Gram-positive bacteria. The working method was diffusimetric. Antibacterial activity was determined by measuring the diameter of the zone of inhibition around the disc. Sample discs of filter paper and cotton (diameter 15mm) were treated with different amounts of basil essential oil: 20 µL, 30 µL and 50 µL, labeled as HFB20, HFB30, HFB50 and as BBCB20, BBCB30, BBCB50. Samples of filter paper and cotton without essential oil was used as a blank.

RESULTS AND DISCUSSIONS

Basil essential oil was obtained by hydrodistillation in Clevenger continuous extractor (El-Nekeety *et al*., 2021). Large variations in the content of essential oils in basil could be attributed to a number of factors, such as varied agroclimatic conditions, harvesting region, diversity of genotypes and basil population, harvest time, methods of drying and storage of basil after harvest (Ilić *et al*., 2022).

The ATR-FTIR spectrum showed in Figure 1 reveals the spectral bands at: 3410 cm^{-1} (O-H in alcohols), 2966 cm^{-1}, 2925 cm^{-1} (aliphatic C-H), 1708 cm^{-1}, 1639 cm^{-1}, 1511 cm^{-1}, 1451 cm^{-1}, 1411 cm^{-1} (C=C stretching vibration), 1375 cm^{-1} (isopropyl methyl group

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symmetric bending vibration), 1246 cm^{-1} (C–O–C stretching), 1174 cm^{-1}, 1112 cm^{-1}, (CH_3 deformations), 1037 cm^{-1}, 994 cm^{-1} (para-substituted phenyl), 887 cm^{-1}, 834 cm^{-1} (C-H bend pattern), 767 cm^{-1}, (aromatic C-H out-of-plane bend) (Valderrama and Rojas De, 2017).

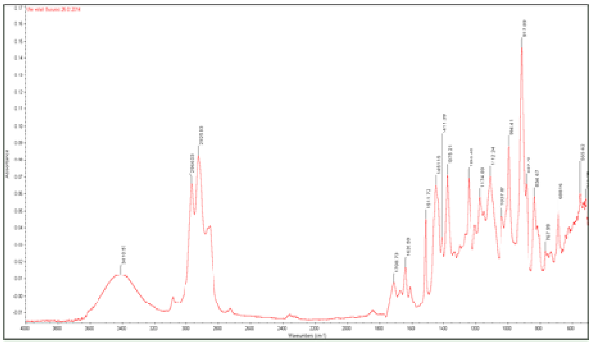

Figure 1. ATR-FTIR spectrum of *Ocimum basilicum* essential oil

By GC-MS analysis of basil essential oil, 53 different volatile organic compounds were identified (Table 1).

Table 1. Compounds identified in basil volatile oil

No.	Retention time, minutes	Name	Formula	Percentage of area, %	Kovats indices, K x10^3
1	7.251	Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl-,	$C_{10}H_{16}$	0.118	0.656
2	8.968	α-pinene	$C_{10}H_{16}$	0.222	0.698
3	9.877	Geranyl bromide	$C_{10}H_{17}Br$	0.190	0.718
4	11.379	Sylvestrene	$C_{10}H_{16}$	0.205	1.06
5	11.441	Eucalyptol	$C_{10}H_{18}O$	3.745	1.062
6	12.507	Ocimene	$C_{10}H_{16}$	0.214	1.086
7	13.537	Methyl 6-nonynoate	$C_{10}H_{16}O_2$	0.111	1.107
8	14.986	Linalol	$C_{10}H_{18}O$	64.569	1.134
9	15.144	3,7-Octadiene-2,6-diol, 2,6-dimethyl	$C_{10}H_{18}O_2$	1.442	1.504
10	16,697	Camphor	$C_{10}H_{16}O$	0.537	1.542
11	17.714	Isobornyl thiocyanoacetate	$C_{13}H_{19}NO_2S$	0.161	1.565
12	17.854	2-(4-Methylcyclohexylidene)-1-propanol	$C_{10}H_{18}O$	0.133	1.568
13	18.105	Carane	$C_{10}H_{18}$	0.130	1.574
14	18.239	5-Isopropyl-2-methylbicyclo[3.1.0]hexan-2-ol	$C_{10}H_{18}O$	0.171	1.576
15	18.815	α-Terpineol	$C_{10}H_{18}O$	1.112	1.589
16	19.165	Anisole, p-allyl	$C_{10}H_{12}O$	5.163	1,596
17	20.367	m-Toluic acid, 4-methylpentyl ester	$C_{14}H_{20}O_2$	0.151	1.968
18	21.331	Nerol	$C_{10}H_{18}O$	1.521	1.996
19	22.252	1,5,5-Trimethyl-6-methylene-1-cyclohexene	$C_{10}H_{16}$	0.412	2.022
20	24.487	Eugenol	$C_{10}H_{12}O_2$	0.689	2.34

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No.	Retention time, minutes	Name	Formula	Percentage of area, %	Kovats indices, K x10³
21	24.570	p-Cresol, 2-amino-6-tert-butyl-	$C_{11}H_{17}NO$	0.045	2.343
22	24.960	Copaene	$C_{15}H_{24}$	0.067	2.356
23	25.205	Methyl cinnamate	$C_{10}H_{10}O_2$	0.546	2.364
24	25.257	Caryophyllene oxide	$C_{15}H_{24}O$	0.229	2.366
25	25.364	Lanceol, cis	$C_{15}H_{24}O$	0.282	2.561
26	25.466	β-Elemene	$C_{15}H_{24}$	1.541	2.565
27	26.166	Alloaromadendren	$C_{15}H_{24}$	0.594	2.593
28	26.695	Bergamotene	$C_{15}H_{24}$	1.176	2.613^3
29	27.102	α-Bisabolene	$C_{15}H_{24}$	0.483	2.629
30	27.365	bicyclosesquiphellandrene	$C_{15}H_{24}$	0.193	2.638
31	27.853	β-Cubebene	$C_{15}H_{24}$	0.697	2.656
32	27.971	α-Selinene	$C_{15}H_{24}$	0.432	2.661
33	28.262	δ-Elemene	$C_{15}H_{24}$	0.474	2.837
34	28.476	Alloaromadendrene oxide-(2)	$C_{15}H_{24}O$	0.245	2.846
35	28.524	δ-Guaiene	$C_{15}H_{24}$	0.890	2.848
36	28.727	τ-Cadinene	$C_{15}H_{24}$	0.719	2.856
37	28.989	δ-Cadinene	$C_{15}H_{24}$	0.301	2.867
38	30.019	Varidiflorene	$C_{15}H_{24}$	0.724	3.909
39	30.286	Spathulenol	$C_{15}H_{24}O$	0.911	2.92
40	30.413	τ -Himachalene	$C_{15}H_{24}$	0.460	2.925
41	31.048	3,7,11-Trimethyl-oxa-cyclotrideca-7,11-diene-2,4-dione	$C_{15}H_{22}O_3$	0.130	3.097
42	31.138	Cedren-13-ol, 8-	$C_{15}H_{24}O$	0.123	3.101
43	31.205	Cubenol	$C_{15}H_{26}O$	0.523	3.104
44	31.817	α-Cadinene	$C_{15}H_{24}$	3.510	3.131
45	31.941	Farnesyl bromide	$C_{15}H_{25}Br$	0.188	3.136
46	32.009	Phen-1,4-diol, 2,3-dimethyl-5-trifluoromethyl	$C_9H_9F_3O_2$	0.443	3.139
47	32.123	α-Guaiene	$C_{15}H_{24}$	0.763	3.144
48	32.331	7-Tetracyclo[6.2.1.0(3.8)0(3.9)]undecanol, 4,4,11,11-tetramethyl-	$C_{15}H_{24}O$	0.180	3.153
49	32.537	Spathulenol	$C_{15}H_{24}O$	0.311	3.162
50	33.358	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	$C_{15}H_{24}O$	0.175	3.328
51	33.547	α-Vatirenene	$C_{15}H_{22}$	0.143	3.337
52	33.638	Ledene oxide-(II)	$C_{15}H_{24}O$	0.336	3. 341
53	38.459	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	0.089	3. 804

The majority compounds in the analyzed basil essential oil are linalool, 64.569%, p-allyl anisol, 5.163%, Eucalyptol, 3.745 and α-Cadinene, 3.510%. The essential oils from basil collected from different regions and in different periods have different compositions in percentages of majority compounds and identified compounds that give different

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qualitative and quantitative bioproprieties. The main components of Serbian sweet basil essential oil were linalool (35.1 %), eugenol (20.7 %) and 1.8-cineol (9.9 %) (Ilić *et al*., 2022). Both essential oils had the main compound linalool that gives antimicrobial characteristics (Hussain *et al*., 2008) and insect-repellent properties, anti-inflammatory activity, antihyperlipidemic, antidepressant, neuroprotective and anticancer properties (Alsaraf *et al*., 2020), antiviral, antifungal (Tursun, 2022). Basil essential oils from European countries contain methyl chavicol and linalool. Methyl cinnamate is the main constituent of tropical basil, while Indian basil essential oil is characterized by a high percentage of methyl eugenol (Ilić *et al*., 2022). Microbiological analyses were done on filter paper and cotton fabric. In order to interpret the measurements, a classification of the samples was chosen, according to a scale used in similar studies, starting from the diameter of the inhibition zone.

$$H = \frac{D-d}{2} \tag{2}$$

H = the zone of inhibition, in millimeters;
D = total diameter of the disc and the inhibition zone, in millimeters;
d = disc diameter, in millimeters.

Table 2. Resistance against *Escherichia coli* of filter paper samples treated with *Ocimum basilicum* L. essential oil

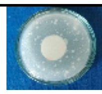
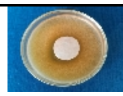
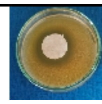
Sample	Control	HFB20	HFB30	HFB50
Images				
Inhibition zone (mm)	Contaminated	2.5	6.5	7.5

Table 3. Resistance against *Staphylococcus aureus* of filter paper samples treated with *Ocimum basilicum* L. essential oil

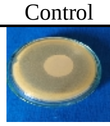
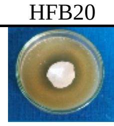
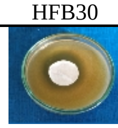
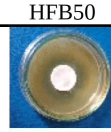
Sample	Control	HFB20	HFB30	HFB50
Images				
Inhibition zone (mm)	Contaminated	6.5	11.5	12.5

Table 4. Resistance against *Escherichia coli* of cotton samples treated with *Ocimum basilicum* L. essential oil

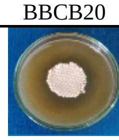
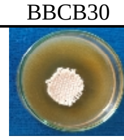
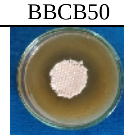
Sample	Control	BBCB20	BBCB30	BBCB50
Images				
Inhibition zone (mm)	Contaminated	6.5	7.5	10

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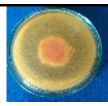
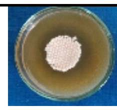
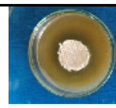
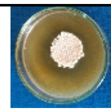
Table 5. Resistance against *Staphylococcus aureus* of cotton samples treated with *Ocimum basilicum* L. essential oil

Sample	Control	BBCB20	BBCB30	BBCB50
Images				
Inhibition zone (mm)	Contaminated	6.5	9.5	13.5

From the microbiological results obtained (Tables 2-5) it is observed that the zone of inhibition increases with the increase of the concentration of basil essential oil used in the treatment of samples. The inhibition zone obtained against *Staphylococcus aureus* samples is higher than in the case of samples against *Escherichia coli*.

CONCLUSION

Basil essential oil was obtained by hydrodistillation in continuous Clevenger extractor. By GC-MS analysis, 53 constituent compounds were identified and the Kovats indices were calculated. The majority compounds of linalool (64,569%), p-allyl anisol (5,163%), eucalyptol (3,745%), α-Cadinene (3,510%) were highlighted. Microbiological analysis showed that basil essential oil has resistance against *Escherichia coli* and against *Staphylococcus aureus* developing a higher inhibition zone with increased concentration of essential oil. The results obtained showed that *Ocimum basilicum* can be a good candidate as a plant-derived antibacterial agent for medical shoes, wound dressings and other medical applications. Basil essential oil has been used in traditional medicine and spices in various dishes since ancient times. Nowadays it is widely used in medical products, pharmaceuticals, cosmetics, perfumes.

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REFERENCES

Alsaraf, S., Hadi, Z., Al-Lawati, W.M., Al Lawati, A.A. and Khan, S.A. (2020), "Chemical Composition, *in vitro* Antibacterial and Antioxidant Potential of Omani Thyme Essential Oil along with *in silico* Studies of Its Major Constituent", *Journal of King Saud University – Science*, 32(1), 1021–1028, https://doi.org/10.1016/j.jksus.2019.09.006.

El-Nekeety, A.A., Hassan, M.E., Hassan, R.R., Elshafey, O.I., Hamza, Z.K., Abdel-Aziem, S.H., Hassan, N.S. and Abdel-Wahhab, M.A. (2021), "Nanoencapsulation of Basil Essential Oil Alleviates the Oxidative Stress, Genotoxicity and DNA Damage in Rats Exposed to Biosynthesized Iron Nanoparticles", *Heliyon*, 7(7), e07537, https://doi.org/10.1016/j.heliyon.2021.e07537.

Hussain, A.I., Anwar, F., Sherazi, S.T.H. and Przybylski, R. (2008), "Chemical Composition, Antioxidant and Antimicrobial Activities of Basil (*Ocimum basilicum*) Essential Oils Depends on Seasonal Variations", *Food Chemistry*, 108(3), 986–995, https://doi.org/10.1016/j.foodchem.2007.12.010.

Ilić, Z.S., Milenković, L., Tmušić, N., Stanojević, L., Stanojević, J. and Cvetkovićb, D. (2022), "Essential Oils Content, Composition and Antioxidant Activity of Lemon Balm, Mint and Sweet Basil from Serbia", *LWT - Food Science and Technology*, 153, 1-12, 112210, https://doi.org/10.1016/j.lwt.2021.112210.

Shalaby, S.M., Darwesh, M., Ghoname, M.S., Salah, Sh.El., Nehela, Y. and Fetouh, M.I. (2020), "The Effect of Drying Sweet Basil in an Indirect Solar Dryer Integrated with Phase Change Material on Essential Oil Valuable Components", *Energy Reports*, 6(S9), 43–50, https://doi.org/10.1016/j.egyr.2020.10.035.

Tursun, A.O. (2022), "Impact of Soil Types on Chemical Composition of Essential Oil of Purple Basil", *Saudi Journal of Biological Sciences*, 29(7), 103314, https://doi.org/10.1016/j.sjbs.2022.103314.

Valderrama, A. and Rojas De, G. (2017), "Traceability of Active Compounds of Essential Oils in Antimicrobial Food Packaging Using a Chemometric Method by ATR-FTIR", *American Journal of Analytical Chemistry*, 8(11), 726-741, https://doi.org/10.4236/ajac.2017.811053.