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Antibacterial and Antifungal Activity of Plant Extracts

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1. Introduction

Spices and herbs have been used for thousands of years by many cultures to enhance the flavor and aroma of foods. Early cultures also recognized the value of using spices and herbs in preserving foods. The numerous experiments since the late 19th century have documented the antimicrobial properties of some spices, herbs, and their components [59, 69]. Many herbs and spice are known to exert antioxidant activity and are useful for preventing lipid oxidation in living organisms as well as in foods [22].

The study of the effects which have natural chemicals compounds released from plants or microorganisms on the growth and spread of plants and microorganisms in natural communities is now engaged in a new field - chemical ecology. It oscillates around allelopathy issues, which so far have the greatest dealt with interaction between weeds, and various species of cereals. Currently, the literature reports, indicate the high bactericidal and fungistatic efficiency of extracts and essential oils prepared from different plant species [21, 39].

The family *Lamiaceae* consists of about 252 genera and more than 6700 species [28]. Some of *Lamiaceae* species are frequently used in cooked dishes and are recognized as important preventive factor of many diseases [5, 13, 39]. Essential oils and extracts of these plants are known to possess antiseptic, antiinflammatory and antimicrobial activities [8, 9, 63]. *Pogostemon cablin*, *Lavandula angustifolia*, *Melissa officinalis*, and *Salvia officinalis* are underutilized species of *Lamiaceae* [30].

Most of the *Pinus* species are trees or shrubs with specific morphological characteristics of leaves (needles) rich in terpene aromatic essential oil. Pine needle essential oils are mainly used in folk medicine for the treatment of respiratory [18]. Up to now, there has been an increased interest in studying chemical composition as well as biological activity of the essentials oils isolated from different pine species [41].

Plant extracts and essential oils are considered to be one of the potential sources of substances with anticancer, antimicrobial and antioxidant properties, and source of free radical scavenging agents [31, 38, 58].

The increasing recognition and importance of bacterial and fungal infections, the difficulties encountered in their treatment and the increase in resistance to antibacterial and antifungal agents have stimulated the search for therapeutic alternatives [50]. The essential oils (EO) and products of plant secondary metabolism have application in folk medicine, fragrance industries, food flavouring and preservation but only in recent years they have started to be recognized for their potential antimicrobial role [40]. Although numerous studies have documented the plant extracts antibacterial and anticandidal effect [9, 14, 24, 36, 49, 27]. There have been few comprehensive *in vitro* studies of the effects exerted by EO on filamentous fungi, probably due to the difficulties encountered in standardized susceptibility methods for these mycetes [27, 35, 37, 43, 61].

In many research centers are conducted intensive *in vitro* studies on the mechanism of action and safety of plant extracts and their individual components. The obtained results encourage to further investigations on the usefulness of the macerates, infusions, decoctions and plant oils in combating a particularly dangerous antibiotic-resistant microorganisms.

The aim of this work was to evaluate the antimicrobial activity of extracts from 3 plant species, obtained by various methods, collected in Poland against Gram-positive and Gram-negative bacteria and fungi. Some spice plants previously examined for biological activity by other investigators were included in this study because different methods and microorganisms or strains were used in the study, which provided a comparison base.

2. Materials and methods

2.1. Plant material

Fresh, free from disease, leaves, flowers and young sprouts of three different plants *Lavandula vera* L. – flowers and leaves, *Melissa officinalis* L. – leaves, *Pinus sylvestris* L. – young sprouts were collected in July 2014 from the area of Koszalin and surrounding areas (Poland). The parts of plants were washed thoroughly 2–3 times with running water and once with sterile distilled water and air-dried at room temperature on sterile blotter under shade for two weeks.

2.2. Preparation of the plant extracts

Used in *in vitro* investigations plant preparations were made in the form of aqueous extracts, as macerates, brews and decoctions. Macerate, brew and decoction were prepared according to the recipe given by Sas-Piotrowska et al. (2005) [55]. Macerate (cold method) – 5 g of dried plant poured over 100 ml of cold water and left for 24 h at 20°C, then filtered; brew (hot method) – 5 g of dried plant poured of 250 ml of boiling water and left covered for 30 minutes, after cooling the extract was filtered. The decoction was prepared according to the recipe given by Tyszyńska-Kownacka and Starek (1989) [68]. For this purpose, weighed 8.75 g of each dried herbs and poured over 1 liter of distilled water. The suspension was mixed thoroughly, allowed to stand for 24 hours and then boiled for 15 minutes. Boiled extracts were sieved through a sieve lined with gauze to glass containers, and after cooling, used for the investigations.

2.3. Essential oil isolation

Essential oil isolation was made by hydrodestillationin all-glass Clevenger apparatus (Ph. Eur 7, 2.8.12.). For that purpose, 20 g of minced plants were distilled for 4 hours. For purification purpose, anhydrous sodium sulfate was added to the isolated essential oil to remove residual water.

2.3. Test microorganisms

The antibacterial activity of the plant extracts were tested *in vitro* against the Gram-positive bacteria: *Bacillus subtilis, Listeria monocy-*

togenes, Micrococcus luteus, Staphylococcus aureus; and the Gram-negative bacteria: *Escherichia coli*. The antifungal activity of plant extracts was studied against 12 different microorganisms, including: *Alternaria alternata* (Fr.) Keissler, *Aspergillus glaucus* (L.) Link, *Aspergillus niger* (Tiegh.), *Botritis cinerea* (Pers), *Cladosporium herbarum* (Pers.) Link ex Fr., *Fusarium culmorum* (Sacc.), *Fusarium oxysporum* (E.F.Sm.),W.C. Snyder & H.N.Hansen, *Fusarium poae* (Peck), *Fusarium sambucinum* (Fuckel f.6 Wollenweber), *Fusarium solani* (Mart.), *Penicillium chrysogenum* (Thom), *Sclerotinia sclerotiorum* (de Bary).

All microorganisms were obtained from the stock cultures of the Microbiology Laboratory (Division of Biological Agriculture Foundations, Koszalin University of Technology).

2.4. Preparation of Inoculum

The inoculum was prepared according to methodology given by Mahesh and Satish (2008) [45]. The Gram-positive and Gram-negative bacteria were pre-cultured in nutrient broth overnight in a rotary shaker in 37°C, centrifuged at 10,000 rpm for 5 min, pellet was suspended in double distilled water and the cell density was standardized spectrophotometrically (A_{610} nm). The fungal inoculum was prepared from 5 day old culture grown on potato dextrose agar medium. The Petri dishes were flooded with 8 to 10 ml of distilled water and the conidia were scraped using sterile spatula. The spore density of each fungus was adjusted with spectrophotometer (A_{595} nm) to obtain a final concentration of approximately 10^5 spores/ml.

2.5. Antimicrobial screening

The antibacterial activity of extracts was determined by diffusion method in Petri dishes (ø 10 cm) with a solid medium Müller - Hinton. Applied to a substrate prepared using a densitometer suspension of 0.5 McFarland units in (10^6jtk / ml), which was uniformly distributed on the surface, and after dried, placed a paper disk (ø 6 mm) soaked botanical extract. The measure of the extracts activity was the size of the zone of inhibition of growth colonies (in millimeters) after 24 hours of incubation in 37°C. The antifungal activity of extracts was determined by diffusion method in Petri dishes (ø 10 cm) with a solid medium PDA. On each substrate 4 drops of an aqueous suspension of spores and mycelial frag-

ments was applied, next uniformly distributed on the surface, then they were dried and placed on a paper disk (ø 6 mm) soaked with botanical extract. The measure of the extracts activity was the size of the zone of inhibition of growth colonies (in millimeters) measured after 5 days incubation in 22°C.

The experiment was established in 6 replicates for each plant, method of extract preparation and the pathogen. Each repetition consisted of four Petri dishes. Results were analyzed using analysis of variance and correlation account.

2.6. Statistical analyses

The results were analyzed statistically by analysis of variance with a single classification (P = 95%), separately for each plant, method of preparation and specific pathogens. The results presented in tables 1–6, were calculated relative to the control sample (the paper discs treated with sterile water). The Fisher's least significant difference ($LSD_{0.05}$) was applied to test the differences between samples at 5% significance level. Additionally, a comparison of averages was made using the correlation coefficient (the limit value of $r = 0.195$ for $\alpha = 0.05$ and $r = 0.254$ for $\alpha = 0.01$). Statistical analysis was performed using the programs ANW (Variance Analysis of Experiments) and ANK (Correlation Analysis of Experiments).

3. Results and discussion

Analysis of variance showed significant differences in the examined factors and their interaction. The reaction of the investigated bacteria on modified environment conditions, depended on the plant species from which the extract was prepared (Table 1) and the method of its preparation (Table 2). The growth of pathogens was most inhibited by extracts from *Lavandula vera* (mean value of inhibition zone ø 10.30 mm). The highest sensitivity to this extract showed *Staphylococcus aureus* (ø 12.08 mm). The most active extracts was applied to the bacterium *Listeria monocytogenes* (ø 12.08 mm). The highest antimicrobial activity was observed for oil from *Lavandula vera*. Among the bacteria the most resistant on the investigated species of plants was *Escherichia coli* (ø 8.22 mm).

Generally, Gram-positive bacteria were more susceptible than Gram-negative bacteria. *Listeria monocytogenes* was the most sensitive,

while *Escherichia coli* was the most resistant strain (against all plant extracts). Our results are in good agreement with the finding of Cantore et al. (2004) [10]. Antibacterial properties of plants extracts depend not only on its chemical characteristics, but also on type of bacteria. Gram-negative bacteria are less susceptible because their membrane contains hydrophilic lipopolysaccharides (LPS), which create a barrier toward macromolecules and hydrophobic compounds [9, 33].

Table 1. Antibacterial activity of plant extracts (1.0 μg Ml $^{-1}$) in disc-diffusion method, inhibition zones (mm)
Tabela 1. Aktywność przeciwbakteryjna wyciągów roślinnych (1.0 μg Ml $^{-1}$) w metodzie dyfuzyjnej, strefa zahamowania wzrostu (mm)

Bacteria	*Lavandula vera*	*Melissa officinalis*	*Pinus sylvestris*
Bacillus subtilis	6.75	10.08	8.28
Eshcerichia coli	9.42	6.5	8.22
Listeria monocytogenes	13.5	9.67	12.08
Micrococcus luteus	9.75	10.25	10.5
Staphylococcus aureus	12.08	9.50	10.19
Mean value	10.30	9.20	10.07
LSD_{plant} = 0.1867	$LSD_{bacteria}$ =0.2410	LSD_{plant} x $LSD_{bacteria}$ =0.4174	

The data show the diameter of inhibition zone growth in mm. The diameter of paper disc was 6 mm. LSD – the least significant difference. When significant (P < 0.05), the value of LSD is indicated.

The growth of bacteria on the surface (Table 2) was the most limited by essential oils. Among the studied bacteria most sensitive to the applied extracts were: *Listeria monocytogenes* (ø 12,08 mm), *Micrococcus luteus* (ø 10,5 mm) and *Staphylococcus aureus* (ø 10,19 mm). The highest resistance was observed for *Escherichia coli* (ø 8.22 mm). The weakest effect on the pathogens growth showed applied macerates (ø 6,60 mm), and bacteria resistant to its use were *Bacillus subtilis* and *Listeria monocytogenes* (ø 6,00 mm).

Many researchers evaluated the antimicrobial activity of plant oils [1, 15, 44, 60, 64]. Analyzing the average values of the zones of growth inhibition of bacteria and fungi by lavender oils, it was found that they differ in the various species. Draws attention to the fact that the Gram-positive bacteria were more sensitive to applied lavender essential oils than Gram-negative bacteria. This is confirmed by the findings of other

authors [1, 3, 6, 15, 16]. *Staphylococcus aureus* species is currently one of the major causes of nosocomial infections [23]. Recent studies show that bacteria do not yet have an adequate defense mechanism to tested oils. Other authors have shown inhibition zone for *S. aureus* in the range of 0,0-18,0 mm [19, 20, 25, 29, 51, 56, 64]. Serban (2011) and Cavanagh et al. (2002) investigations show that the antimicrobial properties depend on the composition of the oil, and also on the species or type of microorganism [1, 11, 56].

Table 2. The reaction of bacteria depending on the method of a plant extract preparation (mean value of diameter inhibition zone growth in mm)
Tabela 2. Reakcja bakterii w zależności od metody przygotowania ekstraktu roślinnego (wartość średnia średnicy zahamowania wzrostu w mm)

Bacteria	Macerate	Brew	Decoction	Oil	Mean value
Bacillus subtilis	6.00	6.00	6.00	15.11	8.28
Escherichia coli	6.67	6.78	7.78	11.68	8.22
Listeria monocytogenes	6.00	15.78	7.44	19.11	12.08
Micrococcus luteus	8.00	7.56	7.00	19.44	10.5
Staphylococcus aureus	6.33	7.11	7.44	19.89	10.19
$LSD_{bacteria}$ =0.2410	$LSD_{preparation}$ =0.2155	$LSD_{bacteria}$x $LSD_{preparation}$ =0.4820		LSD_{plant}x $LSD_{preparation}$ =0.3733	

The data show the diameter of inhibition zone growth in mm. The diameter of paper disc was 6 mm. LSD – the least significant difference. When significant ($P < 0.05$), the value of LSD is indicated

Synthesis of the obtained results showed that the activity of the extracts dependent on the species of plants, a method of preparing plant extracts and the sensitivity of bacteria selected for the test (Table 3). It also showed significantly different response of tested pathogens to the applied extracts of various plant species.

To evaluate the antimicrobial properties of essential oils against strains of bacteria oils of lavender and melissa was examined. *Lavandula vera* and *Melissa officinalis* belong to the family *Lamiaceae*, whose representatives have a particularly valuable therapeutic properties. Lavender oil was the most active against bacteria, which is confirmed by obtained experimental results and it is also well know fact from the literature.

Table 3. Variation and correlation coefficients for the reaction of tested bacteria on plant extracts and methods of their preparation

Tabela 3. Współczynniki zmienności i korelacji dla reakcji badanych bakterii na wyciągi roślinne i sposoby ich przygotowania

Bacteria	$\bar{x}$	Range	V %	Correlation coefficient (r)				
				B. subtilis	*E. coli*	*L. monocytoge-nes*	*M. luteus*	*S. aureus*
Bacillus subtilis	8.28	6 – 22.33	60.67	–	–	–	–	–
Eshcerichia coli	8.22	6 –16.33	36.27	0.19	–	–	–	–
Listeria monocytogenes	12.08	6 – 21.67	56.75	0.56	0.41	–	–	–
Micrococcus luteus	10.50	6 – 23.00	55.27	0.88**	0.57	0.65*	–	–
Staphylococcus aureus	10.19	6 – 26.67	63.11	0.61*	0.84**	0.59**	0.84**	–

$\bar{x}$ – mean value of the diameter of inhibition zone growth in mm; V – coefficient of variation;
***, * – significant compatibility of bacteria response on tested plant extracts*

In the composition of this essential oil predominates compounds such as: pulegone – 40%, menthone – 13%, and menthol – 18%, responsible for inhibiting the growth of reference strains of *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* [4].

Lemon balm and raw material obtained from the essential oil are well known products with wide range of application, because of its valuable antimicrobial activity [47]. Highest activity exhibit monoterpene aldehydes and ketones (neral/geranial, citronellal, menthone and isomenthone) and sesquiterpene hydrocarbons (E-caryophyllene) [47]. In the presented investigations, the highest antibacterial activity showed melissa oil against the strains of *Micrococcus luteus* and *Bacillus subtilis* and antifungial - against the *Sclerotinia sclerotiorum.* Comparison of the antimicrobial activity of the essential oil obtained from melissa and streptomycin against some human pathogenic bacteria, showed that *Melissa officinalis* has a strong antibacterial potential and can be used as a natural protective and fungicide [52]. Very strong protective activity of the essential oil in the process of lipid peroxidation, especially against hydroxyl radical [47], indicates that it can be used not only as a aromatic factor aromatic, but also as a safe dietary antioxidant and antiseptic in health foods and pharmaceuticals. The results of our investigations are reflected in the work of other authors, who confirms that apart from essential oil, also raw materials and extracts of rosemary acid (an important active ingredient *Melissa officinalis L*) exhibit antimicrobial properties [48, 65, 67].

The reaction of tested fungi depended on the plant species from which the extract was prepared (Table 4) and the method of its preparation (Table 4). The growth of pathogens was the most strongly limited by extracts from *Pinus sylvestris* (ø 10,29 mm), and the greatest sensitivity to this extract was observed for fungus *Scleroinia sclerotiorum* (ø 26,33 mm). Simultaneously the use of the aqueous extracts (macerate, infusion, decoction and oils) from *Lavandula vera* not significantly inhibited the growth of microorganisms (ø 8,55 mm). The most resistant fungus on the investigated plant species turned out to be *Aspergillus niger* (ø 7,00 mm) and *Fusarium sambucinum* (ø 7,00 mm).

Fungal growth on PDA medium (Table 4) was the most strongly limited by essentials oils (ø 13.83 mm). Among the analyzed fungi most sensitive to the applied extracts and essential oils have proven to be: *Sclerotinia sclerotiorum* (ø 17,56 mm) and *Fusarium poae* (ø 10,42 mm).

Additionally the weakest effect at the pathogens growth showed decoction (ø 7,20 mm), and fungus particularly resistant to the use of such kind of plant extract was *Aspergillus niger, Botritis cinerea, Fusarium sambucinum, Fusarium solani, Penicillum chrysogenum* (ø 6,00 mm).

Table 4. Antifungal activity of plant extracts (1.0 μg Ml $^{-1}$) in disc-diffusion method, inhibition zones (mm)
Tabela 4. Aktywność przeciwgrzybowa wyciągów roślinnych (1.0 μg Ml $^{-1}$) w metodzie dyfuzyjnej, strefa zahamowania (mm)

Fungi	*Lavandula vera*	*Melissa officinalis*	*Pinus sylvestris*
Alternaria alternata	12.92	9.25	8.67
Aspergillus glaucus	6.68	6.00	9.08
Aspergillus niger	6.68	6.00	8.33
Botritis cinerea	6.75	8.92	9.50
Cladosporium herbarum	7.42	11.75	7.00
Fusarium culmorum	10.5	8.50	8.83
Fusarium oxysporum	9.00	13.33	7.50
Fusarium poae	7.25	10.83	13.17
Fusarium sambucinum	6.58	6.00	8.42
Fusarium solani	6.67	6.33	9.58
Penicillium chrysogenum	11.17	8.08	7.17
Sclerotinia sclerotiorum	11.00	15.33	26.33
Mean value	8.55	9.19	10.29
LSD_{plant} = 0.1186	LSD_{fungi} =0.2372	LSD_{plant}xLSD_{fungi}=0.4108	

The data show the diameter of inhibition zone growth in mm. The diameter of paper disc was 6 mm. LSD – the least significant difference. When significant (P < 0.05), the value of LSD is indicated.

According to the findings of Sas-Piotrowska and Piotrowski (2003), the biological activity of plant extracts depends on several factors, and first of all on content of specific chemical compounds and on their ability to diffuse. Besides that, some those compounds may stimulate a pathogen development and increase a degree of contamination and the others can act as inhibition factors Differences between action of brew, macerate, decoction and oils probably resulting from possible losses caused by evaporation of the solvent during preparation and the difference in the solubility of the extracted compounds [54].

Table 5. The reaction of fungi depending on the method of a plant extract preparation (mean value of diameter inhibition zone growth in mm)
Tabela 5. Reakcja grzybów w zależności od metody przygotowania wyciągów roślinnych (średnia wartość średnicy strefy zahamowania wzrost w mm)

Fungi	Macerate	Brew	Decoction	Oil	Mean value
Alternaria alternata	9.56	11.78	6.56	13.22	10.28
Aspergillus glaucus	6.00	6.00	6.33	10.67	7.25
Aspergillus niger	6.00	6.00	6.00	10.00	7.00
Botritis cinerea	8.89	7.00	6.00	11.68	8.39
Cladosporium herbarum	9.67	10.44	7.33	7.44	8.72
Fusarium culmorum	6.00	6.00	6.00	19.11	9.28
Fusarium oxysporum	8.22	6.00	8.33	17.22	9.94
Fusarium poae	7.44	7.67	8.56	18.00	10.42
Fusarium sambucinum	6.00	6.33	6.00	9.67	7.00
Fusarium solani	7.00	7.11	6.00	10.00	7.53
Penicillium chrysogenum	8.78	6.00	6.00	14.44	8.81
Sclerotinia sclerotiorum	14.22	18.11	13.33	24.56	17.56
$LSD_{fungi} = 0.2372$	$LSD_{preparation} = 0.1369$		$LSD_{fungi} \times LSD_{preparation} = 0.8215$		$LSD_{plant} \times LSD_{preparation} = 0.2372$

The data show the diameter of inhibition zone growth in mm. The diameter of paper disc was 6 mm. LSD – the least significant difference. When significant ($P < 0.05$), the value of LSD is indicated.

Table 6. Variation and correlation coefficients for the reaction of tested fungi on plant extracts and methods of their preparation

Tabela 6. Współczynniki zmienności i korelacji dla reakcji badanych grzybów na wyciągi roślinne i sposoby ich przygotowania

Fungi	$\bar{x}$	Range	V %	Correlation coefficient (r)											
				Aa	*Ag*	*An*	*Bc*	*Ch*	*Fc*	*Fo*	*Fp*	*Fsa*	*Fso*	*Pch*	*Ss*
Aa	10.28	6-27.67	59.41	-	-	-	-	-	-	-	-	-	-	-	-
Ag	7.25	6-18.33	48.69	-0.12	-	-	-	-	-	-	-	-	-	-	-
An	7.00	6-15.33	39.05	0.04	0.99**	-	-	-	-	-	-	-	-	-	-
Bc	8.39	6-20.00	53.39	0.02	0.82**	0.82**	-	-	-	-	-	-	-	-	-
Ch	8.72	6-18.00	50.41	0.31	-0.20	-0.16	0.31	-	-	-	-	-	-	-	-
Fc	9.28	6-24.00	66.88	0.53	0.50	0.61*	0.39	-0.10	-	-	-	-	-	-	-
Fo	9.95	6-21.67	55.27	0.28	0.16	0.25	0.24	-0.01	0.76**	-	-	-	-	-	-
Fp	10.42	6-22.33	54.79	-0.22	0.64*	0.64*	0.42	-0.30	0.62*	0.57	-	-	-	-	-
Fsa	7.00	6-14.67	35.93	0.03	0.98**	0.99**	0.80**	-0.19	0.59*	0.22	0.635*	-	-	-	-
Fso	7.53	6-16.33	38.47	-0.07	0.95**	0.95**	0.77**	-0.19	0.44	0.06	0.605*	0.96*	-	-	-
Pch	8.81	6-26.67	70.50	0.84**	0.21	0.35	0.39	0.30	0.74**	0.53	0.026	0.32	0.16	-	-
Ss	17.56	6-28.00	57.03	0.26	0.14	0.20	0.03	0.01	0.41	0.20	0.528	0.24	0.28	0.18	-

$\bar{x}$ – mean value of the diameter of inhibition zone growth in mm; V – coefficient of variation;

***, * – significant compatibility of fungi response on tested plant extracts.*

Aa – Alternaria alternata, Ag – Aspergillus glaucus, An – Aspergillus niger, Bc – Botritis cinerea,

Ch – Cladosporium herbarum, Fc – Fusarium culmorum, Fo – Fusarium oxysporum,

Fp – Fusarium poae, Fsa – Fusarium sambucinum, Fso – Fusarium solani, Pch – Penicillium chrysogenum,

Ss – Sclerotinia sclerotiorum

Synthesis of the obtained results (Table 6) showed that the activity of the extracts depended on the species of plants, a method of preparing plant extracts and the sensitivity of fungi selected for the test. It also showed significantly different response of tested pathogens to the applied extracts of various plant species.

Some plant extract have demonstrated a broad range of natural fungicidal effects against post-harvest pathogens. The antifungal activities of essential oils could be applied in the vapor phase for food storage. However, more study is required for vapor-phase application because possible deterioration of the food material could still occur [57].

4. Summary

The obtained results showed that the activity of extracts depend on the plant species, method of preparation and the sensitivity of bacteria and fungi selected for testing.

The results of carried investigations showed that the antimicrobial activity of the plant extracts was more effective against bacteria than fungi, similar to the results of Avato et al. (1997) and Zavala et al. (1997) and Erturk (2006) [2, 22, 70].

The macerate, brew, decoction and essential oils were inhibitory to the growth of all the bacteria and fungi under test. It can be concluded that tested extracts were the source of active substances, which (in varying degree) inhibited the growth of selected strains of bacteria and fungi. Additionally in this study the strongest antibacterial and antifungal activity was manifested by the essential oils. The greatest impact on the antimicrobial activity of extracts could have used raw material, the effectiveness of process of plant extracts preparation, as well as the content the active ingredients characterized by antimicrobial activity against bacteria and fungi.

All the tested essential oils showed the antibacterial and antifungal activity. The highest antibacterial activity was observed with the essential oils of *Lavendula vera,* with larger values of inhibition zone. Essentials oil obtained from *Melissa officinalis* exhibited comparatively weaker antibacterial activity. It is generally accepted that essential oils having higher contents of oxygenated terpenes exhibit potent antibacterial potential [32]. There is evidence in the literature that the essential oils

of some *Lamiaceae* plants possess considerable antibacterial activities [7, 12, 17, 26, 34, 42, 46, 47, 53, 66]. However, carried studies have shown that oil of *Lavandula vera*, which most strongly inhibited the growth of bacteria, exhibited the lowest antifungal activity. Among all tested essentials, oil obtained from *Pinus sylvestris* showed the highest antifungal activity.

The most sensitive bacteria against tested plant extracts were *Listeria monocytogenes* and *Micrococcus luteus* (Gram-positive bacteria were more susceptible than Gram-negative bacteria). The tested plant extracts also showed antifungal activity against a wide range of fungi. It strongly inhibited the growth of *Sclerotinia sclerotiorum* and *Fusarium poae*.

In recent years, although technology and medicine have developed extensively, some countries have made it obligatory to use natural products for many different purposes due to decrease in natural richness and drawbacks. Like in many other countries, the plants known by people with health benefits are picked up and used for the treatment of various diseases.

In summary, it can be concluded that the essential oils of different varieties of plants have different spectrum of promising biological properties, which include antibacterial and antifungal properties. The current expansion of research on antimicrobial agents raises hopes that the well-known essential oils, as well as those from new plant varieties may become important class of therapeutically substances.

Due to the apparent increasing resistance of many species of bacteria to antibiotics, the results of the study indicate that it is possible to use plant extracts including essential oils, both in the protection of crop and preservation of food products obtained therefrom.

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Przeciwbakteryjna i przeciwgrzybowa aktywność wyciągów roślinnych

Streszczenie

W doświadczeniach laboratoryjnych oceniono aktywność przeciwdrobnoustrojową wodnych wyciągów roślinnych (wywar, napar, macerat) oraz olejków eterycznych na zahamowanie wzrostu chorobotwórczych kolonii bakterii i grzybów. Do przygotowania ekstraktów i olejków wykorzystano różne części następujących roślin: lawenda wąskolistna (*Lavandula vera* L.), melisa lekarska (*Melissa officinalis* L.), sosna zwyczajna (*Pinus sylvestris* L.). Przeciwdrobnoustrojowe właściwości wyciągów i olejków eterycznych testowano na bakteriach: *Bacillus subtilis, Escherichia coli, Listeria monocytogenes, Micrococcus luteus, Staphylococcus aureus* i grzybach: *Alternaria alternata*, *Aspergillus*

glaucus, *Aspergillus niger*, *Botritis cinerea*, *Cladosporium herbarum, Fusarium culmorum*, *Fusarium oxysporum*, *Fusarium poae, Fusarium sambucinum*, *Fusarium solani*, *Penicillium chrysogenum*, *Sclerotinia sclerotiorum*.

Analiza uzyskanych wyników wykazała, że aktywność wyciągów zależała od gatunku rośliny, sposobu przygotowania wyciągów oraz wrażliwości mikroorganizmów wybranych do badań. Wykazano istotnie różną reakcję testowanych patogenów na wyciągi z poszczególnych gatunków roślin.

Zarówno ekstrakty jak i olejki eteryczne były źródłem substancji aktywnych, które w różnym stopniu hamowały wzrost i rozwój wybranych szczepów bakterii oraz grzybów. Na aktywność przeciwdrobnoustrojową ekstraktów i olejków mogły wpływać: użyty surowiec, efektywność procesów otrzymania wyciągów, a także zawartość składników aktywnych o działaniu antybakteryjnym i przeciwgrzybowym. Działanie hamujące wzrost drobnoustrojów przez ekstrakty roślinne zależało od sposobu przygotowania wyciągów, który prawdopodobnie wpłynął na kształtowanie się różnych profili związków chemicznych.

Olejki eteryczne charakteryzowała największa aktywność przeciwdrobnoustrojowa wobec większości badanych mikroorganizmów, aniżeli wodnych wyciągów roślinnych. Najwyższą aktywnością przeciwbakteryjną, a najniższą przeciwgrzybową wyróżniał się olejek pozyskany z lawendy wąskolistnej. Natomiast najlepszymi właściwościami przeciwgrzybowymi charakteryzował się olejek uzyskany z sosny zwyczajnej.

Spośród badanych mikroorganizmów najwyższą wrażliwość na zastosowane ekstrakty roślinne wykazały szczepy z rodzajów: *Listeria monocytogenes, Micrococcus luteus* oraz *Sclerotinia sclerotiorum*, *Fusarium poae.*

Słowa kluczowe:
aktywność przeciwdrobnoustrojowa, wyciągi roślinne, olejki eteryczne, metoda dyfuzyjna

Keywords:
antimicrobial activity, plant extracts, essential oils, disc diffusion method