

ANTIMICROBIAL POTENTIAL OF DIFFERENT MEDICINAL PLANTS AGAINST FOOD INDUSTRY PATHOGENS

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ABSTRACT

Work aimed to investigate the antimicrobial activity of medicinal plants against selected species of food industry pathogens *in vitro* conditions. The detection of antibacterial properties was examined by the disc diffusion method and the method of the minimum inhibitory concentrations (MIC). The cultivation of microorganisms after the 24 h was performed by disc diffusion method. Petri dishes have grown at 37 °C in which the Mueller - Hinton agar and application it to the sterile paper disc impregnated with the extract. The thickness of the resulting inhibition zone was measured with a ruler after completion of the culture. After the preparation of bacteria and extracts of certain concentrations of a subsequently added to wells microplates we use the method of the minimum inhibitory concentration (MIC) which was conducted out as the second measurement, and we took the readings absorbance spectrophotometer at 570 nm using the Glomax plate spectrophotometer. We found out, that *Equisetum arvense* demonstrated the largest zones of inhibition to the tested Gram-positive and Gram-negative bacteria. The greatest antimicrobial activity achieved *Equisetum arvense*, *Urtica dioica*, and *Taraxacum officinale* against *Salmonella enterica* subsp. *enterica* CCM 3807 and *Yersinia enterocolitica* CCM 5671. *Equisetum arvense* and *Taraxacum officinale* was the most effective against *Escherichia coli* CCM 2024 and the least effective were *Tussilago farfara* and *Mentha piperita* with using the method of minimum inhibitory concentrations.

Keywords: medicinal plants; antibacterial effect; Gram-positive and Gram-negative bacteria; disc diffusion method; MIC

INTRODUCTION

Salvia officinalis belongs to the largest genus of the family Lamiaceae containing approximately 900 different species worldwide. Some of them can be used as an ingredient in the kitchen, others have been used in the cosmetics industry, and their essential oils are also used as ingredients in various fragrances. Analysis of essential oils of different sage species has shown the most important components, which are made up of substances such as eucalyptol, borneol, thujone, or camphor. Eucalyptol or 1,8-cineol is a natural organic compound. These are cyclic ether and monoterpenoid. Positive properties of killing to leukemia cells have been found *in vitro* (Baser et al., 1993; Baser et al., 1997; Ahmadi and Mirza, 1999).

The essential oil of sage extract as reported Hammer et al. (1999) and Marino et al. (2001) has inhibitory properties against the following bacteria: *Bacillus cereus*, *Bacillus megatherium*, *Bacillus subtilis*, *Aeromonas hydrophila*, *Aeromonas sobria*, *Klebsiella oxytoca*, *Candida albicans*, *Shigella*, *Salmonella*, and *Cryptococcus*. Small inhibition zones against *Escherichia coli* and *Staphylococcus aureus* have also been observed. The antimicrobial activity of sage *in vitro* was determined using a minimum inhibitory concentration (MIC) and

achieved a higher activity against Gram-positive bacteria. The most sensitive of these were *S. epidermidis* (MIC = 12.5 µg.mL⁻¹), *B. cereus* and *B. subtilis* (MIC = 25 µg.mL⁻¹). In the case of Gram-negative bacteria, only significant activity was demonstrated against *E. coli*, *S. typhi* and *K. pneumoniae*, which showed the lowest MIC values (50 µg.mL⁻¹). It was moderately active against *P. vulgaris* and *S. enteritidis* (MIC = 100 µg.mL⁻¹) (Tenore et al., 2011).

Melissa officinalis belongs to the family Lamiaceae. It is characterized by having 2 to 8 cm soft, slightly hairy leaves, with older, larger leaves having a heart shape (Bown, 2001). Lemon balm as a medicinal plant has proven antimicrobial and antiviral effects. In addition to these effects, anti-fungal (Darouche et al., 2006), immunomodulatory (Fang et al., 2005), and antioxidant effects comparable to tocopherol have also been shown. In addition to soothing effects, lemon balm extract has anti-bloating effects, convulsions, antibacterial and antiviral effects, as well as anti-inflammatory, antioxidant and neuroprotective effects (Dastmalchi et al., 2008; Pereira et al., 2009).

Mentha x piperita (Lamiaceae) is generally used for various therapeutic purposes. It comes from Europe and

the Middle East, is widespread in Brazilian culture, and can grow in all areas of the country thanks to its modesty. Essential oils show a strong bactericidal effect, in particular against *Escherichia coli*. When inhaled oil was used concomitantly with multiple drugs in patients with lung tuberculosis, the number of bacilli was demonstrably reduced (Dejani et al., 2014). Mint contains a large amount of fragrant (0.1 – 1%) and essential oils. These are mainly monoterpenes, the main components of which are menthol (29 – 48%), menthone (20 – 31%), menthofuran (6.8%), and their derivatives (isomenthone, neomenthol, menthyl acetate, and pulegone). In menthol and peppermint oil, fungicidal and antiviral activity against *Candida albicans*, *Aspergillus albus*, dermatophytic microscopic fungi, and Herpes simplex virus has been demonstrated (Spirling and Daniels, 2001).

Equisetum arvense (Equisetaceae) finds its use in internal use for flushing in bacterial and inflammatory diseases of the urinary tract, kidney sand, gout, and rheumatic diseases; as concomitant treatment in chronic lung diseases, osteoporosis, varicose veins, and immunosuppression. The gilt tea is used to relieve gastric mucosal irritations and heartburn. As the age in the human body increases, the silicon content decreases, so the use of this plant is particularly meaningful in geriatrics (Bühningová, 2010). In determining the antimicrobial activity by the disk diffusion method, the essential oil from the gilt showed strong activity against all strains tested. With the highest inhibition zone, values were found against Gram-negative *S. enteritidis* (35 mm) and *K. pneumoniae* (37 mm). The antimicrobial activity of the essential oil of horsetail oil can be attributed to the presence of various substances, in particular phenol, monoterpenes, and thymol. Also, the combination of thymol and 1,8-cineol may result in an important synergistic fungicidal effect (Vatňák et al., 2014).

Taraxacum officinale belongs to the family Asteraceae. It has a beneficial effect on human health due to its antioxidant and antiallergic properties. The root extract can be used as a probiotic *in vitro* (Yan et al., 2011). A very important part of the plant is the group of sesquiterpene lactones (have anti-inflammatory and anti-cancer effects), phenylpropanoids, polysaccharides and triterpenoids of saponins. The major components of sesquiterpene lactones can often be found in the form of glycosides. These compounds include taraxacine, taraxacerin, dihydrolactucin, ixerin (Schütz et al., 2006a, b). Leaves and dandelion root have beneficial effects in digestion and are considered bitter digestive stimulants. Diuretic effects have also been shown. They probably share a high content of daisies. The root is significant due to mucoprotective, prebiotic, hypoglycemic, and immunostimulatory effects. The leaves in turn have diuretic and anti-inflammatory effects (Trojanová et al., 2004).

Tussilago farfara has found its application in the treatment of cough, bronchitis, and asthmatic diseases. Phytochemical studies have shown that the flower contains several types of metabolites, including essential oils, sesquiterpenes, triterpenes, flavonoids, and phenylpropanoids (Li et al., 2013). Studies have shown that coltsfoot has marked pharmacological and antagonistic effects and also has antioxidant and

antimicrobial activity (Gao et al., 2008). The antimicrobial activity of the coltsfoot *in vitro* was determined using the disk diffusion method. The highest antibacterial activity of *Tussilago farfara* was found on Gram-positive bacteria *Lactobacillus rhamnosus* (6.67 ± 1.53 mm) and lower on yeast *Saccharomyces cerevisiae* (1.67 ± 0.58 mm) (Kačániová et al., 2013).

Urtica dioica (Urticaceae) has been known in Europe and has been used as a medicinal plant for over 2000 years. The leaf and the root are used for these purposes. Leaf extract has been found to be of use in the symptomatic treatment of arthritis, arthrosis, and rheumatic problems, as well as a diuretic for inflammatory diseases of the urinary tract and bladder and overall detoxification of the body. The whole part of the plant can be used for various purposes such as food, feed, medicines, cosmetics, biodynamic agriculture, and textile production (Bodros and Baley, 2008). Clinical studies have shown that the juice obtained from this plant has a diuretic effect in patients suffering from congestive heart failure. It helps in digestion and promotes milk production of nursing mothers. It provides short-term pain relief and is therefore also used to treat rheumatism. It has also been used in the treatment of arthritis, urinary tract diseases, respiratory diseases, body cleansing, etc. It contains silicic acid, which has diuretic effects, chlorophyll with anti-inflammatory and disinfectant properties, anti-bleeding tannins, and glucokinins, which lower blood sugar (Bisht et al., 2012).

Scientific hypothesis

The different antimicrobial effect exists among analysed medicinal plants for individual food industries pathogens. Antimicrobial activity to pathogenic bacterial strains of food industries is variable for individual medicinal plant species.

MATERIAL AND METHODOLOGY

Plant material

Extracts from 7 different medical plants, obtained from Nitra region, were used to demonstrate antimicrobial activity against selected pathogens. The plant parts such as stems, leaves, and flowers were left to dry in a well-aired dark place at room temperature. Ten grams of dried plants were crushed and extracted with 100 mL of ethanol. Extracts from the plants were leached for two weeks at room temperature and subsequently, ethanol was evaporated by vacuum evaporator (Stuart RE300DB, UK). The samples were stored in the freezer at -20 °C.

Tested strains of microorganisms

For determination of antimicrobial activity 5 bacterial species were purchased from Czech Collection of Microorganisms (CCM, Brno). Three of selected strains were Gram-negative (*Salmonella enterica* subsp. *enterica* CCM 3807, *Yersinia enterocolitica* CCM 5671, *Escherichia coli* CCM 2024) and two strains were Gram-positive (*Staphylococcus aureus* subsp. *aureus* CCM 2461, *Listeria monocytogenes* CCM 4699).

Table 1 Antimicrobial activity with disk diffusion method in mm (mean \pm SD).

Medicinal plants	<i>Escherichia coli</i> CCM 2024	<i>Salmonella enterica</i> subsp. <i>enterica</i> CCM 3807	<i>Yersinia enterocolitica</i> CCM 5671	<i>Staphylococcus aureus</i> subsp. <i>aureus</i> CCM 2461	<i>Listeria monocytogenes</i> CCM 4699
<i>Tussilago farfara</i>	11.33 \pm 0.58	13.33 \pm 0.58	12.37 \pm 0.58	5.33 \pm 0.58	4.67 \pm 1.15
<i>Equisetum arvense</i>	14.67 \pm 0.58	14.33 \pm 0.58	15.33 \pm 0.58	6.33 \pm 0.58	6.67 \pm 0.58
<i>Melissa officinalis</i>	12.33 \pm 1.53	10.67 \pm 0.58	10.67 \pm 1.53	4.33 \pm 0.58	5.33 \pm 0.58
<i>Urtica dioica</i>	8.67 \pm 1.15	10.33 \pm 1.15	11.33 \pm 0.58	4.67 \pm 0.58	4.67 \pm 0.58
<i>Taraxacum officinale</i>	8.33 \pm 0.58	8.33 \pm 1.53	10.67 \pm 0.58	3.67 \pm 0.58	4.00 \pm 1.00
<i>Salvia officinalis</i>	7.33 \pm 0.58	9.33 \pm 1.53	9.33 \pm 0.58	3.33 \pm 0.58	4.67 \pm 0.58
<i>Mentha piperita</i>	7.67 \pm 0.58	9.33 \pm 0.58	8.67 \pm 0.58	4.33 \pm 0.58	3.33 \pm 0.58

Disk diffusion method

Five bacterial cultures grown overnight at 37 °C Muller Hinton broth (MBH) were diluted with distilled water to the turbidity 0.5 according to McFarland standard (measured on densitometer). Petri dishes with Mueller-Hinton agar were covered by 100 μ L of cell culture with use of L-shaped cell spreader. Petri dishes were dried in a thermostat.

For each bacteria strain were prepared 9 discs with 6 mm diameter (7 discs with medicinal plants, 1 positive control and 1 negative control). Discs were soaked in plant extracts with use of sterile tweezers and afterwards were placed on Petri dishes with cell cultures. Disc with distilled water was used as negative control and disc with antibiotic (gentamycin for G⁺ bacteria and tobramycine for G⁻ bacteria) was used as positive control.

Prepared samples were incubated for 24 hours at 37 °C. After incubation period, diameter of each inhibition zone was measured in 3 directions, and average size of inhibition zone was calculated.

Minimum inhibitory concentration

The microdilution method is carried out in 0.5 mL microtiter plates. There was A 96 well microtiter plate used in the assay. A stock solution of various concentrations of the medicinal plant is prepared for each well of the plate. There are 12 wells in row A – H into which we pipet 0.5 mL of Mueller – Hinton broth culture medium. The plant extract was pipetted in the next step from row A – H of the first column. Plant extracts were prepared according to the desired concentration and diluted with dimethylsulfoxide. The extracts were pipetted with the given microorganisms with a concentration of 0.5 McFarland. Using an automatic eight-channel pipette with a preset value of 100 μ L, the suspension from the wells of the first column was transferred to the second column. We repeated the procedure until the concentration was reduced in this way. The whole plate concentration was measured with an absorbance spectrophotometer at 570 nm using the Glomax plate spectrophotometer. The cultivation proceeded from 16 to 18 hours. After its completion, we again measured concentration throughout the plate, and from the pre-and post-cultivation readings, we calculated the differences in absorbance. It can be stated that the minimum inhibitory concentration and its value had the lowest extract concentration at which inhibitory bacterial growth was detectable.

Statistical analysis

Mean and standard deviation with Excel were used for the disk diffusion method. Using obtained absorbance before and after the analysis, we were able to express the differences in absorbance between the measurements as a set of binary values. These values were assigned to exact concentrations. The following formula was created for this specific experiment: value 1 (inhibitory effect) was assigned to absorbance values lower than 0.05, while value 0 (no effect or stimulant effect) was assigned to absorbance values higher than 0.05. For this statistical evaluation probit analysis in Statgraphics software was used for minimal inhibitory concentration.

RESULTS AND DISCUSSION

Several studies have described different biological effects of *Equisetum arvense* L., *Tussilago farfara*, *Melissa officinalis*, *Urtica dioica*, *Salvia officinalis* and *Mentha piperita* extract or tea with natural extract, such as antioxidant, anti-inflammatory, antibacterial, antifungal, vasorelaxant, neuro and cardio protectors (Dos Santos et al., 2005; Sandhu et al., 2010; Salehzadeh et al., 2014; Salih, 2014; Rabbani et al., 2015; Lee et al., 2019), and antiproliferative properties (Yamamoto, Inoue and Hamako, 2004; Ćetojevic-Simin et al., 2010; Stanojevic et al., 2010). Disk diffusion method (Table 1) indicated that *E. coli*, *S. enterica*, and *Y. enterocolitica* were more sensitive to extracts from selected medicinal plants than *L. monocytogenes* and *S. aureus*.

The most significant antimicrobial effect was observed at *Equisetum arvense* that exhibited the largest inhibition zones against all bacteria. Considerable results were observed at *Y. enterocolitica*, *E. coli*, and *S. enterica* with sizes of inhibitory zones 15.33 \pm 0.58 mm, 14.67 \pm 0.58 mm and 14.33 \pm 0.58 mm respectively. Also, *L. monocytogenes* and *S. aureus* showed the highest sensitivity against *Equisetum arvense*.

Tussilago farfara and *Melissa officinalis* also showed significantly larger inhibition zones, while *Salvia officinalis* and *Mentha piperita* seemed to be the least effective. Overall this test indicated that Gram-negative bacteria are more susceptible to the antimicrobial activity of medicinal plant extracts.

According to the minimum inhibitory concentration test (Table 2), slight variations in antimicrobial sensitivity were observed against the disk diffusion method.

Table 2 Minimum inhibitory concentration of medicinal plants in $\mu\text{g.mL}^{-1}$.

Medicinal plants		<i>Escherichia coli</i> CCM 2024	<i>Salmonella enterica</i> subsp. <i>enterica</i> CCM 3807	<i>Yersinia enterocolitica</i> CCM 5671	<i>Staphylococcus aureus</i> subsp. <i>aureus</i> CCM 2461	<i>Listeria monocytogenes</i> CCM 4699
<i>Tussilago farfara</i>	MIC50	> 25.6	19.15	19.15	> 25.6	25.60
	MIC90	> 25.6	20.36	20.36	> 25.6	28.57
<i>Equisetum arvense</i>	MIC50	9.59	9.59	9.59	12.80	12.80
	MIC90	10.20	10.20	10.20	14.29	14.29
<i>Melissa officinalis</i>	MIC50	12.80	12.80	19.15	25.60	12.80
	MIC90	14.29	14.29	20.36	28.57	14.29
<i>Urtica dioica</i>	MIC50	12.80	9.59	9.59	12.80	12.80
	MIC90	14.29	10.20	10.20	14.29	14.29
<i>Taraxacum officinale</i>	MIC50	9.59	9.59	9.59	19.15	19.15
	MIC90	10.20	10.20	10.20	20.36	20.36
<i>Salvia officinalis</i>	MIC50	19.15	9.59	10.85	> 25.6	19.15
	MIC90	20.36	10.20	16.67	> 25.6	20.36
<i>Mentha piperita</i>	MIC50	18.03	9.59	25.60	25.60	> 25.6
	MIC90	29.07	10.20	28.57	28.57	> 25.6

However, the same trend with higher sensitivity against plant extract was observed in a group of Gram-negative bacteria *E. coli*, *S. enterica*, and *Y. enterocolitica*. Also, the most conspicuous growth inhibition was caused by *Equisetum arvense* extract with the lowest detected inhibitory concentration at MIC 50: $9.59 \mu\text{g.mL}^{-1}$ and MIC 90: $10.20 \mu\text{g.mL}^{-1}$ for Gram-negative bacteria and MIC50: $12.8 \mu\text{g.mL}^{-1}$ and MIC90: $14.29 \mu\text{g.mL}^{-1}$ for Gram-positive bacteria (*L. monocytogenes* and *S. aureus*). This observation corresponded with the previous test.

In addition, according to MIC test, also *Taraxacum officinale* and *Urtica dioica* extracts achieved comparable values of cell growth inhibition as *Equisetum arvense*. On the other side, *Mentha piperita* and *Tussilago farfara* seemed to be the least effective.

From the perspective of bacteria, the most sensitive to the antimicrobial activity of all tested medicinal plant extracts was *S. enterica* followed by another Gram-positive bacteria *E. coli* and *Y. enterocolitica* that were slightly more resistant especially to plant extracts with inferior antimicrobial effectivity.

A great interest in biologically active substances of plant origin increased in recent years. Many of these substances demonstrated an antimicrobial effect (Essawi and Srour, 2000).

Kačániová et al. (2013) tested the antimicrobial activity of coltsfoot (*Tussilago farfara*) against selected species of microorganisms: *Escherichia coli* CCM 3988, *Enterococcus raffinosus* CCM 4216, *Staphylococcus epidermis* CCM 4418, *Lactobacillus rhamnosus* CCM 1828, *Pseudomonas aeruginosa* CCM 1960, *Serratia rubidaea* CCM 4684, and *Saccharomyces cerevisiae* CCM 8191. Ethanol extract of coltsfoot with two methods, disk diffusion method, and minimal inhibitory concentration method were used for testing. The extract was most effective against *Serratia rubidaea* CCM 4684 and *Saccharomyces cerevisiae* CCM 8191.

Janovská et al. (2003) demonstrated that extracts from *Tussilago farfara*, *Chelidonium majus*, and *Schisandra chinensis* had proven antimicrobial activity. Extract from each plant had significantly different activity against tested

microorganisms, but various extracts showed greater antimicrobial activity against *Bacillus cereus* and *Staphylococcus aureus* than against *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*.

Also, Kokoska et al. (2002) reported that *Tussilago farfara* extract had an antimicrobial effect on *Bacillus cereus* (15.63 mg.mL^{-1}) and *Staphylococcus aureus* (62.50 mg.mL^{-1}) by MIC susceptibility test. The antimicrobial effects of the coltsfoot extract against *Escherichia coli* were not determined, although sensitivity of *E. coli* was observed in other medicinal plants.

According to two statistical processing of disc diffusion method Hleba et al. (2013) indicated that the *Tussilago farfara* extract reached size of inhibition zones $16.7 \pm 3.65 \text{ mm}$ for ampicillin and chloramphenicol resistant *Escherichia coli* isolated from conventional cow breeding and $8.3 \pm 1.41 \text{ mm}$ for antibiotics when we consider that *Escherichia coli* has been isolated from organic mare breeding. In contrast, the methanolic extract of *Aesculus hippocastanum* (diameter of inhibition zones $9 \pm 1.88 \text{ mm}$) had the least antimicrobial effect.

Milovanović et al. (2007) investigated the antimicrobial effects of horsetail (*Equisetum arvense*) extract, with inhibition zones size $12.1 \pm 0.5 \text{ mm}$.

Modarresi-Chahardehi et al. (2012) compared various types of extract preparation of *Urtica dioica* related to antimicrobial activity. Ethyl acetate, hexane and chloroform extracts displayed higher antimicrobial activity than the other extracts. The highest growth inhibition of ethyl acetate extract was observed against *Bacillus cereus*, methicillin resistant *Staphylococcus aureus*, and *Vibrio parahaemolyticus*. Phenols from *Urtica dioica* are one of the major groups associated with the inhibition of microbial infections in cancer (Dar et al., 2012). Also, *Urtica dioica* is a rich source of phytochemicals, such as phenolic compounds and minerals, that can be used as a potential source of useful drugs (Ahmed et al., 2012).

Radulović et al. (2006) examined essential oil from *Equisetum arvense*. The study showed significant antimicrobial properties against all tested strains. Diameter of inhibition zone ranged from 23 to 37 mm with the

highest inhibition zone against Gram-negative bacteria *Salmonella enteritidis* (35 mm) and *Klebsiella pneumoniae* (37 mm). Significant reduction of bacterial growth was demonstrated in medically important pathogens, such as *Staphylococcus aureus* (28 mm). The antimicrobial activity was greater or similar to conventional antibiotics. In addition, activity against fungi *Candida albicans* and *Aspergillus niger* was observed. The study also suggested that Gram-negative bacteria *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Salmonella enteritidis* were more susceptible to tested essential oils than Gram-positive *Staphylococcus aureus*. Exception was Gram-negative *Escherichia coli* which were the most resistant of all tested bacteria.

According to the MIC and MBC method used, the sage extract oil showed an interesting activity against Gram-positive pathogens. The most sensitive was *Staphylococcus epidermidis* (MIC = 12.5 µg.mL⁻¹), but the oil also showed very good activity against *Bacillus cereus* and *Bacillus subtilis* (MIC = 25 µg.mL⁻¹ for both). As regards Gram-negative bacteria, the sample showed significant efficacy only against *Escherichia coli*, *Salmonella typhi* and *Klebsiella pneumoniae*, which showed the lowest MIC values (50 µg.mL⁻¹), while being moderately active against *Proteus vulgaris* and *Salmonella enteritidis* (MIC = 100 µg.mL⁻¹) (Tenore et al., 2011).

Results of Mazandarani et al. (2013) show that oil from *Achillea millefolium* extract may be an alternative to antibiotics in the control of some Gram-positive and Gram-negative pathogens. The antibacterial activity of some *Agrimonia eupatoria* extracts against pathogenic bacteria (*Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*) and their action on wound healing in rats has been confirmed. The presence of certain active substances in both aqueous and ethanol extracts has also been demonstrated, indicating that *Agrimonia eupatoria* may exhibit antimicrobial activity. The results of this study showed that the ethanol extract was more effective at inhibiting the bacteria tested than the aqueous extract. *Pseudomonas aeruginosa* was most resistant to the action of ethanol extract, while *Escherichia coli* with the highest zone of inhibition of 20 mm was the most susceptible. There was moderate activity against *Staphylococcus aureus* with 15 mm inhibition zone after application of ethanol extract (10 mg.mL⁻¹).

With regard to the antimicrobial activity of *Melissa officinalis* oil, Romeo et al. (2008) and Hussain et al. (2011) indicated its antibacterial activity against *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis*, *Bacillus pumilus*, *Pseudomonas aeruginosa*, *Salmonella Poona*, *Escherichia coli* and *Listeria innocua*. Antimicrobial activity (expressed as µg.mL⁻¹) from four ethanolic extracts of *Achillea millefolium*, *Agrimonia eupatoria*, *Melissa officinalis* and *Tilia platyphyllos* against various strains of Gram-positive and Gram-negative bacteria. It was found that more susceptible to *Agrimonia eupatoria* extract with a MIC₅₀ value of 0.80 µg.mL⁻¹ of *Bacillus cereus*, *Lactobacillus brevis* was less susceptible to *Agrimonia eupatoria* with a MIC₅₀ value of 1.48 µg.mL⁻¹.

The bacteria *Lactobacillus hilgardii*, *Enterococcus faecalis*, and *Escherichia coli* were less sensitive to *Agrimonia eupatoria* extract and the values were higher

MIC₅₀ (MIC 2.56 – 17.06 µg.mL⁻¹). *Lactobacillus brevis* was more sensitive to *Melissa officinalis* with a MIC₅₀ of 6.39 µg.mL⁻¹. In addition, the activity of *Achillea millefolium* against Gram-positive and Gram-negative bacteria was contrary to previous reports that antibacterial activity was limited to Gram-positive bacteria from medicinal herbs (Ghaima, 2013).

CONCLUSION

Medicinal plants have been used for treating various diseases due to their beneficial effects and sources of bioactive secondary metabolites for long times. In recent years, research has been increasingly investigating their antimicrobial activity against various pathogens. The emphasis should be placed on further research and monitoring of their effects. The plants that we used in the research showed an antimicrobial effect against Gram-positive and Gram-negative bacteria.

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Acknowledgments:

This work has been supported by the grants of the APVV SK-BY-RD-19-0014 “The formulation of novel compositions and properties study of the polysaccharides based edible films and coatings with antimicrobial and antioxidant plant additives.”

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