

## Antimicrobial Activity of Aqueous Herbal Extracts

LENKA DOSTALOVA, LENKA DETVANNOVA, LIBOR KALHOTKA  
Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition  
Mendel University in Brno  
Zemedelska 1, 613 00 Brno  
CZECH REPUBLIC

lenka.dostalova@mendelu.cz

**Abstract:** Some plants contain various compounds, which can influence microbial growth, reproduction or basic cell functions. Phenols, polyphenols, micronutrients, essential oils and other compounds belong to them. These compounds are primarily present in various herbs. Extracts from these plants could be used as natural food preservatives, which could inhibit growth of undesirable microorganisms. In this work, antimicrobial activity of aqueous extracts of *Thymus vulgaris*, *Lavandula angustifolia*, *Melissa officinalis*, *Ocimum basilicum*, *Allium schoenoprasum* and *Petroselinum crispum* was tested on five gram negative bacteria – *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Hafnia alvei* and *Raoultella terrigena*, which were isolated from dairy products except *Escherichia coli* (CCM 7929). Antimicrobial activity was tested by disc diffusion method. It was found out the less effective extracts were from thyme and lavender. Basil, chive and parsley extracts showed higher potential to inhibit bacterial growth.

**Key-Words:** antimicrobial activity, herbal aqueous extracts, gram negative bacteria

### Introduction

Natural botanical sources contain a diverse array of compounds such as phenolic acids, flavonoids, tannins, vitamins and terpenoids that account for their biological properties. It was suggested that the antibacterial activity of a total of 46 extracts from spices and herbs was closely associated with the presence of their phenolic constituents. The authors have reported that all the tested spices have a strong antibacterial effect against *B. cereus*, *L. monocytogenes*, *S. aureus*, *E. coli*, and *Salmonella Anatum* [1]. Microbial cells are negatively affected by plant-derived substances via various mechanisms of actions that attack the phospholipid bilayer of the cell membrane and disrupt enzyme systems [2]. They have an influence on enzyme activity associated with energy production or they can cause denaturation of proteins, modify cell wall permeability, causing the loss of macromolecules [3]. Therefore, it is difficult for microorganisms to create resistance against these [4, 5]. Herbal extracts are used as natural preservatives for prolonging shelf-life of food and maintaining of food safety [6, 7]. Antimicrobial activity depends on herb species, concentration of active substances, species and concentration of microorganisms, substrate composition and conditions of treatment and storage of food [8, 9].

### Material and Methods

In this work, antimicrobial activity of herbal aqueous extracts was tested by disc diffusion method on gram negative bacteria. *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Hafnia alvei* and *Raoultella terrigena* were isolated from dairy products by a sequence of cross smears. *Escherichia coli* was used as pure culture from Czech Collection of Microorganisms (CCM 7929). Suspensions of density 0.1 and 1 McF were prepared from 24 hours culture of each bacterium. Herbal aqueous extracts were prepared from fresh *Thymus vulgaris*, *Lavandula angustifolia*, *Melissa officinalis*, *Ocimum basilicum*, *Allium schoenoprasum* and *Petroselinum crispum* in two different concentrations – 1:5 (1 part of herbs, 5 parts of distilled water) and 1:10 (1 part of herbs, 10 parts of distilled water). Herbs were weighed out into distilled water and leaved to infuse for one hour at 95°C. Suspension of bacterium was inoculated on Petri dishes with nutrient medium (Plate Count Agar with Skimmed Milk, Biokar diagnostics, France). Sterile paper discs of diameter 9 mm were impregnated with 30 µl of aqueous extract and placed onto nutrient medium with inoculated bacterium. On each Petri dish, 3 discs were placed. All variants with bacteria and extracts were performed in duplicate. Prepared Petri dishes were placed in thermostat at 37°C. After 24 hours cultivation, zones of inhibition were evaluated by a ruler.

**Results and Discussion**

Diameters of zones of inhibition are stated in the Table 1 and Table 2. Extracts did not show any antimicrobial activity since the diameter was 9.00 mm. It is the diameter of paper disc. In the Table 1,

there are stated results of testing antimicrobial activity of basil, chive and parsley, in the Table 2, there are shown inhibitory zones resulting from testing of thyme, lavender and lemon balm.

Table 1 Diameters of inhibitory zones – basil, chive and parsley

MO	Concentration of MO [McF]	Diameters of inhibitory zones [mm]					
		Basil		Chive		Parsley	
		1:10	1:5	1:10	1:5	1:10	1:5
<i>E. coli</i> CCM 7929	1	10.00	9.33	9.00	9.00	9.17	10.00
	0.1	9.00	9.67	9.50	9.67	9.33	9.00
<i>Hafnia alvei</i>	1	9.67	10.17	10.17	10.20	10.50	11.80
	0.1	9.17	9.00	10.17	10.20	9.00	9.50
<i>Raoultella terrigena</i>	1	10.00	10.17	9.83	10.00	10.00	9.83
	0.1	9.00	9.00	9.33	9.67	9.00	9.00
<i>Klebsiella pneumoniae</i>	1	9.17	9.67	9.00	9.00	9.17	9.00
	0.1	9.00	9.00	9.00	9.00	9.00	9.00
<i>Klebsiella oxytoca</i>	1	10.20	10.20	9.33	9.67	10.33	10.83
	0.1	9.50	9.00	9.17	9.50	10.00	9.00

MO – Microorganism

McF – McFarland Units

Parsley showed relatively strong antimicrobial activity. Basil and chive showed some antimicrobial activity as well. In some cases, the less concentrated

extract was more effective. *Klebsiella pneumoniae* was less susceptible to effect of these extracts.

Table 2 Diameters of inhibitory zones – thyme, lavender and lemon balm

MO	Concentration of MO [McF]	Diameters of inhibitory zones [mm]					
		Thyme		Lavender		Lemon balm	
		1:10	1:5	1:10	1:5	1:10	1:5
<i>E. coli</i> CCM 7929	1	9.17	9.83	9.83	9.17	9.00	9.33
	0.1	9.17	9.00	9.00	9.00	9.67	9.67
<i>Hafnia alvei</i>	1	9.00	9.00	9.00	9.00	9.00	9.00
	0.1	9.17	9.00	9.00	9.00	9.00	9.00
<i>Raoultella terrigena</i>	1	9.00	9.17	9.00	9.17	9.00	9.00
	0.1	9.00	9.17	9.00	9.00	9.00	9.33
<i>Klebsiella pneumoniae</i>	1	9.00	9.00	9.00	9.17	9.33	9.50
	0.1	9.00	9.00	9.00	9.00	9.33	9.00
<i>Klebsiella oxytoca</i>	1	9.00	9.33	9.00	9.00	9.17	10.33
	0.1	9.00	9.00	9.00	9.17	9.00	9.00

These three herbs did not show any significant antimicrobial activity. Lemon balm was the most effective. Aqueous extracts from thyme and lavender influenced growth of microbes a little.

Parsley extract proved to be the most effective. It inhibited all of bacteria; most susceptible were

*Hafnia alvei* and *Klebsiella oxytoca*. On the other side, *Raoultella terrigena* and *Klebsiella pneumoniae* were the least susceptible. Basil inhibited all of bacteria also; chive did not suppress growth of *Klebsiella pneumoniae*. *K. pneumoniae* was very resistant to impact of all tested extracts. Gyawali et Ibrahim [10] present that crude chive

extract containing sulfur compounds can be effective against the growth of *Salmonella* and could be used in food products to prevent the growth of this pathogen. The antimicrobial effect of chive against *E. coli* and yeast (*Pichia membranaefaciens* CCRC 20859) has been also reported [9]. Wahba et al. [11] proved antibacterial activity of different types of pepper, parsley and dill against natural microflora, coliforms, yeasts and moulds and *S. aureus* in Kareish cheese. The most susceptible bacterium to thyme was *E. coli*, contrarily growth of *Klebsiella pneumoniae* was not suppressed; *Hafnia alvei*, *Raoultella terrigena* and *Klebsiella oxytoca* were inhibited only by one concentration of thyme extract. Oregano and thyme possess significant in vitro colicidal and colistatic properties, and this bactericidal concentration irreversibly damaged *E. coli* O157:H7 cells within 1 minute [12]. Lavender and lemon balm did not suppress the growth of *Hafnia alvei*; remaining bacteria were inhibited only in limited way at least by one concentration of extracts. Jianu et al. [13] tested antibacterial properties of lavender, which showed significant bactericidal effects against microorganisms such as *Shigella flexneri*, *Staphylococcus aureus* and *E. coli*.

## Conclusion

Some herbs tested in this work could be used as natural preservatives for prolonging shelf-life of food. Some of them showed moderate antimicrobial effect, thus they rather could be used in mixture with other herbs. Susceptibility of individual bacteria to extracts was very dissimilar. *H. alvei*, *R. terrigena* and *E. coli* 7929 were the most susceptible in the most of cases. It is important to research antimicrobial effects of herbs and other plants in many various forms, like different extracts or essential oils, as a natural source of antimicrobial compounds.

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