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THE APPLICATION OF ESSENTIAL OILS AGAINST MICROORGANISMS

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Abstract. *According to the definition of the International Organization for Standardization, essential oil is „a product obtained from a natural raw material of plant origin, by steam distillation, by mechanical processes, from the epicarp of citrus fruits, or by dry distillation, after separation of the aqueous phase – if any – by physical processes". Essential oils contain secondary plant metabolites with microbiostatic or microbicidal activity on microbial growth. Although herbs and medicinal plants always had a notable use in folk medicine, recently the production and application of essential oils have gained a significant role in the production of foodstuffs, and cosmetic and pharmaceutical products. The increasing resistance of microorganisms to synthetic drugs is a global problem. It imposes the need to test and find new effective substances of a wide spectrum of action against pathogenic microorganisms. Depending on the chemical components present, essential oils have different mechanisms of action against pathogenic microorganisms, and only experimental tests are reliable proof of their activity against microorganisms. There are frequent results of tests and effects of essential oil on certain strains of microorganisms without complete identification of the chemical components present in the essential oil. This paper provides an overview of tests related to the effect of different essential oils on pathogenic microorganisms and microorganisms that are important for the microbiological quality of food.*

Keywords: *essential oils, pathogenic microorganisms, microbiological food quality*

Introduction

Infection is the invasive growth of microorganisms in or on the host causing health problems. On the other side, antimicrobial drug use and antimicrobial resistance (AMR) is a global health problem. Resistance of microorganisms mostly lies in the overuse and spreading of resistant mutants. The resistance of microorganisms to drugs has induced research to find new antimicrobial products that could treat human and animal diseases.

In the field of food technology, there is evidence that usually, food preservation methods such as cooling, drying, pasteurisation, canning, etc. can not always protect the foodstuff during shelf life, and consequently is occurring the growth of saprophytic and/or pathogenic microorganisms. In recent years, consumers have preferred food of good quality and with longer shelf-life. Additionally, customer demands for food without the chemical preservatives of the food preserved with natural means have increased. So any clarification on the antimicrobial use of essential oil is welcome and this is a review of the author's own contribution to the field.

Antimicrobial resistance

AMR is a serious global problem for public health in 21st century. It is estimated that bacterial AMR was directly responsible for global deaths in 2019 and contributed to 4.95 million deaths [1]. In February 2017 World Health Organisation (WHO) published a list of pathogens that includes the pathogens designated by the acronym ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) with highest "priority status" since they represent the great treat to humans [2].

The impact of applied antimicrobial drugs is very difficult to estimate because microorganisms have several resistance mechanisms (Figure 1).

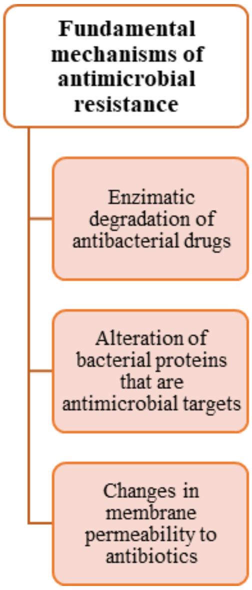

Figure 1. Mechanism of bacterial resistance to antibiotics [3]

Many studies include results from the field, i.e. medical laboratories and hospitals covering the registered patients. So, Jovanović et al. [4] reported that in the territory of Šabac (Serbia) microorganisms *Escherichia coli* (59.51%), *Klebsiella* spp. (14.1%) and *Enterococccus* spp. (8.97%) have the largest share among isolates' uropathogenic representatives. In that study is a high rate of isolates of *E. coli* (54.77%) and *Klebsiella* spp (100%) resistant to ampicillin. In the next study, Jovanović et al. (2024) [5] reported for the same territory that *Staphylococcus aureus*

had dominant participation in the infections of the throat (56.06%), skin (37.96), nose (36.32%), wounds (24.43%), sputum (21.66%).
Another problem is the use of antibiotics in primary animal and agricultural production. AMR in the food chain is a growing problem creating risk for the quality of food products and consequently the risk for human health. For example, the resistance tetracyclin has been detected in probiotic bifidobacteria, including seven *Bifidobacterium animalis* subsp. *lactis* and *Bifidobacterium bifidum* strains [6]. *Lactobacillus bulgaricus and Streptococcus thermophilus* used in dairy products can transfer antibiotic-resistance genes to the food chain [7].
A special problem is the classification of microorganisms into pathogens. For example, coagulase-negative staphylococci are part of the normal microflora of the skin and mucous membranes of humans. In the mid-1980s, it was discovered that coagulase-negative types of staphylococcus also caused diseases in humans, especially intrahospital (nosocomial) infections [8] with novel information about their clinical impact [9] and bacteremia [10]. Kalaba et al. [11] found multi-resistant coagulase-negative staphylococci in fresh milk, which can pose a potential risk to consumers due to possible gene transfer of resistance through the food chain. The results obtained in this study showed that all isolates were resistant to imipenem and cefuroxime, 90% of the isolates were resistant to sulfamethoxazole, 80% of isolates to penicillin, nalidixic acid and lincomycin, and to ampicillin 70% of isolates; resistance to cephalexin, amikacin, gentamicin and ceftriaxone resistance showed 40% of isolates, and tetracycline and amoxicillin resistance showed 20% of isolates; cefazolin resistance shows only one isolate.

Essential oils

Essential oils are easily volatile, aromatic substances, naturally present in the plant and are found in every plant with a distinct smell. They can be found in all parts of plants or can be concentrated in one of its parts (flower, leaf, seed, root, bark). There are 400000 known plant species of both, aromatic and medicinal plants, among which about 2000 species come from nearly 60 botanical families of essential oils-bearing plants [12].
Essential oils vary greatly, sometimes due to genetic causes, but also because of climate, rainfall, or geographic origin. Most essential oils are complex mixtures of hydrocarbons, alcohols, ketones, acids, esters and other aliphatic, acyclic and heterocyclic compounds. The most common and main constituents of essential oils are terpenes, which are characteristic of certain types of plants such as myrcene from laurel, ocimene from basil, menthol from mint, or bisabolene from chamomile. In addition to terpenes, they are widely used as constituents of essential oils compounds with an aromatic structure are also represented. These are most often phenols, aromatic aldehydes and their derivatives. Of many compounds from this group, thymol and carvacrol are significant and are found in thyme oil, eugenol from clove oil, etc. [13-16]. Recently, there has been a growing interest in the clarifying antimicrobial mechanisms of space essential oils and their application in the food industry (Li et al., 2022) [17].

Antimicrobial activity of essential oil

Essential oils as natural, biologically active substances are of great interest to the pharmaceutical industry in the control of human diseases caused by microorganisms. These substances also have insecticidal and antimicrobial activity, which is very important in the food industry for preserving food and preventing the development of pathogens and microorganisms. Literature data show that the essential oils of various species and medicinal plants can prevent the growth of certain pathogens [18].

Many scientific papers reported the activity of essential oils as antibacterial, antifungal and antiviral (Figure 1). The different methods of testing antimicrobial activity can give different results making it difficult to compare the findings in various studies. So, Kalaba et al. [19] modified the disc diffusion method and tested the antibacterial effect of Norway spruce (Picea abies) essential oil on different microbiological media (neutral agar, blood agar, and Mueller Hinton agar). Zones of inhibition showed that the antibacterial effect of spruce essential oils was less pronounced on Mueller Hinton agar than on the other two media. The mentioned paper also compared the antibacterial activity of spruce essential oil concerning the antibacterial activity of five antibiotics (penicillin, amikacin, amoxicillin, ceftriaxone and streptomycin). The essential oil showed a greater antimicrobial effect than penicillin against *Salmonella enteritidis* and *Escherichia* coli and better results than ceftriaxone against *Bacillus subtilis*.

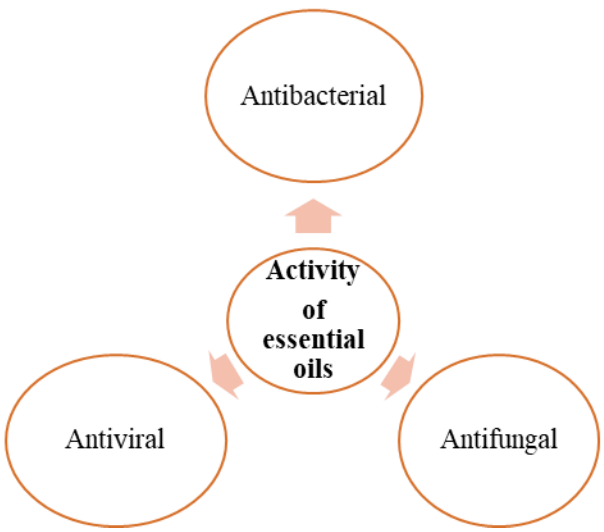

Figure 2. Antimicrobial activity of essential oils

Also, Kalaba et al. [20] noted that an increase in the volume of applied essential oils leads to an increase in the inhibition zone. The antibacterial activity of 20 µl of juniper berry (Juniperus communis) essential oil inhibits clinical isolates of coagulase-negative staphylococci, and 100 µl of the essential oil is antibacterial to

eight of ten tested pathogens. The strains of *Pseudomonas aeruginosa* and *Escherichia coli* showed total resistance to juniper berry essential oil.
Based on the disc diffusion method, Stanojevic et al. [21] reported the antibacterial activity of chamomile flowers essential oil on *Listeria monocytogenes*, *Escherichia coli*, *Salmonella enterica*, *Staphylococcus aureus*, with inhibition zone 13.33±0.25, 31.0±0.37, 25±0.26, and 40.0±0.51, respectively. Also, the authors found the absence of antibacterial activity of chamomile flower essential oil on *Pseudomonas aeruginosa*. The results of antibacterial activity favour the essential oil compared to the effect of antibiotic amoxicillin.
Besides stronger antibacterial activity on bacteria (*Salmonella enterica*, *Providencia stuartii*, coagulase-positive staphylococci, *Streptococcus* group D) compared to commercial antibiotic ciprofloxacin, basil essential oil showed stronger antifungal activity to yeast *Candida albicans* compared to metronidazol (Stanojević et al., 2017) [22].
Marjanović-Balaban et al. (2018) [23] reported results of antimicrobial analysis which indicate a good antimicrobial effect of the peppermint oil (*Menthae piperitae* L.) on all tested bacterial strains (*Listeria monocytogenes* WDCM 00020, *Pseudomonas aeruginosa* WDCM 00024, *Escherichia coli* WDCM 00013, *Salmonella enterica* WDCM 00030). *Staphylococcus aureus* WDCM 00032 was the most sensitive bacterial strain with an inhibition zone of 37.66 mm.
Kalaba et al. (2023) [24] showed that both extracts, *Allium sativum* oil extract and *Allium ursinum* tincture, prepared in "home conditions" according to a traditional recipe, have a high spectrum of antibacterial potential against the examined gram-positive (*Staphylococcus aureus*) and gram-negative (*Salmonella* Epidermidis and *Salmonella* spp) bacteria. Another study by Kalaba et al. (2014) [25] showed that fresh *Allium sativum* extract has higher antifungal activity against *Candida albicans*, *Saccharomyces cerevisiae*, and *Aspergillus brasiliensis* compared to clotrimazole, a standard antifungal drug.
Popović et Đurđević-Milošević [16] tested twelve essential oils on *Escherichia coli* ATCC 25922. All tested essential oils show some antimicrobial activity, except essential oils of anise and cedar. However, effectiveness varies with plant type and concentration of oils. The essential oils of cloves, thyme, cinnamon, mint and rosemary showed the greatest activity. The pure essential oils of these plants have shown a bactericidal effect (thyme, cloves, laurel and oregano), while etheric oils of other tested plants (sage, sailor and lemon) showed a bacteriostatic effect. Besides the results obtained, the essential oils that showed the best effect on the growth of *Escherichia coli* can be recommended for use in the pharmaceutical and food industries, to prevent the development of *Escherichia coli* and the diseases it can cause. Variation of the efficiency of essential oil can be reached by diluting with alcohol or mixing with oils. Same authors Jovanović (ex. Popović) et Đurđević-Milošević [26] reported the results obtained by examining the influence of ten essential oils of different origins (*Thymus vulgaris, Cinnamomum Zeylanicum, Eugenia aromatica, Eucalyptus melliodora, Rosamarinus officinalis, Citrus sinensis, Mentha piperita, Salvia officinalis, Foeniculum vulgare, Pimpinela anisum*) on the growth of *Staphylococcus aureus* ATCC 25923. There is a noticeable difference in the activity of pure essential oils and alcoholic solutions of essential oils. In the case

of thyme, cinnamon and anise, the greatest activity against bacteria was shown by the applied essential oil: alcohol in the ratio of 2:1. Pure essential oil and essential oil: alcohol 1:1 had the same effect with alcoholic solutions of the essential oil, regardless of the proportions. Eucalyptus and orange essential oils showed reductions in the inhibition zone when applied as an alcoholic solution, regardless of the proportion. Based on the above results, Figure 3 compares results of the same essential oils tested in the same test conditions against *Escherichia coli* ATCC 25922 by Popović et Đurđević-Milošević (2008) [16] and against *Staphylococcus aureus* ATCC 25923 by Popović et Đurđević-Milošević (2009) [26]. It is a visible variation of essential oil's efficiency depending on the oil origin and test microorganisms.

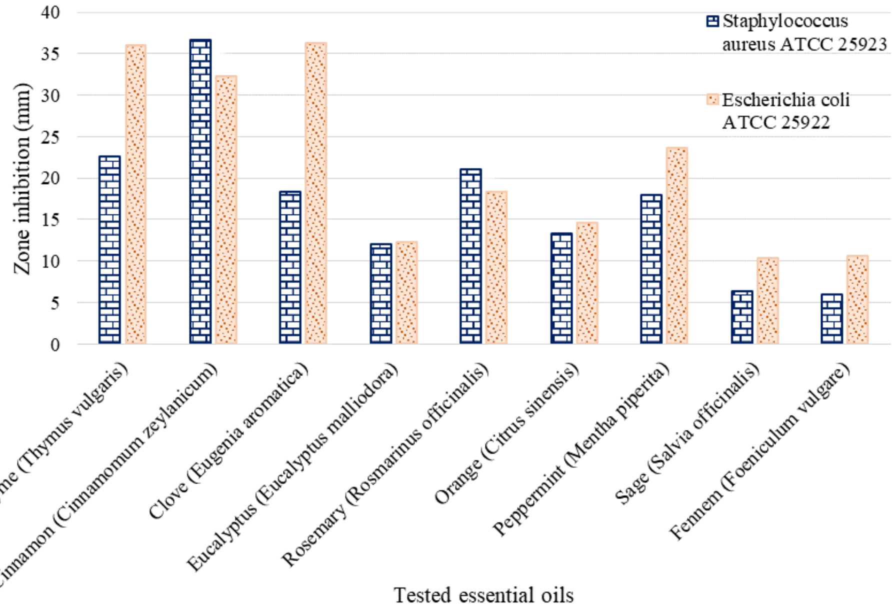

Figure 3. The comparison of tested essential oils by Popović et Đurđević-Milošević (2008) [16] and by Popović et Đurđević-Milošević (2009) [26]

Results presented by Kalaba et al. showed [27] that the essential oil of St. John's Wort (*Oleum Hyperici*) showed a weak inhibitory effect on the growth of *Staphylococcus aureus*, but no inhibitory effect on the growth of *Salmonella* Typhimurium. The results showed that the essential oil of St. John Wort diluted with olive oil has a good antimicrobial effect on the growth of *Pseudomonas aeruginosa*, but a weak effect was observed with pure Oleum Hyperici and also diluted with ethanol (1:1 and 2:1).

Recently there have been presented studies about the antiviral activity of essential oils [28]. In addition, essential oils can be used as a complementary treatment of symptoms where Eucalyptus essential oil exerts anti-inflammatory, mucolytic, and spasmolytic effects in the attenuation of inflammatory responses caused by viruses, in particular, respiratory diseases [29].

Conclusions

At a time of great global concern about the resistance of microorganisms to antibiotics, essential oils represent a significant alternative to synthetic chemicals and additives. Many studies are proving the antimicrobial efficiency of essential oils.
The clarification of the antimicrobial properties of essential oils is giving the direction for their use in the food, pharmaceutical and chemical industries. Standardisation of methodology could be an important contribution to a better understanding of the efficiency of various essential oils.

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PRIMENA ESENCIJALNIH ULJA PROTIV MIKROORGANIZAMA

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Sažetak. *Prema definiciji Međunarodne organizacije za standardizaciju, esencijalno ulje je „proizvod dobijen od prirodne sirovine biljnog porekla destilacijom vodenom parom, meahničkim procesima iz epikarpa citrusa ili suvom destilacijom, nakon odvajanja vodene faze, ako postoji, fizičkim procesima“. Esencijalna ulja sadrže sekundarne metabolite biljaka koji su sposobni da zaustave ili uspore rast mikroorganizama. Iako su začinske i medicinske biljke oduvek imale zapaženu primenu u narodnoj medicini, proizvodnja i primena esencijalnih ulja u novije vreme dobija značajnu ulogu u proizvodnji prehrambenih, kozmetičkih i farmaceutskih proizvoda. Sve veća otpornost mikroorganizama na sintetičke lekove je globalni problem i nameće potrebu ispitivanja i pronalaženja novih efikasnih supstanci širokog spektra djestva protiv patogenih mikroorganizama. Zavisno od prisutnih hemijskih komponenata, esencijalna ulja imaju različite mehanizme dejstva prema mikroorganizima, te su eksperimentalna ispitivanja pouzdan dokaz njihove aktivnosti prema mikroorganizmima. Česti su rezultati ispitivanja i efekata esencijalnog ulja na određene sojeve mikroorganizama, bez potpune identifikacije u esencijalnom ulju prisustnih hemijskih komponenata. U ovom radu je dat pregled ispitivanja vezanih za dejstvo različitih esencijalnih ulja na patogene mikroorganizme i mikoorganizme koji su od značaja za mikrobiološki kvalitet prehrambenih proizvoda.*

Ključne riječi*: esencijalna ulja, patogeni mikroorganizmi, mikrobiološki kvalitet hrane*