

Antibacterial activities of rosemary essential oils and their components against pathogenic bacteria

Abstract

There are many known essential oils, of which only 300 are commercially important for different types of industry. Antimicrobials are used in food for two main reasons: to control natural spoilage processes and to prevent/control growth of micro-organisms, including pathogenic microorganisms. The aim of this review was to investigate antibacterial effects of rosemary essential oils (EOs) on some Gram-positive and Gram-negative bacteria: *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Salmonella enteritidis*, *Salmonella typhimurium*. *Rosmarinus officinalis* essential oil is very important for its medicinal uses and its powerful antibacterial, cytotoxic, anti-mutagenic, antioxidant, anti-phlogistic and chemopreventive properties. The antimicrobial and other biological activities of rosemary essential oil are directly correlated with the presence of bioactive volatile components. To this end, one possibility has been the use of rosemary essential oils (EOs) and the compounds found there in as alternative antimicrobial food preservatives. The future will see investigation of food applications of the naturally occurring antimicrobials, especially the effectiveness of rosemary EOs, individually and in combination with other parts of plant extract, other effective EOs and other food-processing techniques.

Keywords: antibacterial effect, essential oil, rosemary, pathogenic bacteria

Volume 3 Issue 4 - 2018

Jasmina Stojiljkovic,¹ Metodija Trajchev,²
Dimitar Nakov,² Milena Petrovska³

¹Food Technology, College of Applied Studies, Republic of Serbia

²Faculty of Agricultural Science and Food, University St. Cyril and Methodius, Republic of Macedonia

³Faculty of Medicine, University St. Cyril and Methodius, Republic of Macedonia

Correspondence: Jasmina Stojiljkovic, MSc of Technological Microbiology, Coordinator of the department Food Technology, College of Applied Studies, Filipa Filipovica 20, Vranje, Republic of Serbia, Tel +3816 3108 6667, Fax +3811 7404 661, Email jasmina_vranje@yahoo.com

Received: February 05, 2018 | **Published:** September 18, 2018

Abbreviations: EOLs, essential oils; ATP, adenosine three phosphate; MIC, minimum inhibitory concentrations; IZ, inhibition zone; ATCC, American type culture collection; NCTC, national collection of type cultures, a culture collection of public health England

Introduction

Plants producing essential oils belong to various genera in about 60 families, including *Alliaceae*, *Apiaceae*, *Asteraceae*, *Lamiaceae*, *Myrtaceae*, *Poaceae*, and *Rutaceae*.¹ Plant essential oils are a potentially useful source of antimicrobial compounds.² Aromatic plants and their essential oils (EOs) have been used since ancient times in food, agriculture, medicine, cosmetic applications, as condiments and spices, in therapeutic uses, as antimicrobials, as flavoring agents, and in storage as insecticidal agents.³ The use of plant essential oils has been the new focus of agricultural and food industries as an alternative to synthetic food additives for inactivation of microorganisms such as pathogenic and spoilage bacteria that cause food safety and public health-related issues during transportation, storage, shelf-life, and packaging.⁴ Antimicrobials are used in food for two main reasons: to control natural spoilage processes (food preservation), and to prevent/control growth of microorganisms, including pathogenic microorganisms (food safety).⁵ Natural antibacterial essential oils have gained an increased interest and are considered as safe and eco-friendly alternative to control foodborne bacteria and other pathogenic microorganisms, notably drugs resistant ones.^{6,7} In food processing, it is important that proper measures are taken to ensure the safety and stability of products for the duration of their shelf life.⁸

Discussion

Mechanisms of action of the EOs on pathogenic bacteria

Some studies have demonstrated that whole EOs usually have higher antibacterial activity than the mixtures of their major components, suggesting that the minor components are critical to the synergistic activity, though antagonistic and additive effects have also been observed.^{9,10} EOs derived from spices and plants have antimicrobial activity against *Listeria monocytogenes*, *Salmonella typhimurium*, *Escherichia coli* O157:H7, *Shigella dysenteriae*, *Bacillus cereus* and *Staphylococcus aureus* at levels between 0.2 and 10 µL/mL.¹¹ Determination of the antimicrobial activity of 17 essential oils against *Escherichia coli* O157:H7 and *Salmonella enterica* in apple juices indicated that the reduction in the number of bacteria can reach 50%.¹² Considering the large number of different groups of chemical compounds present in EOs, it is most likely that their antibacterial activity is not attributable to one specific mechanism but that there are several targets in the cell. An important characteristic of EOs and their components is their hydrophobicity, which enables them to partition in the lipids of the bacterial cell membrane and mitochondria, disturbing the structures and rendering them more permeable. Leakage of ions and other cell contents can then occur and causes the death of bacterial cell. Generally, the EOs possessing the strongest antibacterial properties against food borne pathogens contain a high percentage of phenolic compounds such as carvacrol, eugenol and thymol.¹¹ The mechanisms of action of the EOs include the degradation of the cell wall, damaging the cytoplasmic membrane,

cytoplasm coagulation, damaging the membrane proteins, increased permeability leading to leakage of the cell contents, reducing the proton motive force, reducing the intracellular ATP (Adenosine Three Phosphate) pool via decreased ATP synthesis and augmented hydrolysis that is separate from the increased membrane permeability and reducing the membrane potential via increased membrane permeability. The cell wall of Gram-negative bacteria is more resistant to the activity of EOs and their components. The Gram-negative cell wall does not allow for the entrance of hydrophobic molecules as readily as Gram-positive bacteria; thus, EOs are less able to affect the cell growth of the Gram-negative bacteria.¹³ Interaction of essential oils with microbial cell membranes results in growth inhibition of some Gram-positive and Gram-negative bacteria.¹⁴ It has been reported that Gram-positive bacteria appear to be more susceptible to the antibacterial properties of essential oil compounds than Gram-negative bacteria. This is expected, as Gram-negative bacteria have an outer layer surrounding their cell wall, limiting the access of hydrophobic compounds. Gram-negative bacteria are usually more resistant to the plant-origin antimicrobials and even show no effect, compared to Gram-positive bacteria.^{15,16}

Essential oil of rosemary

Rosemary (*Rosmarinus officinalis* L.) is of considerable importance in term of its great an important medicinal and aromatic value. This plant belongs to *Lamiaceae* family. Rosemary is a perennial evergreen herb with fragrant needlelike leaves.¹⁷ Rosemary herbs have been widely used in the traditional medicine and cosmetics. They are also used as flavouring agents in foods.¹⁸ *Rosmarinus officinalis* essential oil is also important for its medicinal uses and its powerful antibacterial, cytotoxic, antimutagenic, antioxidant, antiphlogistic and chemopreventive properties.¹⁹ The major components determined in *R. officinalis* essential oil were 1,8-cineol (38.5%), camphor (17.1%), α -pinene (12.3%), limonene (6.23%), camphene (6.00%) and linalool (5.70%).²⁰ The chemical analysis of rosemary essential oil by GC has identified 11 compounds which they make 78.25% rosemary essential oil. Major components of the essential oil include alpha-pinene (23.93%), camphen (8.7%), camphor (10.97%), verbenon (15.44%), p-cymene (7.48%) and 3-Octanone (5.63%).²¹

The antimicrobial activity of rosemary oil

In the Italian territory, Abruzzo is one of the central area regions characterized by a multitude of environments and microclimates and with the richest flora of Italy and the Mediterranean basin.²² In the Abruzzo territory, different plant species are cultivated and exploited for their therapeutic and alimentary properties;²³ among them, *Rosmarinus officinalis*, *Origanum vulgare*, *Salvia officinalis*, *Mentha piperita*, *Allium sativum*, *Foeniculum vulgare*, *Satureja montana*, *Thymus vulgaris*, and *Coriandrum sativum*.

The study of²⁴ assessed the effect of the combined application of essential oils (EOs) from *Origanum vulgare* L. - oregano and *Rosmarinus officinalis* L. - rosemary, alone or in combination at subinhibitory concentrations, against three pathogenic bacteria that are associated with fresh leafy vegetables: *Listeria monocytogenes* (*L. monocytogenes*), *Escherichia coli* (*E. coli*) and *Salmonella enterica* Serovar Enteritidis (*S. Enteritidis*). The minimum inhibitory concentration MIC of oregano was 0.6mL/mL against the test strains either in single and mixed inoculum. The MIC of rosemary was 5mL/mL against *L. monocytogenes* and *E. coli* and 10mL/mL against *S. Enteritidis* in single inocula, whereas it was 10mL/mL against the mixed inoculum. The fractional inhibitory concentration index of the combined EOs was 0.5 against the mixed bacterial inoculum,

which suggested a synergic interaction. The incorporation of oregano and rosemary alone (MIC) or combined at different subinhibitory concentrations in vegetable broth resulted in a decrease in the viable cell counts of all test strains over 24h. After a 10-min treatment, the oregano and rosemary, either alone or in combination at different sub inhibitory concentrations, caused similar decrease ($p > 0.05$) in the counts of all assessed groups (or family) of spoilage microorganisms. The decrease in counts of mesophilic bacteria, enterobacteria and fungi was always close to 3.0, 2.5 and 3.1 log cycles, respectively. Overall, the exposure of the EOs for 10 min caused a greater decrease ($p < 0.05$) in spoilage native flora counts in comparison to 5 min. The essential oil of rosemary were tested against eight strains of bacteria: *Staphylococcus aureus* (NCTC 6571), *Bacillus cereus* (ATCC 11778), *Bacillus subtilis* (NCTC 10400), *Bacillus pumilis* (wild type), *Pseudomonas aeruginosa* (NCTC 1662), *Salmonella poona* (NCTC 4840), *Escherichia coli* (ATCC 8739) and ampicillin resistant *Escherichia coli* (NCTC 10418). The results from the disc diffusion assay followed by modified resazurin assay indicated that the tested essential oil showed higher antibacterial activity against Gram-positive bacteria (IZ 18.0- 24.2; MIC 0.20-0.48mg mL⁻¹) than against Gram-negative bacteria (IZ 12.8-17.5; MIC 1.16-1.72 mg mL⁻¹).²⁵ *Rosmarinus officinalis* Linn. (rosemary) hydroalcoholic extract was assayed against *Streptococcus mitis*, *Streptococcus sanguinis*, *Streptococcus mutans*, *Streptococcus sobrinus* and *Lactobacillus casei* standard strains, and its antimicrobial activity was proven in all tests, except against *S. mitis*.²⁶ The aim of the study of,²⁷ was to evaluate antibacterial properties of commercial essential oils (rosemary, caraway and fennel) to reduce the number of *Staphylococcus aureus* and *Escherichia coli*. The result of experiments showed that essential oils contained in microbiological media significantly reduced the number of *S. aureus* and *E. coli* cells. The best antibacterial properties possessed caraway oil: 1mg/g for *S. aureus* and 10mg/g for *E. coli*, weaker rosemary (5mg/g) and fennel (20mg/g) oils. The combination of clove and rosemary essential oils produced an additive effect against the Gram-positive and Gram-negative bacteria, namely *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Escherichia coli*, *Proteus vulgaris* and *Pseudomonas aeruginosa*.²⁸ Essential oils *Origanum vulgare* and *Rosmarinus officinalis* combined at sub-inhibitory concentrations were effective in inhibiting the growth and survival of pathogenic and spoilage microorganisms associated to minimally processed vegetables, although the underlying mode of action remains to be explored in the future. Sensory evaluation suggested that application of the essential oils in mixture at sub-inhibitory concentrations as sanitizer in vegetables would be acceptable to consumers, mainly when regarded a more extended storage time.²⁹ The inhibitory effects of the hydrosols of thyme, black cumin, sage, rosemary, and bay leaf against *S. typhimurium* and *Escherichia coli* O157:H7 were shown in carrots and apples. Thyme hydrosol had the highest antibacterial effect on both *Salmonella typhimurium* and *Escherichia coli* O157:H7 counts.³⁰ Among Gram-negative, *Salmonella* spp. was inhibited mostly by EOs of thyme (500ppm), rosemary (5000ppm) and garlic (20000ppm), *E. coli* was mostly inhibited by thyme (250ppm), cinnamon (2500ppm) and cumin (2500ppm).³¹

Conclusion

Natural antimicrobial extracts from herbs, spices, and plants can be used in food industry to prevent growth of foodborne pathogens and food spoilage microorganisms and to enhance the shelf life and stability. Due to the antioxidant and antimicrobial effects of natural ingredients, they can be a good alternative to classical food

preservation methods and usage of chemical preservatives and food additives. These plant products have shown to reduce the growth of both Gram-positive and Gram-negative microorganisms including foodborne pathogens.

Three main factors can influence the results of a test of the antimicrobial activity of plant oil: the composition and solubility of the oil, the microorganism, and the method of growing and enumerating the surviving bacteria. *Rosmarinus officinalis* essential oil is also important for its medicinal uses and its powerful antibacterial, cytotoxic, anti-mutagenic, antioxidant, anti-phlogistic and chemopreventive properties.

The major components determined in *R. officinalis* essential oil were 1, 8-cineol, camphor, α -pinene, limonene, camphene and linalool. *Rosmarinus officinalis* essential oil showed higher antibacterial activity against Gram-positive bacteria than against Gram-negative bacteria.

Acknowledgements

None.

Conflict of Interest

The author declares that there is no conflict of interest.

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