EXPERIMENTAL PAPER

Antibacterial activity of rosemary, caraway and fennel essential oils

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Summary

Introduction: Recently, interest in essential oils used in natural medicine, has been increasing. Essential oils are still being tested for their potential uses as an alternative remedies for the treatment of many infectious diseases. **Objective:** The aim of the study was to evaluate antibacterial properties of commercial essential oils (rosemary, caraway and fennel) to reduce the number of *Staphylococcus aureus* and *Escherichia coli*. **Methods:** The antibacterial activity of essential oils was investigated by agar dilution method. **Results:** 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: 1 mg/g for *S. aureus* and 10 mg/g for *E. coli*, weaker rosemary (5 mg/g) and fennel (20 mg/g) oils. **Conclusion:** Results confirmed the inhibiting effect of commercial essential oils on *S. aureus* and *E. coli* and provide a scientific ground for future research.

Key words: *Escherichia coli*, *Staphylococcus aureus*, rosemary, caraway, fennel

INTRODUCTION

Down the ages essential oils and other extracts of plants have evoked interest as sources of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases [1]. Many plants contain extensive variety of phytochemical compounds with antimicrobial activity [2].

Essential oils such as rosemary (*Rosmarinus officinalis* L.), caraway (*Carum carvi* L.) and fennel (*Foeniculum vulgare* Mill.) have shown antibacterial and antifungal activities [3-5]. Rosemary oil also possesses analgesic, anti-inflammatory, anti-oxidative, anti-tumor, anti-ulcerogenic and hepatoprotective properties [6]. The volatile oils from *C. carvi* have also been used as an anti-ulcerogenic, anti-tumor, anti-proliferative and anti-hyperglycemic agent [7]. Fennel essential oil has shown anti-oxidant, cytotoxic, anti-inflammatory, hypotensive, hepatoprotective, anti-thrombotic and anti-mutagenic activity [8]. These oils are used in many industries, mostly in natural medicine. The abovementioned properties indicate that natural essential oils may be used in the prevention and treatment of some disorders, especially as topically applied substances.

Staphylococcus aureus and *Escherichia coli* are two opportunistic pathogens that are responsible for moderate to severe and life-threatening infections, especially in immunocompromised patients [9]. *S. aureus* is a major pathogen in both community-acquired and nosocomial infections. The primary site of infection is often a breach in the skin that may lead to minor skin and wound infections but *S. aureus* can also infect any tissue [10]. *E. coli* is present in human and animal intestine. Pathogenic strains are responsible for intestinal disorders, e.g. diarrhea and extraintestinal infections in both humans and animals, including urinary tract infections (UTI), septicemia and meningitis [11]. Nowadays, the spread of drug resistant microbial pathogens is one of the most serious threats to successful treatment of infectious diseases [12].

The main objective of this study was to examine the antibacterial activities of the essential oils of rosemary, caraway and fennel against representatives of Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria by agar dilution method.

MATERIAL AND METHODS

Bacterial strains, media and growth condition

Gram-positive *S. aureus* (DSMZ 346) and Gram-negative *E. coli* (DSMZ 1576) used in this study were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ). Before the assay strains were incubated at 37°C for 24 h: *S. aureus* on trypticase soy agar (TSA) (Merck KGaA, Germany) containing 10% sodium chloride (Sigma-Aldrich, Poland), *E. coli* on MacConkey agar (Merck KGaA, Germany).

Essential oils

In this study, three essential oils were used: rosemary oil (*R. officinalis* L.), caraway oil (*C. carvi* L.) and fennel oil (*F. vulgare* Mill.). These oils were purchased from Vera-Nord Company, Poland (commercial producers of plant essential oils and aromatic substances). Quality of the essential oils was ascertained to be more than 98% pure. The oils exhibited a strong and characteristic odor.

Agar dilution method

The agar dilution method was performed with the following modifications: MacConkey agar and TSA were used instead Mueller Hinton agar [13]. A series of two-fold dilutions of each oil, ranging from 2% (w/w) to 0.05% (w/w), were prepared in TSA (for *S. aureus*) and MacConkey agar (for *E. coli*) with 0.5% (w/w) Tween 20 (incorporated into agar after autoclaving to enhance oil solubility). The mixtures were stirred using mechanical stirrer (Lka-Werke GmbH, Germany) to evenly distribute essential oils throughout the whole medium. Cultures were adjusted to 1 McFarland standard with sterile saline solution. Then serial dilution (1:10) of bacterial suspensions were performed. Plates were dried at 37°C for 30 min prior to inoculation with 150 µl bacterial suspension of the appropriate concentration, using an automatic pipette (Eppendorf Research Plus, Poland). Bacterial suspensions of different concentrations were spread over the plates containing modified microbial media using a sterile cotton swab in order to get a uniform microbial growth on both control and test plates. TSA, with 0.5% (w/w) Tween 20 but no oil, was used as a positive growth control for *S. aureus* bacteria. MacConkey agar, with 0.5% (w/w) Tween 20 but also no oil, was used as a positive growth control for *E. coli* bacteria. Inoculated plates were incubated at 37°C for 48 h. Three repetitions were performed for each concentration of oil dilution and for each of the two suspensions. After incubation period, colonies were counted using colony counter (Lka2002 POL-EKO, Poland) and their average values (of three repeats) were tabulated.

Ethical approval: The conducted research is not related to either human or animal use.

RESULTS AND DISCUSSION

In recent decades, interest in essential oils, which are used for centuries in natural medicine, increased. Many essential oils are claimed to possess antimicrobial activity and they have been used for the prevention and treatment of many infectious diseases as alternative remedies. It is known that the efficacy of the essential oil depends on many environmental and genetic factors. It has been proven that the main factors responsible for the diverse chemical composition of essential oils are climatic conditions, geographic origin, time of collection, distillation conditions, correct farming practices and part of the plant from which oil is

extracted [14-16]. In our study, commercial essential oils derived from rosemary, caraway and fennel were used. These oils did not have a precise characteristic all the factors influencing on bactericidal activity but they are used in aromatherapy, aromatization of premises, rubbing, sauna, bath or shower.

Based on experiments, it was found that essential oils: caraway oil, rosemary oil and fennel oil contained in TSA significantly limited the growth of *S. aureus* (tab. 1, fig. 1). It was shown that the least studied concentration of caraway oil (0.5 mg/g) reduced the growth of *S. aureus* cells and the concentration of 1 mg/g completely inhibited the growth of these bacteria. The decrease of microbial cells was approximately 96%, as compared to the control samples. Slightly worse results were obtained for the fennel oil and the rosemary oil, which turned out to be better fennel oil. It was found that for these two oils concentration of 1 mg/g did not completely inhibit the growth of microorganisms, and it was limited by 99.6% (fennel oil) and 75% (rosemary oil). Both essential oils in the concentration of 5 mg/g completely inhibited the growth of *S. aureus*.

Table 1.

Influence of essential oil concentrations in growth medium on the number of *S. aureus* cells

Type of essential oil	The number of bacterial cells [10^7 cfu/ml]					
	Control	0.5 mg/g	1 mg/g	5 mg/g	10 mg/g	20 mg/g
Caraway oil	32.22±4.91	1.28±0.16	b	b	b	b
Rosemary oil	10.96±1.44	a	2.6±0.57	b	b	b
Fennel oil	32.22±4.91	a	0.13±0.03	b	b	b

Legend: a – uncountable colonies; b – no growth. Values are expressed as means ± standard deviation.

Figure 1.

Values of essential oil concentrations influencing on the number of *S. aureus*

Additionally, it was found that bacterial colonies isolated on TSA with essential oils lost their natural golden pigment opposite to the control growth on TSA without essential oils. Staphyloxanthin is responsible for this characteristic carotenoid pigment. It has an antioxidant action that help the bacteria to survive oxidative stress during neutrophil killing [17]. It cannot be excluded that essential oils weaken production of the staphyloxanthin and this way can lead to decrease virulence *S. aureus*. However, this observation needs further studies.

Performed experiments have shown that caraway, rosemary and fennel essential oils in MacConkey agar also significantly limited the growth of *E. coli* (tab. 2, fig. 2). The minimum concentration of caraway oil reducing the growth of *E. coli* cells was 5 mg/g. The decrease in the number of cells for these rods was about 73% compared to the control. Only concentration 10 mg/g completely inhibited the growth of these microorganisms. Similarly, to Gram-positive bacteria, worse effect on Gram-negative bacteria was obtained for the fennel and rosemary essential oils. The concentration 10 mg/g inhibited the growth of microorganisms in 95.9% for fennel oil and in 73% for rosemary oil. Only the concentration of 20 mg/g for both essential oils completely inhibited the growth of *E. coli*.

After comparing the activity of the three essential oils to both bacterial species, it was observed that *S. aureus* was more sensitive to studied oils than *E. coli*. All essential oils inhibit in lower concentrations Gram-positive cocci than Gram-negative rods. The cell wall of Gram-negative bacteria is more resistant to the toxic effects of essential oils than Gram-positive bacteria [18]. The structure of the Gram-positive bacteria cell wall allows hydrophobic molecules to easily penetrate the cells [19]. Additionally, we noted that caraway oil was the most effective and gave high percentage of microbial reduction for both *S. aureus* and *E. coli*.

In last few years, studies on the effect of these essential oils on the growth of bacteria were conducted by many authors. The majority of research studies present the beneficial impact of essential oils, extracted in laboratory setting, from various parts of plants. In such conditions, antimicrobial activity of essentials oils is controlled from the outset. Studies have demonstrated that fennel and caraway essential oils effectively reduced the growth of *S. aureus* in concentration of 1%, and *E. coli* at 2% concentration [20]. In other studies, a minimum concentration of fennel essential oil inhibiting the growth of *S. aureus* and *E. coli* was <0.025% [21]. The experiment showed that the minimum inhibitory concentration of *S. aureus* and *E. coli* depends on the location of extraction of essential oil from the plant *F. vulgare*. These researchers noted that the better antibacterial properties were obtained from seed. Seed oil completely inhibited growth of tested microorganisms at a concentration of 0.039 mg/ml (*E. coli*) and <0.039 mg/ml (*S. aureus*) [22]. Rosemary oil added to Mueller-Hinton agar had antimicrobial properties against *E. coli* and *S. aureus*. The authors noticed the overall reduction of these bacteria after application of 0.5% concentration of essential oil from *R. officinalis* [20].

Table 2.

Influence of essential oil concentrations in growth medium on the number of *E. coli* cells

Type of essential oil	The number of bacterial cells [10^5 cfu/ml]					
	Control	0.5 mg/g	1 mg/g	5 mg/g	10 mg/g	20 mg/g
Caraway oil	31.33 ± 6.95	a	a	8.44 ± 2.78	b	b
Rosemary oil	31.33 ± 6.95	a	a	a	8.44 ± 4.28	b
Fennel oil	38.44 ± 9.89	a	a	a	1.58 ± 0.19	b

Legend: a – uncountable colonies; b – no growth. Values are expressed as means $\pm$ standard deviation.

Figure 2.
Values of essential oil concentrations influencing on the number of *E. coli*

The relatively smaller number of scientific papers refers to the essential oils purchased in companies. In these case the oils are usually obtained by steam distillation [23]. Studies have demonstrated that commercial caraway oil had antibacterial properties against *S. aureus* and *E. coli*. The minimum inhibitory concentrations of bacteria were 0.1 μ l/ml and 2 μ l/ml, respectively [24]. In our research effective concentrations of caraway oil were 1 mg/g for *S. aureus* and 10 mg/g for *E. coli*. Other studies showed antibacterial effects of commercial rosemary oil which inhibited the growth of *S. aureus* at a concentration of 0.195% and *E. coli* at 0.39% [25]. In our study inhibition of the growth by rosemary oil was obtained at concentration of 5 mg/g (0.5%) for *S. aureus* and 20 mg/g (2%) for *E. coli*. Research associated with commercial fennel essential oil did not show antibacterial properties against above-mentioned microorganisms [26]. In contrast, in our study completely inhibited growth concentrations of fennel essential oil were 5 mg/g for *S. aureus* and 20 mg/g for *E. coli*.

CONCLUSION

Our results confirmed that commercial essential oils cause significant growth inhibiting effects on Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria and have similar antimicrobial properties as essential oils obtained from plants in the laboratory condition. In this study we used a standard strain of *S. aureus* DSMZ 346 (known as strain P-209) and standard strain of *E. coli* DSMZ 1576 (known as Crooks). The efficiency of rosemary, caraway and fennel essential oils against the representatives of most important human pathogens provides a scientific ground for future research with using routinely isolated from infected patients strains presenting different mechanisms of resistance (e.g. methicillin-resistant, ESBL-positive).

Conflict of interest: Authors declare no conflict of interest.

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WŁAŚCIWOŚCI ANTYBAKTERYJNE OLEJKÓW ETERYCZNYCH Z ROZMARYNU LEKARSKIEGO, KMINKU ZWYCZAJNEGO ORAZ KOPRU WŁOSKIEGO

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Streszczenie

Wstęp: W ostatnich latach notuje się wzrost zainteresowania olejkami eterycznymi, które są stosowane w medycynie naturalnej. Ich potencjalne zastosowanie jako alternatywnych środków w leczeniu chorób infekcyjnych wciąż jest badane. **Cel:** Celem niniejszej pracy była ocena właściwości antybakteryjnych komercyjnych olejków eterycznych (rozmarynu lekarskiego, kminku zwyczajnego i kopru włoskiego) w stosunku do *Staphylococcus aureus* i *Escherichia coli*. **Metody:** Aktywność antybakteryjną olejków badano z wykorzystaniem metody rozcieńczeń w agarze. **Wyniki:** Wykazano, iż olejki eteryczne zawarte w podłożach mikrobiologicznych działają hamująco na wzrost *S. aureus* i *E. coli*. Najlepsze właściwości przeciwbakteryjne posiadał olejek eteryczny z kminku zwyczajnego w rozcieńczeniu 1 mg/g dla *S. aureus* i 10 mg/g dla *E. coli*. Nieco słabszy efekt uzyskano dla olejku rozmarynowego i olejku z kopru włoskiego: 5 mg/g (*S. aureus*) i 20 mg/g (*E. coli*). **Wnioski:** Wyniki potwierdziły hamujący wpływ komercyjnych olejków eterycznych na wzrost *S. aureus* i *E. coli* i stanowią podstawę do przeprowadzenia dalszych badań.

Słowa kluczowe: *Escherichia coli*, *Staphylococcus aureus*, rozmaryn lekarski, kminek zwyczajny, koper włoski