

Leptospermum petersonii Bailey Essential Oil as Antagonist against Plant Pathogen Bacteria

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Abstract

Natural ingredients have been used in folk medicine since ancient times. These naturally occurring substances, which can come from plants, are often considered a priori harmless. Certain species of plants called *Leptospermum* are said to have considerable benefits in traditional medicine. The essential oils of *L. scoparium* are often used as antibacterial agents. The determination of the antibacterial activity of *L. scoparium* essential oil against representatives of plant bacteria was the aim of our experiment. The disc diffusion method was used for testing under *in vitro* conditions and the vapor phase method was used against five different bacteria under *in situ* conditions. Carrot was used as a model food for *in situ*. The best antibacterial activity of *L. scoparium* essential oil against *B. subtilis* was found using the disc diffusion method. This method was also used to test the antibiotic resistance of the model bacterial species, which was higher than the antibacterial activity of *L. scoparium* essential oil. *L. scoparium* essential oil showed the best antibacterial effect against *Xanthomonas arboricola* at a concentration of 62.5 µg/L in the carrot model. Therefore, it can be concluded that the essential oil has adequate antibacterial capacity and can be used as a suitable natural preservative to prolong the shelf life of carrots.

Keywords: disc diffusion method, vapor phase, antimicrobial activity, lemon tea tree essential oil

1. Introduction

Because of their strong pharmacological activity, low toxicity, and various health benefits, phytochemicals have attracted a lot of attention in recent years for their potential medical uses [1]. Due to their numerous special biological and pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, and anticancer qualities, essential oils (EOs), a class of phytochemicals derived mainly from aromatic and medicinal plants, are garnering more and more attention [2].

Leptospermum petersonii F.M. Bailey is a member of the family Myrtaceae. This family comprises at least 3000 species of plants, widely

spread over numerous tropical and warm-temperate regions, including Australia, Central America, and South America [3]. Many EOs derived from Myrtaceae species are utilized as antimicrobial agents in cosmetic goods and have been shown to possess insecticidal, nematicidal, anti-inflammatory, and antifungal properties [4–6].

Leptospermum petersonii is a rare small tree that grows naturally in lowland or floodplain areas of Northern New South Wales. It is also known as Australian Rose or lemon-scented tea-tree. Previous research, according to Brophy et al. [7], suggested that this species might be divided into three chemical variants depending on the makeup of its EO.

Researcher interest in EOs has increased recently, and efforts to create active-packaging systems that incorporate EOs into polymeric materials and release the volatile active compounds onto food surfaces gradually have been made [8–11]. Hyun

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et al. [12] discovered that the vapor from thyme, lemongrass, and oregano EOs strongly inhibited the growth of *E. coli* and *L. monocytogenes*. They also observed that the use of EO vapor in conjunction with modified atmosphere packaging greatly increased the shelf life of fresh cabbage.

Consumers prefer foods without artificial additives, which are safer to consume, opening the door for the use of natural preservatives like EOs. Extensive study is being conducted on the potential of spices and EOs as natural agents for food preservation. It has been shown that a wide variety of naturally occurring chemicals derived from plants and microbes can be helpful for both increasing the shelf life of food and reducing or eliminating diseases. There may be other uses for essential oil components in food processing to suppress food microorganisms [13].

The objective of our study was to evaluate the antibacterial qualities of *Leptospermum petersonii* Bailey against a variety of plant infections using both *in vitro* investigation and *in situ* investigation using a carrot model.

2. Materials and methods

2.1 Essential oil

Steam distillation was used to create the essential oil (EO) from *Leptospermum petersonii* Bailey (LPEO) leaves that were acquired from Hanus s.r.o. in Nitra, Slovakia. Australia provided the *L. petersonii* leaves, and the EO was carefully preserved for later use at 4 °C in the dark. Citral (neral 35%, geranial 35%) up to 70%, citronellal 21%, and citronellol 3% were the primary components.

2.2. Microorganisms tested

The antibacterial activity of the studied LPEO was evaluated using several bacterial strains. These strains included *Bacillus subtilis* CCM 2217, *Priestia (Bacillus) megaterium* CCM 2007 from the Gram-positive bacteria (G⁺) group. Gram-negative (G⁻) bacteria included *Xanthomonas arboricola* CCM 1441, *Pectobacterium carotovorum* CCM 1008, and *Pseudomonas putida* CCM 7156. The Czech Collection of Microorganisms, based in Brno, Czech Republic, provided all of the bacterial strains. Prior to

analysis, the bacterial inoculum was cultured in Mueller Hinton Broth (MHB, Oxoid, Basingstoke, UK) for a 24 h at 37 °C. On the day of the experiment, the optical density of the bacterial inoculum was calibrated to 0.5 McFarland.

2.3 Disc diffusion method

In the disk diffusion susceptibility experiment, the microbial strains mentioned above were employed. Bacterial strains were inoculated into Mueller Hinton broth (MHB, Merck, Germany) for 24 h. A disk diffusion assay was performed using Mueller Hinton agar on which 100 µL of bacteria of known density (MHA; Merck, Germany) was pipetted. Six-millimeter sterile discs were moistened with 10 µL of the investigated LPEO and then placed on the agar medium. For twenty-four hours, the bacterial cultures were incubated at 37 °C. The inhibitory activity was measured and the results were represented in millimeters following the 24-hour incubation period. Gentamicin and chloramphenicol (30 µg/disc, Oxoid, Basingstoke, UK) were the two antibiotic treatments (ATBs), which additionally functioned as positive controls for the microorganisms. Three runs of each measurement experiment were conducted.

2.4 Antimicrobial activity in vapor phase

The effectiveness of LPEO as an antibacterial agent was evaluated *in situ* against a range of bacterial species, including both G⁺ and G⁻ bacteria. Carrots were one type of model vegetable that was used as a substrate to encourage the growth of bacteria. The evaluation's experimental strategy adheres to the methods described by Kačániová et al. [14]. The carrot was dried, then cut into 0.5 mm pieces and cleaned with distilled water. Bacteria were then added to 60 mm Petri plates that had prepared substrates supported by agar. The tested LPEO sample was put on sterile filter paper after being dissolved in ethyl acetate at concentrations of 500, 250, 125, and 62.5 µg/L. Filter sheets that were solely exposed to ethyl acetate were used as a control.

The ready-made Petri dishes needed to be incubated at 37 °C for seven days. Using conventional operating protocols, the *in situ* bacterial growth evaluation was carried out. To determine the volume density of bacterial colonies

(vv), the National Institutes of Health, Bethesda, Maryland, USA, provided the ImageJ program. The following formula was used to determine the volume density of bacterial colonies: $vv (\%) = P/p$ where P denotes the stereological grid points that intersect the colonies and p denotes the locations inside the reference space of the growth substrate. Here is a representation of the percentage (%) of BGI (bacterial growth inhibition) caused by the EO vapor phase treatment: $BGI = (C-T)/C \times 100$ where the letters T and C represent the treatment and control groups, respectively. The two groups represent the bacterial growth represented as v/v. findings obtained; negative values imply the encouragement of growth.

2.5 Statistical analyses

To assess statistically significant variances, one-way analysis of variance (ANOVA) was employed, and at a significance level of $p < 0.05$, Tukey's significant difference (HSD) test was performed. For this experiment, Astatsa Anova One Way, an online tool, was utilized.

3. Results and discussion

Even with the current magnitude of the tea tree oil market, lesser-known tea tree species like *L. petersonii* still have a ton of untapped potential for study and commercial development [15]. The range of LPEO's antibacterial activity was 3.33 to 7.67 mm (Table 1). The G⁺ bacteria's *B.*

subtilis and the G⁻ bacteria *P. carotovorum* were discovered to be the targets of LPEO's greatest antibacterial action.

Numerous disc diffusion studies have been conducted on *L. petersonii* as part of previous antimicrobial investigations; Demuner et al. [16] has reported on the most current of these studies. These prior investigations are somewhat redundant due to a number of disc diffusion study limitations and the suggestion to employ a quantitative (MIC) technique of antibacterial analysis [17]. Certain species have been the subject of several studies, such as the study of phytopathogenic fungus [18–20]. *L. petersonii* oil was reported to be more than 90% efficient against all strains tested in a recent study focusing on dermatophytes (*Microsporum canis*, *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Epidermophyton floccosum*, and *Microsporum gypseum*) [20].

In the past, Van Vuuren [21] advised that activities with MIC values ≤ 2.00 mg/mL be taken into consideration when analyzing the antibacterial activity of essential oils. Thus, for 11 of the 16 pathogens examined, *L. petersonii* showed noteworthy action, especially against *B. agri*. Some of the most notable sensitivities were also displayed by the two *Brevibacteria* species, *B. brevis* and *B. laterosporum* (1.00 and 0.25 mg/mL).

The most sensitive bacteria against antibiotic Gentamycin was *P. carotovorum* and against chloramphenicol was *X. arboricola*.

Table 1. Disc diffusion method antimicrobial activity of *Leptospermum petersonii* Bailey in mm

Microorganism	Inhibition zone	Gentamycin	Chloramphenicol
Gram positive bacteria			
<i>Bacillus subtilis</i>	7.67±0.58 ^a	30.33±0.58 ^a	29.67±0.58 ^a
<i>Priestia megaterium</i>	3.67±0.58 ^b	26.67±0.58 ^b	27.67±0.58 ^b
Gram negative bacteria			
<i>Xanthomonas arboricola</i>	5.67±0.58 ^c	30.33±0.58 ^a	30.67±0.58 ^a
<i>Pectobacterium carotovorum</i>	6.33±0.58 ^c	32.33±0.58 ^c	30.33±0.58 ^a
<i>Pseudomonas putida</i>	3.33±0.58 ^b	29.67±0.58 ^a	29.67±0.58 ^a

Data are the mean (\pm SD) of 3 samples. Different letters in each column refer to significant differences (Tukey, $p \leq 0.05$).

Table 2. *In situ* analysis of the antimicrobial activity (in %) of *Leptospermum petersonii* Bailey in the vapor phase on carrot

Food model	Microorganisms	Concentration of EO in µg/L			
		62.5	125	250	500
Carrot					
Gram-positive					
	<i>Bacillus subtilis</i>	66.97±3.35 ^a	14.63±1.06 ^a	55.14±1.52 ^a	13.96±2.52 ^a
	<i>Priestia megaterium</i>	33.43±1.48 ^c	24.47±3.16 ^c	13.34±1.25 ^c	4.70±1.00 ^c
Gram-negative					
	<i>Pectobacterium carotovorum</i>	43.63±2.00 ^b	35.21±2.54 ^b	65.74±2.22 ^b	25.73±2.16 ^b
	<i>Pseudomonas putida</i>	85.99±2.21 ^d	35.14±2.60 ^b	44.35±2.18 ^d	65.11±2.55 ^d
	<i>Xanthomonas arboricola</i>	96.66±2.01 ^e	25.14±3.62 ^c	85.66±2.93 ^e	75.80±2.86 ^e

Data are the mean (± SD) of 3 samples. Different letters in each column refer to significant differences (Tukey, $p \leq 0.05$).

The antibacterial ability of a range of natural compounds, such as EOs, against infections and spoilage fungi in different vegetable products has been investigated. Recently, edible films have incorporated EOs to enhance their physicochemical, antioxidant, and antibacterial qualities [22,23]. Studies using EOs (lemon, sweet orange, and lime peel essential) have shown variable antibacterial action at various doses as well as an overall improvement in shelf life and quality maintenance [24]. Various bioactive compounds, primarily EOs or aromatic plant extracts were combined to improve the performance of edible films. These compounds not only improve antimicrobial properties but also reduce biochemical deteriorations caused by processing, such as texture collapse, enzymatic browning, and the development of off-flavors [25].

An additional goal of this investigation was to examine the tested LPEO's antibacterial effects in the vapor phase, given its intriguing antibacterial capabilities. Regarding the G⁻ and G⁺ bacteria that thrive on carrots, the effectiveness of LPEO was assessed (Table 2).

After analyzing the inhibitory effects on G⁺ bacterial strains in the carrot model, it was shown that *P. megaterium* displayed the highest levels of suppression at concentrations of 62.5 µg/L (33.43 %), and that LPEO was most effective against *B. subtilis* at concentrations of 62.5 µg/L (66.97 %). Notably, at a lower dosage (62.5 µg/L), the vapor phase of LPEO showed the maximum efficiency against G⁻ bacteria, with reported inhibitory effects of 96.66% against *X. arboricola* in the carrot model.

Nonetheless, fresh veggies have a limited postharvest life and are naturally perishable. These horticultural products are susceptible to contamination during handling, temperature and humidity fluctuations during handling, and mechanical damage, all of which can result in postharvest deterioration. It is predicted that during postharvest processing, pathogens decompose 20–25% of the harvested vegetables [26–28]. Preventative measures are being investigated as a means of preventing this.

According to Skrinjar and Nemet [29], a number of EOs have antibacterial capabilities against common bacteria that cause spoilage, such as *Escherichia coli*, *Listeria monocytogenes*, and *Pseudomonas fluorescens*. These oils include rosemary, turmeric, clove, pepper, fennel, round cardamom, and angelica root. EOs have been utilized for controlling illnesses and pests, reducing losses after harvest, extending the shelf life of food, and enhancing food flavor and smell. According to Serrano et al. [30], EOs derived from mints, lemongrass, thyme, rosemary, lavender, bergamot, dill, and coriander, among other plants, are effective at prolonging the shelf life and preserving the quality of fruits and vegetables.

4. Conclusions

Although adding natural compounds directly to food is the most common application strategy, numerous attempts have been made to develop other strategies that minimize undesired inactivation. Currently, food products are treated with viable active solutions through dipping,

spraying, and coating before packing. It is advisable to conduct further studies using various food models and storage conditions to optimize the application of *Leptospermum petersonii* EO as a natural substitute for synthetic preservatives. A novel method of extending product shelf life and improving microbiological safety by managing microbial contamination of food surfaces has captured the interest of the packaging industry. Antimicrobial substances are combined with food packaging materials in this treatment. Natural antibacterial compounds are becoming increasingly common, particularly in the food and healthcare sectors, in line with the current emphasis on the utilization of natural and renewable resources.

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