

Antibacterial Activity of *Picea mariana* Essential Oil against Selected Animal Pathogens and Insecticidal Activity against *Megabruchidius dorsalis*

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Abstract

Black spruce has been used healing balms, due to the powerful antimicrobial and antiviral properties of the oil. These benefits have made black spruce an extremely popular ingredient for detergents, meaning that using the essential oil in your own personal cleaning routine can be an effective way to eliminate microorganisms. The objective of our work was to evaluate the antimicrobial and insecticidal activity of *Picea mariana* essential oil. The antimicrobial activity was evaluated against the Gram-negative bacteria *Escherichia coli*, *Salmonella enterica*, and *Yersinia enterocolitica*, and the Gram-positive bacteria *Enterococcus faecalis*, *Listeria monocytogenes*, and *Staphylococcus aureus* by the disc diffusion method. In addition, antimicrobial activity was determined under *in situ* conditions on pear and potato models. Insecticidal activity was determined against *Megabruchidius dorsalis* using different concentrations of the *P. mariana* essential oil. The best antimicrobial activity of *P. mariana* essential oil was found against *Escherichia coli* under *in vitro* and *in situ* conditions. Insecticidal activity was detected at the highest concentrations of 50 and 100 %. The results of our experimental work indicated that *P. mariana* essential oil has good antimicrobial and insecticidal properties and is a suitable agent against Gram-positive and Gram-negative bacteria, as well as against insect *M. dorsalis*.

Keywords: *Picea mariana* essential oil, *Megabruchidius dorsalis*, *in vitro*, vapour phase, antimicrobial and insecticidal effect.

1. Introduction

Picea mariana (Miller) B.S.P., commonly referred to as black spruce, represents a pivotal industrial species within the context of the Canadian boreal forest ecosystem. The processing of timber during the logging and subsequent wood transformation stages has been demonstrated to generate significant levels of biomass residue, comprising 13 % foliage, 6 % branches and 8 % bark per tree, on an oven dry basis [1]. The foliage is currently

utilised in the production of the essential oil of black spruce, a substance which is widely employed in the field of aromatherapy. Shaw provided the first comprehensive description of the composition of this substance, identifying it as a mixture of various compounds, including bornyl acetate, which accounted for 37 % of the total, followed by α -pinene (16 %), camphene (10 %), β -pinene (6.5 %), and limonene (6.5 %) [2]. The hydrosol from black spruce needles obtained during hydrodistillation (also known as distilled water) has been the subject of research by Garneau et al. [3]. Its composition is characterised by a significant presence of oxygenated monoterpenes, predominantly comprising α -terpineol (14.8 %), borneol (13.5 %), bornyl

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acetate (7.8 %), and terpinen-4-ol (6.5 %). In relation to bark, investigations have been made into the production of a hot water extract rich in polyphenols [4,5] (Diouf et al., 2009; Garcia-Perez et al., 2012), yet no study has yet dealt with its hydrodistillation to produce an essential oil. In addition, there is currently a paucity of reports available regarding the chemical composition of essential oils derived from coniferous stem bark, and no reports were identified pertaining to the *Picea* genus [2].

Our study's goal was to assess *Picea mariana* essential oil's antimicrobial and insecticidal properties.

2. Materials and methods

2.1. Essential oil

Picea mariana essential oil was purchased from Inevita SK (Bratislava, Slovakia). The essential oil was stored at 4 °C before use. The country of origin was India.

2.2. Bacterial strains

The antibacterial activity of *Picea mariana* essential oil (PMEO) was evaluated against a range of bacteria *Escherichia coli* CCM 3988, *Salmonella enterica* subsp. *enterica* CCM 3807, *Yersinia enterocolitica* CCM 5671, *Enterococcus faecalis* CCM 4224, *Listeria monocytogenes* CCM 4699 and *Staphylococcus aureus* subsp. *aureus* CCM 4223. All bacterial strains were obtained from the Czech Collection of Microorganisms in Brno, Czech Republic. Bacterial inocula were cultured in Mueller Hinton broth (MHB, Oxoid, Basingstoke, UK) for 24 hours at 37 °C before analysis. The optical density of the inocula was adjusted to 0.5 McFarland standard on the day of the experiment.

2.3. Disc diffusion method

In an effort to assess the antimicrobial activity, we opted for the disc diffusion method, a technique that we have previously outlined. We proceeded with the preparation of small discs (6 mm in diameter) that were saturated with PMEO and placed them on Mueller Hinton agar (MHA) for the bacterial strains. The strain were then incubated at 37 °C for a duration of 24 hours. To

conclude the process, we measured the inhibition zones in mm. The blank discs were used as negative controls, while the antibiotic discs (gentamicin and ciprofloxacin from Oxoid, Basingstoke, UK) served as positive controls [6].

2.3. In situ antimicrobial activity

The present study set out to assess the *in situ* antifungal activity of PMEO. A range of substrates were tested for this purpose, and these included commercial pear and potato, as well as specific bacteria. The substrates were sliced into pieces measuring 0.5 mm, thoroughly cleaned, and placed in 60 mm Petri dishes. These dishes had previously been inoculated with bacterial samples. PMEO was dispersed in ethyl acetate at concentrations of 500, 250, 125, and 62.5 µg/L. Ethyl acetate filter sheets served as controls. The plates were hermetically sealed and then placed within an incubator set at 37 °C for a period of seven days. Assessment of microbial colony growth was facilitated using the ImageJ software to calculate bacterial volume densities. This was undertaken alongside standard methods for measuring *in situ* colony development [6].

2.4. Insecticidal activity

The model organism, *Megabruchidius dorsalis* Fahreus, 1839, was used to evaluate the insecticidal activity of PMEO. Each Petri plate was lined with sterile filter paper, and groups of fifty *M. dorsalis* insects were put within. The PMEO was diluted with a 0.1 % polysorbate solution to create a range of concentrations, including 100 %, 50 %, 25 %, 12.5 %, 6.25 %, and 3.12 %. One hundred µL of each PMEO concentration was added to sterile filter paper discs, and the plates were then covered with parafilm and allowed to sit at room temperature for a full day. A volume of 100 µL of the 0.1 % polysorbate solution was given to the control group. Insects were counted both alive and dead after a whole day. In three different investigations, this experimental process has been successfully repeated.

3. Results and discussion

The best antibacterial activity of PMEO was found against *Escherichia coli* (12.33 mm). The lowest

antibacterial was found against *Staphylococcus aureus* (7.67 mm) (Figure 1). *In situ* analyses show the best antibacterial activity in lower concentration 62.5 µg/mL on both models against *Escherichia coli*. Inhibition of *Escherichia coli* on pear model was 76.45 % and on potato model was 78.76 % (Figure 1,2).

The essential oils (EOs) were steam-extracted from the needles and twigs of balsam fir, black

spruce, white spruce, tamarack, jack pine and eastern white cedar that remained after harvesting in eastern Canada. The antimicrobial activities demonstrated in this study were found to be comparable to those exhibited by exotic essential oils (EOs). These activities were observed to be effective against common pathogenic bacteria and fungi [7].

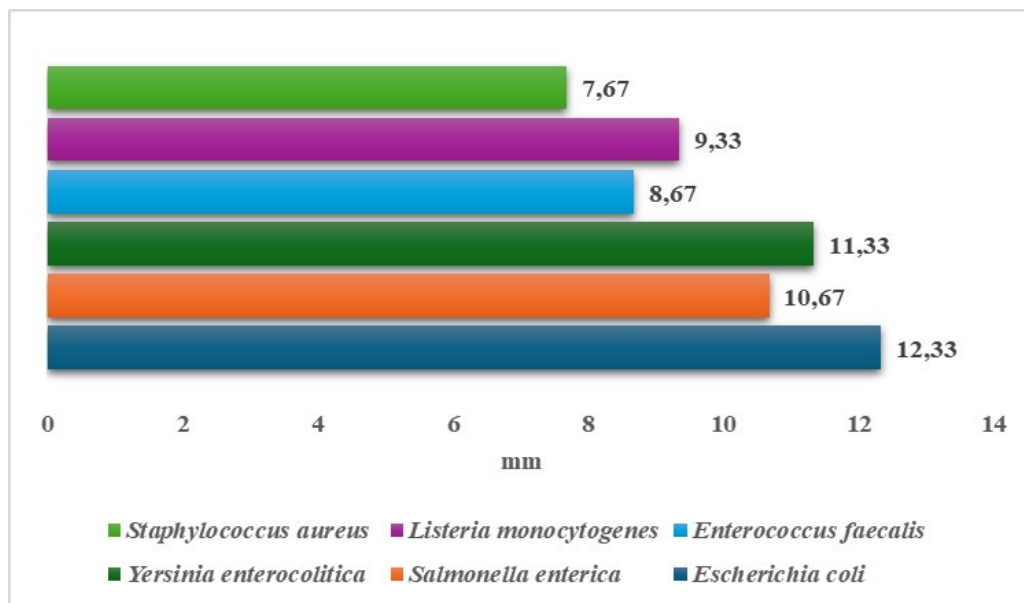


Figure 1. Antifungal activity of PMEO with disc diffusion method in mm

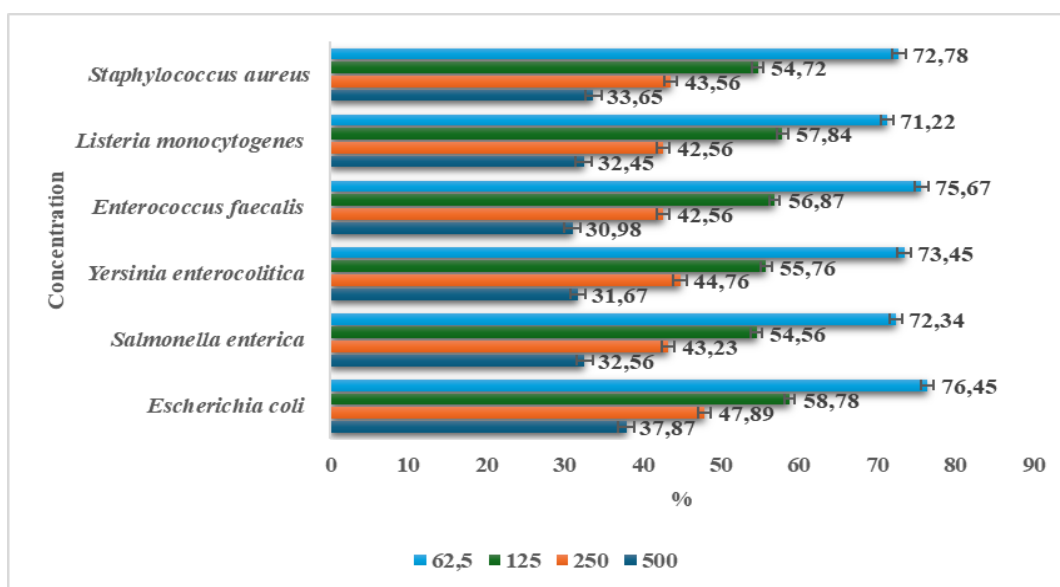


Figure 2. Antimicrobial activity of PMEO *in situ* on pear model in %

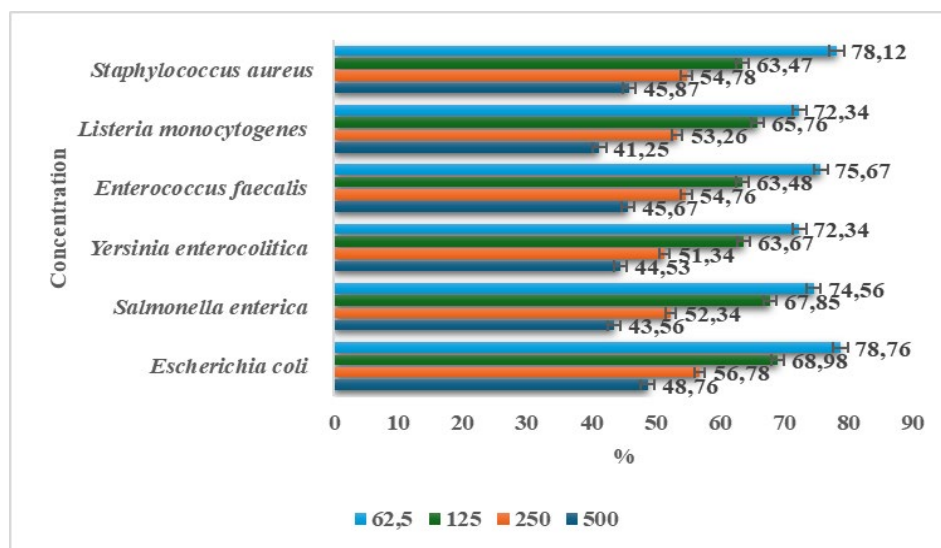


Figure 3. Antimicrobial activity of PMEO *in situ* on potato model in %

In light of the pervasive nature of common public health concerns, including respiratory infections, meningitis, gastroenteritis, and dermatoses, which are partially attributable to the ubiquity of ambient microorganisms [8-10], it is imperative to deliberate the incorporation of boreal volatile oils into personal hygiene and care products. These products, which include hand sanitizers, deodorants, face cleansers, shaving foams, skin scrub creams, and shampoos, generally contain alcohol in its native or denatured form as their primary active ingredient. This is often found to be undesirable due to its olfactory properties, flammability, skin drying properties, and potential for irritation [11-13]. The utilisation of boreal oils in lieu of alcohol, a practice that is appealing from olfactory and emollient perspectives, could also prove to be advantageous. It has the potential to benefit public health, enhance the production and manufacturing of EOs, and offer a novel approach for valorising conifer foliage waste from logging activities. Such applications would also depend greatly on the appropriate evaluation of their antimicrobial and antioxidant properties. Adjustments in the methods required for the evaluation of the volatility and both the lipophilic and hydrophilic behaviours of EOs have been reported [14-16].

In recent years, there has been a growing interest in researching and developing new antimicrobial agents from EOs due to drug resistance in foodborne bacterial enteric pathogens. As is evidenced by the numerous publications that have presented data on the antimicrobial properties of

EOs [17, 18], it is clear that there is a significant body of research on this topic. A plethora of laboratory methods are at disposal for the purpose of evaluating the *in vitro* antimicrobial activity of an essential oil (EO). The most widely known and elementary methods are disk diffusion and broth or agar dilution [19]. In recent years, there has been a marked increase in the prevalence of antimicrobial resistance (AMR) to antimicrobial drugs against *Salmonella Enterica* and *Campylobacter* spp. *Campylobacter* spp. is one of the most prevalent causative agents of gastroenteritis on a global scale, whereas salmonellosis is a significant foodborne disease on a global scale. The transmission of bacteria to humans can be facilitated through the consumption of contaminated poultry, eggs, beef products, milk, juices, fruits and vegetables. Numerous studies have indicated the potential of EOs as a novel therapeutic modality for the treatment of antibiotic-resistant *Salmonella*. In this regard, *Ruilopezia bracteosa* Essential Oil (EO) has been described as effective against *S. aureus* and *E. faecalis* in comparison with several antibiotics [20]. In a similar vein, Ashraf et al. [21] investigated the efficacy of *Nigella sativa* (black seed) oil in combating antibiotic-resistant isolates, employing a well diffusion and microbroth dilution method. Their findings indicated that *N. sativa* exhibited *in vitro* activity against *Salmonella Enterica* [20]. In a study by Chiboub et al. [22], the biological activity of the

Essential Oils (EOs) of two varieties of *Foeniculum vulgare* was evaluated with regard to their impact on the growth of *Salmonella Enterica*, and a significant antimicrobial effect was demonstrated.

The present study examined the insecticidal efficacy of PMEO against *M. dorsalis*, as illustrated in Table 1. The results of the study demonstrated that the highest levels of insecticidal activity were observed in trials where 25 %, 50 % and 100 % of the PMEO concentrations were

applied. Nevertheless, concentrations of 3.125 % of the PMEO did not demonstrate repellent effect against *M. dorsalis*. Antibacterial, insecticidal, fungicidal, nematocidal, herbicidal, antioxidant, and anti-inflammatory chemicals are among the physiologically active substances found in essential oils [23-26]. Three hundred of them are marketed and widely utilized in the food, flavor, and cosmetics industries [27]. In the food industry, they are also utilized as spices or to make drinks [28].

Table 1. Insecticidal activity of PMEO against *Megabruchidius dorsalis* (n=50)

Concentration (%)	Number of Living Individuals	Number of Dead Individuals	Insecticidal Activity (%)
100	10	90	90.00 ± 0.00
50	20	80	80.00 ± 0.00
25	30	70	70.00 ± 0.00
12.5	80	20	20.00 ± 0.00
6.25	90	10	10.00 ± 0.00
3.125	100	0	0.00 ± 0.00
Control group	100	0	0.00 ± 0.00

4. Conclusions

Due to their significant antibacterial qualities, EOs can be used as ingredients in functional meals, as well as food preservation and antibiotic substitutes. Although the aforementioned results are not always extrapolated, certain essential oils (EOs) have shown their effectiveness against a number of foodborne pathogens in vitro and in model food systems. They can also be used in foods to increase their microbiological safety.

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