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ANTIMICROBIAL ACTIVITIES OF THREE COMMERCIAL ESSENTIAL OILS DERIVED FROM PLANTS BELONGING TO FAMILY PINACEAE

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The aim of this study was to compare the antibacterial effects of several essential oils derived from plants belonging to family Pinaceae and subfamily Abietoideae (cedar oil, fir oils derived from Silver fir *Abies alba* Mill. and Siberian fir *Abies sibirica* L., respectively) against some Gram-positive and Gram-negative bacteria. The Kirby-Bauer disk diffusion test for measuring zone diameters of bacterial growth inhibition was used. The fir oil derived from Silver fir *Abies alba* showed considerably more profound activity than the cedar oil and fir oil derived from Siberian fir *Abies sibirica*. Maximum antibacterial activity was shown by essential oil of silver fir oil against *Escherichia coli*, methicillin-sensitive *Staphylococcus aureus* (MSSA), and *Pseudomonas aeruginosa*. Silver fir essential oil was found to be active against *K. pneumoniae* while Siberian fir essential oil showed the mild activity of clear inhibition zone against *P. aeruginosa* and methicillin-sensitive *Staphylococcus aureus*. Silver fir essential oil has the highest antibacterial potential from all tested essential oils and could be a promising candidate concerning possible applicability in the prevention of bacterial growth. Moreover, essential oils may play an important role in the discovery of new drugs for the treatment of a wide range of pathogenic microorganisms in the near future. This study provides insight into the *in vitro* antibacterial activity of a wide variety of essential oils derived from many different plant genera against pathogenic bacteria. The data contributes to the ongoing scientific investigation regarding the application of essential oils as natural antibacterial agents.

Keywords: *Abies alba* Mill., *Abies sibirica* L., essential oils, antibacterial activity, Gram-positive and Gram-negative bacteria, Kirby–Bauer disk diffusion test

Introduction

Pinaceae, the largest family of conifers, comprises more than 230 species in 11 genera – *Abies* Mill., *Cathaya* Chun & Kuang, *Cedrus* Trew, *Keteleeria* Carrière, *Larix* Mill., *Nothotsuga* Hu ex C.N.Page, *Picea* A. Dietr., *Pinus* L., *Pseudotsuga* Carrière, *Pseudolarix* Gordon, and *Tsuga*

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(Endl.) Carrière (Sudianto et al., 2016; Ran et al., 2018). The pine family is one of the most ecologically and economically important groups of living plants. Many of the species that are highly valuable for their timber include firs (*Abies*), cedars (*Cedrus*), larches (*Larix*), spruces (*Picea*), pines (*Pinus*), Douglas firs (*Pseudotsuga*), and hemlocks (*Tsuga*) (Lin et al., 2010). The Pinaceae are exclusively distributed in the northern hemisphere, except for one species, *Pinus merkusii* Jungh. & de Vriese, whose habitat crosses the equator in Sumatra (Thieret, 1993). Twelve genera (i.e., *Abies*, *Cathaya*, *Cedrus*, *Hesperopeuce*, *Keteleeria*, *Larix*, *Nothotsuga*, *Picea*, *Pinus*, *Pseudolarix*, *Pseudotsuga*, and *Tsuga*) have been recognized in the family since the pioneering work of Van Tieghem published in 1891 (Lin et al., 2010).

The morphology and anatomy of Pinaceae are well studied relative to other plant groups (Gernandt et al., 2018). Hart (1987) divided the family into two groups: the presence of resin canals in the seeds and cleavage polyembryony supported the monophyly of *Abies*, *Cedrus*, *Keteleeria*, *Pseudolarix* and *Tsuga* and the presence of resin canals in the secondary xylem and leaves having an endodermis with thickened Casparian strips supported the monophyly of *Cathaya*, *Larix*, *Picea*, *Pinus*, and *Pseudotsuga*.

Pinaceae species often form the dominant component of boreal, coastal, and montane forests in the Northern Hemisphere (Farjon, 1990; Liston et al., 2003). For instance, *Pinus*, the largest genus of the family, with more than 110 species, occupies an extended geographic range – North America, the northern part of Asia, and Europe (Farjon, 1990).

The cedar genus *Cedrus*, consisting of 4–5 species (Farjón, 1990), is native to the mountains of the western Himalayan and Mediterranean regions (Lin et al., 2010). *Cedrus* is traditionally placed in the Abietoideae along with other four genera, *Abies*, *Keteleeria*, *Pseudolarix*, and *Tsuga*. *Cedrus* (true cedar) is one of 11 commonly accepted genera in Pinaceae, first described by Trew in 1757 (Farjón, 2001). It comprises four species with a highly disjunctive distribution in circum-Mediterranean and western Himalayas (Farjón, 1990, 2001), i.e. *Cedrus deodara* (Roxb.) G. Don in the Hindu Kush, Karakoram and Indian Himalayas, *Cedrus libani* A. Rich. in Turkey, Lebanon and Syria, *Cedrus brevifolia* (Hook. f.) Henry in Cyprus, and *Cedrus atlantica* (Endl.) Manetti ex Carriére in North Africa (Algeria, Morocco) (Qiao et al., 2007).

The *Cedrus* species have many medicinal properties. The extract of essential oil from tree wood has a significant anti-inflammatory effect, severe analgesic, and anticonvulsant action. The wood ethanol extract shows antihyperglycaemic activity, anxiolytic and anticonvulsant effect, while the branch's bark air-dried aqueous extract has an antiarthritic activity. Petroleum ether extract of the heartwood presents severe diuretic and anti-urolithiasis effect (Tewari, 1994; Bhatnagar and Moitra, 1996; Dirr, 2016). In the traditional Ayurveda therapeutic system, all plant parts of the Deodar Cedar are used against various diseases. Among others, they are used to treat inflammation, dyspepsia, insomnia, cough, and cold. They are also given against fever, urinary discharges, bronchitis, leucoderma, elephantiasis, tuberculosis glands, mental disorder, and skin and blood diseases. Wood is used as a diuretic, expectorant, for relieving rheumatism, as well as for treating epilepsy and urinary tract diseases. The bark is used in formulations that are administered as astringents, antipyretics, and antidiarrheals. Finally, Cedar oleoresin is applied for the healing of wounds and the treatment of skin rashes

(Tewari, 1994; Bhatnagar and Moitra, 1996; Dirr, 2016). Cedar of Lebanon has ethnobotany and pharmaceutical value while its aromatic wood is used in the construction, woodworking, and instrumental industries. Also, the tree is widely grown for ornamental purposes and is used both in gardening and in landscape architecture (Chaney, 1993).

Silver fir (*Abies alba* Mill.) is a large conifer that can be found in central Europe and some parts of Southern and Eastern Europe. It is one of the tallest tree species of the genus *Abies* in Europe. This tree is considered an important ecological and functional balancer of European forests and a fundamental species for maintaining high biodiversity in forested ecosystems (Mauri et al., 2016). Siberian fir (*Abies sibirica* L.), a large evergreen conifer with a high part of greenery, is one of the dominant species in European Russia, west and east Siberian taiga, using for therapy and prophylaxis in official and folk medicine for ages (Koctesha et al., 1997). The essential oils obtained from the leaves were also used in the past to heal bruises as well as for treating coughs and colds (Farjon, 2010) and to help respiratory system and have easing and soothing effect for muscle (Yang et al., 2009).

Up to 2008, 277 compounds were isolated from 19 plants of *Abies* species. The chemical constituents are mostly terpenoids, flavonoids, and lignans, together with minor constituents of phenols, steroids, and others. The crude extracts and metabolites have been found to possess various bioactivities including insect juvenile hormone, antitumor, antimicrobial, anti-ulcerogenic, anti-inflammatory, antihypertensive, antitussive, and CNS (central nervous system) activities (Yang et al., 2008).

Essential oils are complex mixtures of compounds, mainly monoterpene and sesquiterpene hydrocarbons (10 and 15 carbon atoms, respectively) and their oxygenated derivatives (alcohols, aldehydes, esters, ketones) as well as phenylpropanoids (Lee et al., 2009; Saad et al., 2013), which have antitumor, antioxygen, anti-aging, anti-mutation, and sedative effects (Kwak et al., 2006; Lee et al., 2007).

Essential oils (EO) that are lipophilic liquids, extracted from diverse plants containing different natural, biologically active components have antimicrobial and antioxidant properties (Khorshidian et al., 2018). The essential oils are obtained from plant materials (leaves, buds, fruits, flowers, herbs, twigs, bark, wood, roots and seeds). Having a density generally lower than that of water, essential oils are volatile, liquid, limpid, lipid-soluble, rarely colored, and soluble in organic solvents (Chouhan et al., 2017). Essential oils from different plant species contain more than 200 constituents which are comprised of volatile and non-volatile components. The application of essential oils as antimicrobial, anticancer, anti-inflammatory and anti-viral agents is due to their effective and efficient properties, inter alia (Aziz et al., 2018).

Due to the hydrophobicity of essential oils' components, they easily pass through the bacterial cell membrane interfering with molecular transport mechanisms leading to cell inactivation (Burt, 2004; Chouhan et al., 2017; Khorshidian et al., 2018). This eventually results in the death of a bacterial cell due to leakage of critical molecules and ions from the bacterial cell to a great extent. Some compounds modulate drug resistance by targeting efflux mechanisms in several species of Gram-negative bacteria (Chouhan et al., 2017).

The effect of the antibacterial activity of essential oils may inhibit the growth of bacteria (bacteriostatic) or destroy bacterial cells (bactericidal). Nevertheless, it is difficult to distinguish these actions. In relation to this, antibacterial activity is more frequently measured as the minimum bactericidal concentration (MBC) or the minimum inhibitory concentration (MIC) (Burt, 2004; Swamy et al., 2016).

The long-known antimicrobial actions of essential oils are now being extensively scientifically reviewed and applied in health and industry fields (Sienkiewicz et al. 2012). Numerous experimental studies have confirmed the inhibitory action of essential oils against bacteria, fungi, yeasts, viruses, and protozoa. They have also been reported to exhibit anti-inflammatory and immunostimulatory activities (Dorman and Deans, 2000; Król et al., 2013).

In this regard, the antibacterial properties of three commercial essential oils derived from plants belonging to family Pinaceae and subfamily Abietoideae (cedar oil, fir oils derived from Silver fir *Abies alba* and Siberian fir *Abies sibirica*, respectively) against some Gram-positive and Gram-negative bacteria were studied in the present research. To this intent, the antimicrobial susceptibility test was used (the Kirby–Bauer disk diffusion test for measuring zone diameters of bacterial growth inhibition).

Material and methods

Essential oils

Essential oils were provided by Polish essential oil manufacturers (Etja, Elbląg, Poland). Overall, essential oils from 3 plant species (cedar oil, fir oils derived from Silver fir *Abies alba* and Siberian fir *Abies sibirica*, respectively). The investigated samples did not contain additives or solvents and were confirmed to be natural by the manufacturers. The samples were stored in resalable vials at 5 °C in the dark but were allowed to adjust to room temperature prior to investigation. Geographical origins were excluded as information was mostly not available.

Clinical Isolates

Clinical specimens submitted for routine culture and antibiotic susceptibility testing of hospitalized patients during the period of April to May 2019, at the microbiology laboratory of the Koszalin Regional Hospital were processed. The sources of the clinical isolates were pus, wound, and urine of seven different patients. The purity, as well as the identity of each isolate, was confirmed in the laboratory conditions by standard microbiological methods. The isolates were further identified on the basis of 16S ribosomal RNA (rRNA) gene sequence homology.

Antimicrobial susceptibility testing

First, the samples were examined by a microscopic Gram stain examination. Samples with Gram-negative results were inoculated on plates of nutrient agar, Clede agar, MacConkey's, and blood agar (Merck, Germany) and then incubated at 37 °C for 24 hours. The colony that showed fermenting of lactose on MacConkey agar and Cled agar media were purified and identified according to their morphology as circular, rose-pink to red colonies on MacConkey

agar medium and yellow colonies on Cled agar. The isolates were identified by some biochemical reactions (e.g. catalase enzyme, potassium hydroxide test, Indole and methyl red test, Voges Proskauer reaction, urease and citrate, H_2S and oxidase test).

P. aeruginosa isolates were tested for MBL production by the imipenem-EDTA disk diffusion test (Yong et al., 2002). Susceptibility testing of the isolates was performed by disk diffusion according to the Guidelines of Clinical and Laboratory Standard Institute (CLSI). The following antimicrobial agents were used: amikacin (30 μg), ampicillin (10 μg), aztreonam (30 μg), ceftazidime (30 μg), ciprofloxacin (5 μg), cephalexin (30 μg), gentamicin (10 μg), erythromycin (15 μg), imipenem (10 μg), meropenem (10 μg), clindamycin (2 μg), aztreonam (30 μg), norfloxacin (10 μg), oxytetracycline (30 μg), oxacillin (1 μg), enrofloxacin (5 μg), tetracycline (30 μg), amoxicillin (25 μg), piperacillin (100 μg), piperacillin-tazobactam (100/10 μg), tobramycin (10 μg), ceftazidime (30 μg), chloramphenicol (30 μg), doxycycline (30 μg), cefadroxil (30 μg), pefloxacin (5 μg), and cefepime (30 μg). MIC was determined by E-test strips (according to manufacturer's instruction) and agar dilution method on all MBLs-producing isolates (the Guidelines of Clinical and Laboratory Standard Institute). The resistance breakpoints were the same as the ones defined by the *National Committee for Clinical Laboratory Standards* (NCCLS, 2000).

Phenotypic Disc Confirmatory Test was performed as recommended by the CLSI (Clinical and Laboratory Standards Institute). Disks of ceftazidime (CA, 30 μg) and ceftazidime-clavulanic acid (CAC, 20 + 10 μg) or ceftriaxone (CE, 30 μg) and ceftriaxone clavulanic acid (CEC, 20 + 10 μg) were placed on Muller Hinton Agar (MHA). An increase in the zone diameter (= 5 mm) for CAC versus CA or CEC versus CE is confirmed as β-lactamase (ESBL)-producing strain *E. coli* strain. The *Staphylococcus aureus* isolates were characterized by Gram-staining and their ability to produce coagulase and clumping factor using Slidex Staph Plus (BioMerieux). Additionally, the species were identified using the biochemical identification system ID 32 Staph (BioMerieux).

The antibacterial susceptibility profile of the isolates revealed that many isolated strains were classified as multi-drug resistant (MDR) bacteria.

Isolate 1 – *Pseudomonas aeruginosa* was resistant to gentamicin (10 μg), cefotaxime (10 μg), and amikacin (30 μg);

Isolate 2 – *Enterococcus faecalis* was resistant to gentamicin (10 μg);

Isolate 3 – *Pseudomonas aeruginosa* was resistant to piperacillin-tazobactam (100/10 μg), ceftazidime (30 μg), piperacillin (100 μg), and cefepime (30 μg);

Isolate 4 – *Enterococcus faecium* was resistant to gentamicin (10 μg) and ampicillin (10 μg);

Isolate 5 – *Klebsiella pneumoniae* was resistant to piperacillin-tazobactam (100/10 μg), gentamicin (10 μg), tobramycin (10 μg), and ciprofloxacin (5 μg);

Isolate 6 – *Escherichia coli*, not β-lactamase (ESBL)-producing strain, was a sensitive strain to antibiotics tested;

Isolate 7 – methicillin-sensitive *Staphylococcus aureus* (MSSA) was resistant to tobramycin (10 μg), piperacillin (100 μg), clindamycin (2 μg), and erythromycin (15 μg).

Bacterial Growth Inhibition Test of Essential Oils by the Disk Diffusion Method

Strains tested were plated on TSA medium (Tryptone Soy Agar) and incubated for 24 hr at 25 °C. Then the suspension of microorganisms was suspended in sterile PBS and the turbidity adjusted equivalent to that of a 0.5 McFarland standard. Muller-Hinton agar plates were inoculated with 200 µl of standardized inoculum (10^8 CFU/mL) of the bacterium and spread with sterile swabs. Sterile filter paper discs impregnated by sample were applied over each of the culture plates, 15 min after bacteria suspension was placed. The antimicrobial susceptibility testing was done on Muller-Hinton agar by the disc diffusion method (Kirby-Bauer disk diffusion susceptibility test protocol) (Bauer et al., 1966). A negative control disc impregnated by sterile ethanol was used in each experiment. After culturing bacteria on Mueller-Hinton agar, the disks were placed on the same plates and incubated for 24 hr at 37 °C. The assessment of antimicrobial activity was based on the measurement of the diameter of the inhibition zone formed around the disks.

The diameters of the inhibition zones were measured in millimeters and compared with those of the control and standard susceptibility disks. The activity was evidenced by the presence of a zone of inhibition surrounding the well. Each test was repeated six times. The following zone diameter criteria were used to assign susceptibility or resistance of bacteria to the phytochemicals tested: Susceptible (*S*) ≥15 mm, Intermediate (*I*) = 11–14 mm, and Resistant (*R*) ≤10 mm (Okoth et al., 2013).

Results and discussion

Three commercial essential oils derived from plants belonging to subfamily Abietoideae (cedar oil, fir oils derived from Silver fir *Abies alba* and Siberian fir *Abies sibirica*, respectively) were tested against Gram-positive and Gram-negative pathogenic bacteria locally isolated from the patients. The fir oil derived from Silver fir *Abies alba* showed considerably more activity than the cedar oil and fir oil derived from Siberian fir *Abies sibirica* (Figure 1). Maximum antibacterial activity was shown by essential oil of silver fir oil against *E. coli* with the inhibition zone size (25.7 ±1.13) mm, methicillin-sensitive *Staphylococcus aureus* (MSSA) – (23.8 ±1.25) mm, and *Pseudomonas aeruginosa* – (22.4 ±1.1) mm (Figure 1, 2). Silver fir essential oil was found to be active with the inhibition zone diameter of (19.4 ±0.98) mm against *K. pneumoniae* while Siberian fir essential oil showed mild activity with (9.4 ±0.1) mm and (9.4 ±0.25) mm of clear inhibition zone against *P. aeruginosa* and *S. aureus* MSSA (Figure 1).

These findings are in line with the results from previous works and enhance the often requested need for chemical characterizations of antimicrobial essential oils to identify the active compounds and their interdependencies. Antimicrobial properties of essential oils against a wide range of microorganisms have been reported in various studies. Due to the hydrophobicity of essential oils' components, they easily pass through the bacterial cell membrane interfering with molecular transport mechanisms leading to cell inactivation (Burt, 2004; Khorshidian et al., 2018).

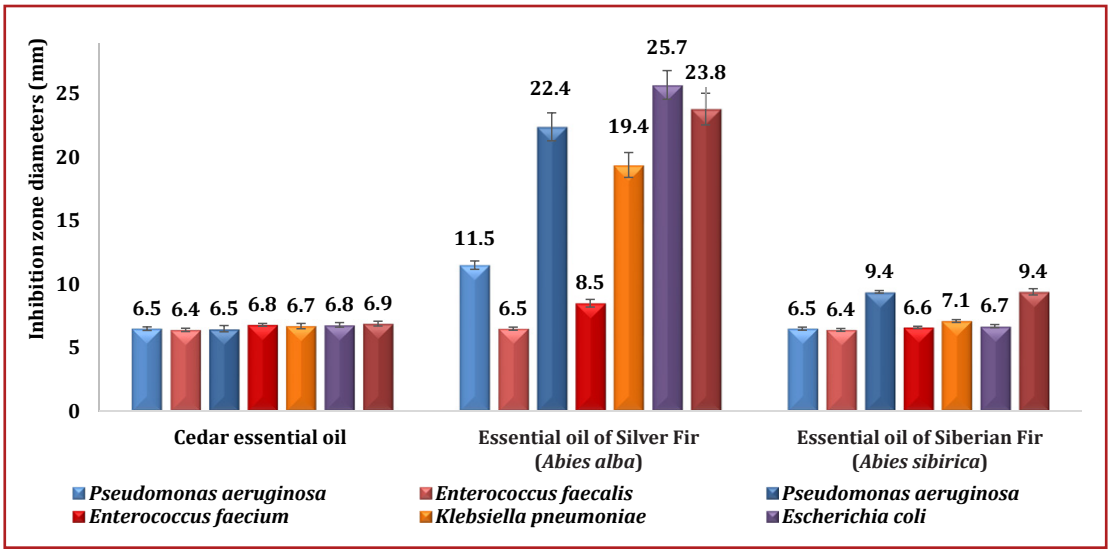

Figure 1 Antibacterial activity of examined essential oils (cedar oil, fir oils derived from Silver fir *Abies alba* and Siberian fir *Abies sibirica*) against Gram-positive and Gram-negative pathogenic bacteria locally isolated from the patients. The data were presented as the mean ± the standard error of the mean (S.E.M.)

The chemical composition, including the enantiomeric excess of the main terpenes, the antimicrobial and antiradical activities, as well as the cytotoxicity of *Abies alba* and *A. koreana* seed and cone essential oils, were investigated in the study by Wajs-Bonikowska et al. (2015). In the examined oils and hydrolats, a total of 174 compounds were identified, which comprised 95.6–99.9% of the volatiles. The essential oils were mainly composed of monoterpene hydrocarbons, whereas the composition of the hydrolats, differing from the seed oils of the corresponding fir species, consisted mainly of oxygenated derivatives of sesquiterpenes. The seed and cone essential oils of both firs exhibited DPPH-radical-scavenging properties and low antibacterial activity. Moreover, they evoked only low cytotoxicity towards normal fibroblasts and the two cancer cell lines MCF-7 and MDA-MBA-231. At concentrations up to 50 μg/ml, all essential oils were safe in relation to normal fibroblasts. Although they induced cytotoxicity towards the cancer cells at concentrations slightly lower than those required for the inhibition of fibroblast proliferation, their influence on cancer cells was weak, with IC_{50} values similar to those observed towards normal fibroblasts (Wajs-Bonikowska et al., 2015).

Studies by Yang et al. (2009) dedicated to investigation of the chemical composition, cytotoxicity and its biological activities of Silver fir (*Abies alba*) essential oil have revealed that the composition of the oil was follow: bornyl acetate (30.31%), camphene (19.81%), 3-carene (13.85%), tricyclene (12.90%), dl-limonene (7.50%), α-pinene (2.87%), caryophyllene (2.18%), β-phellandrene (2.13%), borneol (1.74%), bicyclo[2.2.1]hept-2-ene,2.3-dimethyl (1.64%) and α-terpinene (1.24%). The results also indicated that the oil showed no cytotoxic effect, at concentrations of 1 and 5%, for as long as 24 and 3 h, respectively. The antiradical capacity was evaluated by measuring the scavenging activity of the essential oil on the

2.2-diphenylpicrylhydrazyl (DPPH) and 2.2'-azino-bis 3-ethyl benzothiazoline-6-sulfonic acid (ABTS) radicals. The oil was able to reduce both radicals dose-dependently, and the concentration required for 50% reduction against DPPH radicals (2.7 ±0.63%) was lower than ABTS radicals (8.5 ±0.27%). The antibacterial activity of the oil was also evaluated using a disc diffusion method against *Staphylococcus aureus*, *Streptococcus mutans*, *Listeria monocytogenes*, *Acinetobacter baumannii*, *Escherichia coli*, and *Vibrio parahaemolyticcus*. The oil exhibited no antibacterial activity against all the bacterial strains tested except *S. aureus* of mild activity (Yang et al., 2009).

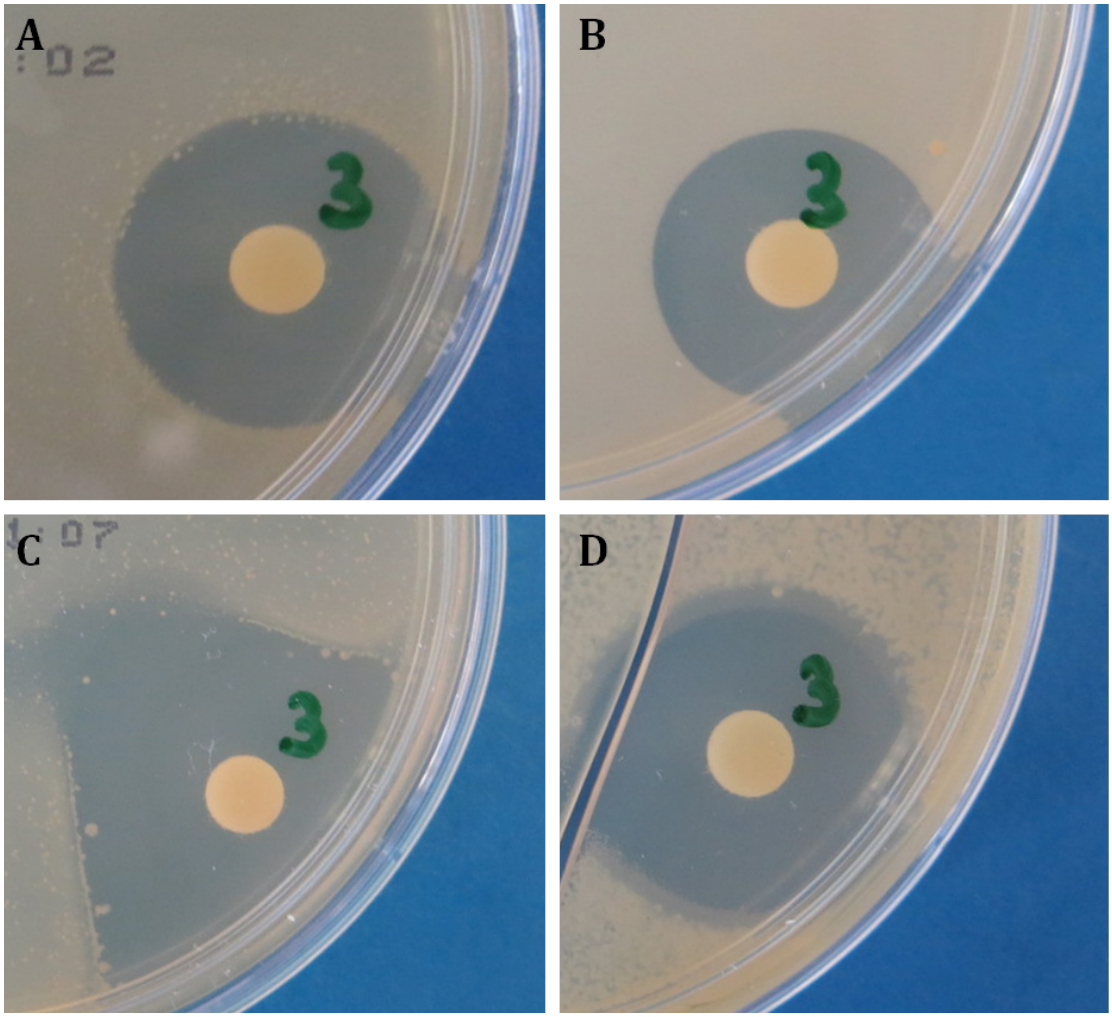

Figure 2 Antibacterial activity of essential fir oils derived from Silver fir *Abies alba* in disc diffusion assays. Example of a disc diffusion assay plate showing the halos in the bacterial lawn resulting from the antibacterial activity of Silver fir oil against *Pseudomonas aeruginosa* (isolate 3, A), *Klebsiella pneumoniae* (isolate 5, B), *Escherichia coli* (isolate 6, C), and methicillin-sensitive *Staphylococcus aureus* (isolate 7, D)

Lanzerstorfer et al. (2019) have evaluated the efficacy of the dispersion of selected essential oils in reducing microbial contamination in two hospital wards. The study was carried out at two wards of a 1,227-bed acute-care hospital in Austria. The concentration of airborne bacteria and fungi was measured in the patient rooms before and after the dispersion of a mixture of *Citrus limon* and *Abies alba* essential oils. Before dispersion of the essential oils in both wards, the mean concentration of bacteria was in a typical range (123 colony forming units (CFU)/m^3 and 10^4 CFU/m^3) while the mean concentration of fungi differed substantially (155 CFU/m^3 and 28 CFU/m^3). After the dispersion of the essential oils, a-reduction in both bacterial and fungal contamination was observed. In the first two hours, the mean concentration of airborne bacteria and fungi was reduced by approximately 40 and 30–60% respectively (Lanzerstorfer et al., 2019).

The antiproliferative, antimicrobial and antioxidative effects of fir (*Abies alba* Mill.) honeydew honey from the mountain region of Croatia (Gorski Kotar) as a potential replacement for standard antibiotics and chemotherapeutic agents were studied by Broznić et al. (2018). Cell viability, annexin V assay, and flow cytometry analysis served to analyze the antiproliferative effect on, apoptosis induction in and cell death of cancer cell lines: HeLa, MCF-7, SW620, CFPAC-1, MIA PaCa-2 and normal diploid human fibroblasts (BJ). Antimicrobial activity was tested against *Staphylococcus* and *Acinetobacter* strains by agar well diffusion and microdilution assays. The $DPPH^{\cdot}$ assay determined the radical scavenging activity, while mathematical models helped to evaluate the kinetic data of $DPPH^{\cdot}$ inhibition. Antiproliferative effect on all tested cell lines and the prominent effect on normal diploid human fibroblasts (BJ), colorectal adenocarcinoma (SW620, metastatic) and breast epithelial adenocarcinoma (MCF-7, metastatic) was observed. The mechanisms of the antiproliferative effect included the accumulation of cells in the sub-G1 phase in all tested cells and induction of apoptosis in SW620 and MCF-7 cells predominantly. The antibacterial assays showed that antibiotic-resistant strains of both bacteria, including multi-resistant strain *A. baumannii* ATCC® BAA-1605™, were sensitive to all tested honey samples (Broznić et al., 2018). Results of well diffusion tests showed that all tested *Staphylococcus* strains were more sensitive than *Acinetobacter* strains to all honey samples with inhibition zones between 13 and 21 mm, while the inhibition zone of *Acinetobacter* strains varied between 7 and 14 mm. There were no significant sensitivity differences between the antibiotic-resistant and susceptible bacterial *Staphylococcus* and *Acinetobacter* strains to the tested honey samples (Broznić et al., 2018).

Multiple studies have been dedicated to investigating the chemical compositions and antibacterial and antifungal activities of essential oils extracted from *Abies* plants (Lee and Hong, 2009; Satou et al., 2011). Various bioactive compounds such as lignans and triterpenoids have been isolated from several *Abies* species (Roshchin et al., 1998; Kim et al., 1999). Recently, the chemical composition of the essential oil prepared from the needles of A. koreana and its antibacterial activity against nine bacterial strains were reported (Jeong et al., 2007).

The antimicrobial activity of essential oils isolated from nine *Abies* species (*Abies koreana* Wills, *A. alba* Mill., *A. pinsapo* Boiss., *A. concolor* (Gord. et Glend.) Lindl. ex Hildebr., *A. firma* Sieb. et Zucc. (all of these exotic for Turkey), *A. nordmanniana* (Stev.) Spach. subsp *nordmanniana* which are plantation forms and *A. cilicica* (Ant. et Kotschy.) Carr. subsp. *cilicica*, *A. cilicica*

(Ant. et Kotschy.) Carr. subsp. *isaurica* Coode et Cullen, *A. nordmanniana* (Stev.) Spach. subsp. *bornmüelleriana* Mattf. which are natural forms against nine bacteria (*Escherichia coli*, *Bacillus megaterium*, *B. cereus*, *B. subtilis*, *B. brevis*, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Staphylococcus aureus*) and two yeasts (*Saccharomyces cerevisiae* and *Candida albicans*) have been investigated by Bagci and Diğrak (1996) using a disc diffusion method. The results suggested that the essential oils tested for antimicrobial activity can be classified into three groups according to the strength of their antimicrobial activities. The essential oils of *A. pinsapo* and *A. concolor* does not have any antimicrobial activity, while the essential oils, isolated from *A. alba* and *A. firma* had a modest activity. The essential oils of *A. koreana*, *A. cilicica* subsp. *cilicica*, *A. cilicica* subsp. *isaurica*, *A. nordmanniana* subsp. *nordmanniana*, and *A. nordmanniana* subsp. *bornmüelleriana* had possessed the highest antimicrobial activity against the tested bacteria and yeast species. The essential oils of the nine *Abies* species tested for antimicrobial activity were more active against yeast species than against bacteria, and the antimicrobial activity of essential oils was variable, depending on the bacterial strains and the source of the essential oil (Bagci and Diğrak, 1996).

The study of Coté et al. (2016) supports the use of oleoresin of *Abies balsamea* (L.) Mill. by the used by Native Americans of the boreal forest of Canada and French Canadians to treat various infections, suggesting that oleoresin has antibacterial properties. The antibacterial activity of the oleoresin was investigated against *E. coli*, *S. aureus* and two methicillin-resistant *S. aureus* (MRSA) strains using a new sensitive assay developed to evaluate hydrophobic matrix and compounds. The results showed that whole oleoresin was inactive against Gram-negative *E. coli* (MIC_{90} >90 µg/ml) but active against Gram-positive *S. aureus* and MRSA with MIC_{90} ranging from 18.2 to 30 µg/ml. The oleoresin is mainly composed of monoterpene (28%), sesquiterpenes (2%), and diterpenes (45%). Resin acids were found, in part, responsible for the antibacterial activity of the whole oleoresin. Isopimaric acid and levopimaric acid are the most active with an MIC_{90} of respectively 9.7 µg/ml and 10µg/ml.

This study also revealed results that differ greatly from those reported by others. The lacking growth inhibition by cedar oil and Siberian fir oil may be due to the comparably low concentrations used. Authors, who found these oils to be inhibitory, used way higher concentrations. In this case, clarification can only be achieved by chemical analysis of the respective oils which has not been performed as part of our investigations, due to the more broadened approach.

The essential oils extracted from the leaves and the shoots of five *Abies* species (Pinaceae) growing in Japan, i.e., *A. firma*, *A. homolepis*, *A. veitchii*, *A. mariesii*, and *A. sachalinensis*, were characterized by GC-FID and GC/MS analyses (Satou et al., 2011).

The antimicrobial activities of several parts of various trees grown in the Kahramanmaraş region of Turkey were investigated by Diğrak et al. (1999). Chloroform, acetone and methanol extracts of leaves, resins, barks, cones and fruits of *Pinus brutia* Ten., *Juniperus oxycedrus* L., *Abies cilicia* Ant. & Kotschy Carr., *Cedrus libani* A. Rich. and *Pinus nigra* Arn. were prepared and tested against *Bacillus megaterium* DSM 32, *Bacillus subtilis* IMG 22, *Bacillus cereus* FMC

19, *Escherichia coli* DM, *Klebsiella pneumoniae* FMC 3, *Enterobacter aerogenes* CCM 2531, *Staphylococcus aureus* Cowan 1, *Mycobacterium smegmatis* RUT, *Proteus vulgaris* FMC 1, *Listeria monocytogenes* Scoot A, *Pseudomonas aeruginosa* DSM 5007, *Candida albicans* CCM 314, *Candida tropicalis* MDC 86 and *Penicillium italicum* K. The antifungal effects were not observed for the whole extracts, *E. coli* was not inhibited by any of the plant extracts except by the chloroform and acetone extracts of the leaves of A. cilicia, which showed inhibition zones of 16-18 mm, respectively. All the plant extracts used in the study of Diğrak et al. (1999) inhibited the growth of the other bacteria studied.

Increasingly, it has been recognized that the water-soluble extract from pine needles of *Cedrus deodara* (WEC) might be a new potential source of natural antibacterial agents applicable to food. Zeng et al. (2012) have studied the antibacterial activity of WEC against five food-borne bacteria, and its related mechanism was investigated by a transmission electron microscope. *In vitro* antibacterial assay showed that WEC possesses a remarkable antibacterial activity against tested food-borne bacteria including *Escherichia coli*, *Proteus vulgaris*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Bacillus cereus*, with the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values in the ranges of 0.78–12.5 and 1.56–25 mg/ml, respectively. In a food system of fresh-squeezed tomato juice, WEC was observed to possess an effective capacity to control the total counts of viable bacteria. Shikimic acid was isolated from WEC and identified as the main antibacterial compound.

Extracts of woods commonly used for animal bedding were tested for antimicrobial activity. Essential oils from Alaska cedar (*Chamaecyparis nootkatensis*), western juniper (*Juniperus occidentalis*) and old-growth Douglas fir (*Pseudotsuga menziesii*), as well as methanol extracts of wood from these trees plus western red cedar (*Thuja plicata*) and ponderosa pine (*Pinus ponderosa*), were tested for antimicrobial activity against anaerobic bacteria and yeast in study by Johnston and co-workers (2001). The test microbes included *Fusobacterium necrophorum*, *Clostridium perfringens*, *Actinomyces bovis* and *Candida albicans* which are common to foot diseases and other infections in animals. The essential oils and methanol extracts were tested using a standardized broth assay. Only extracts of Alaska cedar and western juniper showed significant antimicrobial activity against each of the microbes tested. The essential oil of Douglas fir did show antimicrobial activity against *A. bovis* at the concentrations tested. The methanol extracts of the heartwood of Douglas fir and the sapwood of ponderosa pine showed no antimicrobial activity. The major chemical components of western juniper (cedrol and alpha- and beta-cedrene) and Alaska cedar (nootkatin) were also tested. In western juniper, alpha- and beta-cedrene were found to be active components. Nootkatin showed activity only against *C. albicans*. The inhibitory activity in Alaska cedar oil was high enough to justify further efforts to define the other chemical components responsible for the antimicrobial activity.

Chaudhary et al. (2009) reported 36 constituents in the oil of woodchips of *C. deodara*. In the pentane fraction, 27 compounds were identified representing 90.89% of the constituents detected. A total of 11 sesquiterpene hydrocarbons and 16 oxygenated sesquiterpenes were identified constituting 59.68 and 31.21%, respectively. In the acetonitrile fraction, 31 compounds were identified representing 89.24% of the constituents detected assigning to three different classes: oxygenated monoterpene (3.73%), sesquiterpene hydrocarbons

(14.44%), and oxygenated sesquiterpenes (71.07%). The major constituents in the pentane fraction were himachalenes (52.35%) and atlantones (15.45%). The other constituents were himachalene oxide (9.12%), himachalol (2.04%), α-dehydro-ar-himachalene (1.94%), cis-α-bisabolene (1.58%), and γ-dehydro-ar-himachalene (1.44%). The major constituents in the acetonitrile fraction were atlantones (41.40%) followed by himachalenes (11.70%). Further chromatography led to an increase in the percentage of himachalenes and atlantones from pentane and acetonitrile fraction to 90.89 and 95.74%, respectively (Chaudhary et al., 2009, 2011). Generally, the *Cedrus* oils contain high percentages of the himachalenes (Chaudhary et al., 2011).

Takao et al. (2012) have prepared essential oil (EO) from waste wood chips made from used sake barrels (USBs) of Japanese cedar (i.e., EO-USB) by steam distillation. They have found that EO-USB and three commercially purchased EOs derived from xylem tissue of Japanese woods, such as Japanese cedar (*Cryptomeria japonica*), Japanese cypress (*Chamaecyparis obtusa*) and false arborvitae (*Thujopsis dolabrata*), suppressed fungal growth activity against Trichophyton rubrum, which is the cause of tinea disease. The magnitude of the suppressive effects of the EOs ranked as follows: *T. dolabrata* > USB = *C. japonica* > *C. obtusa*. These EOs also inhibited the activity of DNA polymerase in an extract from *T. rubrum* mycelia with the following ranking: *T. dolabrata* > USB = *C. japonica* > *C. obtusa*. In addition, 50 μg/ml of EO-USB showed antifungal properties, killing *T. rubrum* mycelia at 27–42 °C in 20 min. Three prepared sesquiterpenes, δ-cadinene, epi-cubenol, and β-eudesmol, inhibited the fungal growth and DNA polymerase activities of *T. rubrum*, and epi-cubenol showed the strongest inhibition among the compounds tested. These sesquiterpenes had no inhibitory effects on the activities of other DNA metabolic enzymes, such as DNA topoisomerase II, IMP dehydrogenase, polynucleotide kinase, and deoxyribonuclease from *T. rubrum*. These results suggest that EO-USB containing epi-cubenol may be useful for its anti-tinea disease properties, which are based on DNA polymerase inhibition (Takao et al., 2012).

A comprehensive overview of new discoveries in essential oil research with new insights on antimicrobial activity, as well as immunomodulatory, anti-apoptotic, anti-angiogenic and antitumoral properties of these compounds was provided by Saad et al. (2013). The interest in using essential oils and their active components as modulators of cellular physiology, homeostasis, and fate is due to the high diversity in the composition of essential oils in addition to the presence of a high molecular assortment in each composition. It was assumed that this natural and sustainable resource of diverse molecular composition and structure will open an opportunity for targeting a wide spectrum of cellular processes (Saad et al., 2013).

Additionally, the numerous modes of essential oils action have been proposed involving, for example, degradation of the bacterial cell wall, modification of proteins of the cytoplasmic membrane, alteration of membrane permeability, inactivation of extracellular enzymes, reduction of intracellular ATP, leakage of cellular contents, coagulation of cytoplasm, and interruption of electron flow and active transport (Burt, 2004; Llana-Ruiz-Cabello et al., 2015; Radaelli et al., 2016).

Conclusions

In summary, this study provides insight into the *in vitro* antibacterial activity of a wide variety of essential oils derived from many different plant genera against pathogenic bacteria. The data contributes to the ongoing scientific investigation regarding the application of essential oils as natural antibacterial agents. After benchmarking essential oils from plants belonging to subfamily Abietoideae, silver fir essential oil is identified as a promising candidate concerning possible applicability in the prevention of bacterial growth.

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