ТЕЗИ ДОПОВІДЕЙ

УЧАСНИКІВ
МІЖНАРОДНОЇ НАУКОВО-ПРАКТИЧНОЇ КОНФЕРЕНЦІЇ

«ВІДНОВЛЕННЯ, ОХОРОНА Й ЗБЕРЕЖЕННЯ РОСЛИННОГО СВІТУ ЛІСІВ УКРАЇНИ В УМОВАХ ТЕХНОГЕННОГО НАВАНТАЖЕННЯ ТА ЗМІН КЛІМАТУ»

(15-16 жовтня 2019 року)

Київ - 2019

UDK 665.528.1:579.61:579.222:615.32:615.33

THE ANTIMICROBIAL ACTIVITIES OF THE COMMERCIAL ESSENTIAL OIL DERIVED FROM SILVER FIR *ABIES ALBA*

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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 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 (Saad et al., 2013), which have antitumor, antioxygen, anti-aging, anti-mutation, and sedative effects (Kwak et al., 2006; Lee et al., 2007).

In this regard, the antibacterial properties of the commercial essential oil derived from Silver fir *Abies alba* 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.* The essential oil from Silver fir *Abies alba* was provided by Polish essential oil manufacturer (Etja, Elbląg, Poland). 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 and Antimicrobial susceptibility testing. 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.

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 disc diffusion method (Kirby-Bauer disk diffusion susceptibility test protocol) (Bauer et al., 1966). After culturing bacteria on Mueller-Hinton agar, the disks were placed on the same plates and incubated for 24 h 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.

Results and conclusions. 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. Silver fir essential oil was found to be active with the inhibition zone diameter of (19.4±0.98) mm against *K. pneumoniae*. 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).

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 and co-workers (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 50μg/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 and co-workers (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 2,20-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 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).

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. Silver fir essential oil is identified as a promising candidate concerning possible applicability in the prevention of bacterial growth.

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