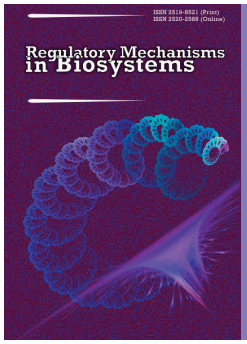

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Antibacterial and fungicidal effect of ethanol extracts from *Juniperus sabina*, *Chamaecyparis lawsoniana*, *Pseudotsuga menziesii* and *Cephalotaxus harringtonia*

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We determined a high antibacterial effect of ethanol extracts of four species of gymnosperms (*Juniperus sabina*, *Chamaecyparis lawsoniana*, *Pseudotsuga menziesii* and *Cephalotaxus harringtonia*) against 23 strains of bacteria of families Enterobacteriaceae (*Escherichia coli*, *Enterococcus faecalis*, *Salmonella typhimurium*, *S. adobraco*, *Proteus vulgaris*, *P. mirabilis*, *Serratia marcescens*, *Klebsiella pneumoniae*), Staphylococcaceae (*Staphylococcus aureus*, *S. epidermidis*), Yersiniaceae (*Yersinia enterocolitica*), Bacillaceae (*Bacillus subtilis*, *B. cereus*), Listeriaceae (*Listeria ivanovi*, *L. innocua*, *L. monocytogenes*), Corynebacteriaceae (*Corynebacterium xerosis*), Campylobacteraceae (*Campylobacter jejuni*), Nocardiaceae (*Rhodococcus equi*), Pseudomonadaceae (*Pseudomonas aeruginosa*) and one strain of fungi of the Saccharomycetaceae family (*Candida albicans*). The experiment *in vitro* revealed zone of inhibition of growth of colonies, measuring over 8 mm, produced by ethanol extracts from *J. sabina* against seven species of bacteria (*S. aureus*, *B. subtilis*, *B. cereus*, *L. innocua*, *C. xerosis*, *Rh. equi* and *P. aeruginosa*), *Ch. lawsoniana* – against five species (*E. coli*, *B. subtilis*, *L. innocua* and *Rh. equi*), *P. menziesii* –two species (*Rh. equi* and *P. mirabilis*), *C. harringtonia* – ten species of microorganisms (*E. coli*, *S. aureus*, *S. epidermidis*, *L. ivanovi*, *L. monocytogenes*, *C. xerosis*, *C. jejuni*, *P. vulgaris*, *S. marcescens* and *C. albicans*). As a result of the research, the most promising plants for further *in vivo* study of antibacterial activity were *C. harringtonia* and *J. sabina*.

Keywords: growth inhibition zone; bacterial colonies; multi-resistant strains; gymnosperms; candidiasis.

Introduction

Over recent years, due to spread of antibiotic-polyresistant strains of bacteria which are poorly susceptible to treatment, more and more often reports emerge, describing the potential of the search for efficient antibacterial substances in ethanol plant extracts (Boyko et al., 2016; Zazharskyi et al., 2019a, b; Palchykov et al., 2019, 2020). Potentially, a subject of significant interest in this aspect is various groups of gymnosperms used for the greening of settlements.

Cephalotaxus harringtonii (Forbes) K. Kokh known as Japanese plum-yaw is an evergreen coniferous shrub or small tree from the Taxaceae family. Scioheliophyte, eutrophic, mesohyte. Quite winter-hardy (second category), quite drought tolerant (second category) species. Needs protection against wind over winter. The plants grow in deep rich soils. *C. harringtonii* can be used in group plantations in parks. The species is native to Japan, but sometimes is used in Western couuntires. Several forms are grown for this purpose. In Japan, *C. harringtonii* grows from Kyuschu in the south to Hokkaido in the north. Particularly, the species is found in Hondo in Chiba Prefecture on Kiyosumi Mountain located in Awa district of Awa province. It is also common in Nagasaki and Hiroshima Prefectures. The nana variety occurs in the eastern part of Honshu, and also Hokkaido, especially on coastal rocks and highland areas (Tripp, 1995). In Europe, *C. harringtonii* has been cultivated since 1829. Many gardeners are familiar with this species named after Charles Harrington, the fourth duke Harrington, one of the first who grew the plant in a European garden in Elvaston. Omacetaxine, a substance obtained from the leaves of the plant is a new preparation aginst leukemia.

Pseudotsuga menziesii (Mirbel) Franco – Douglas fir is a tall coniferous tree from the Pinaceae family, which relates to the first and the second layers of forests. Also known as Douglas fir, Douglas pine, Oregon pine, and Columbian pine. Evergeen, phytoncidal, decorative fruit plant. Heliophyte, xeromesophyte, neutrophil, mesotrophic-eutrophic. Prefers acidic and neutral soils. Winter-hardy (second category), drought-tolerant (first category) plant. Grows better in well-drained, neutral loams. For greening it is used in parks, garden squares, embankments, more rarely in the streets. It is native to the western part of North America. Morphologically, *P. menziesii* is flexible, has many forms. *P. menziesii* var. *menziesii* forms a deeper root system. *P. menziesii* var. *glauca* manifests more flexibility, occurring in relic forests of British Columbia (Canada); in semi-arid vermouth steppe in the greater part of its range, forms the deepest roots. *P. menziesii* contains poriol – flavanone (one of the flavonoids) produced in response to *Poria weirii* (Barton) infection. Different groups of Indians used bark, resin and needles of this plant for treating various diseases.

Chamaecyparis lawsoniana (A. Murray) Parl. is a tree of the second layer, belonging to the Curpessaceae family. Evergreen, phytoncidal, quite winter-hardy (second category) and drought-hardy (second category) plant. Mesophyte, but badly widstands air dryness, heliophyte, mesotrophic. The plant is not demanding in soil fertility requirements, but prefers light (light-loamy and sabulous) conditions. *Ch. lawsoniana* grows better in places sufficiently protected from wind and with good exposure to sunlight. It grows well in urban conditions; its blue-grey and blue forms are the most common. The species is characteristic for Oregon and North-West California. It grows at the height of 1,500 m above the sea level in the valleys of the Klamath Mountains,

along streams. The species is very sensitive to infection by *Phytophthora lateralis* Tucker & Milbrath.

Juniperus sabina L. or savin juniper, savin juniper of the Cupressaceae family is a dioecious, humifuse shrub 1.0–1.5 m high. It rapidly grows in width and forms dense thickets. More rarely, small trees 4 m in height with curved trunks are seen. The prostrate or shrub-like tree form occurs in many cities of Europe. Evergreen, phytoncidal, ground cover, soil protective, winter-hardy (first category) and drought-hardy (first category) species. Heliophyte, mesophyte, xeromesophyte. It is widely used for decorating rocky hills, slopes, in singular or group plantations on lawns or forest edges. Smoke and gas-resistant. Resilient in urban conditions, can be used for greening streets, except in industrial areas. Light-loving, undemanding in soil requirements, has soil-protecting abilities. Forests and groves of *J. sabina* are common in the steppe zone, on rocky mountain slopes and sandy dunes; sometimes the plant extends from the lower to the higher mountain belt (1,000–2,300 m above the sea level), where it forms thickets. The range includes Asia Minor, the Caucasus, Russia (Urals, Siberia and Primorje), South-East Asia, South and Central Europe. *J. sabina* grows in very rare relic communities, and has a limited distribution, being at the verge of extinction (García-Cervigón et al., 2018; Lambevska-Hristova & Bancheva, 2019). Shoots contain essential oil and are poisonous (San Feliciano et al., 1991; Batsatsashvili et al., 2017). Shoots and juniper berries contain glycosides, saponins, flavonids, tannins and up to 17% sabinol, which causes severe poisoning and miscarriages in cattle. Toxic properties of juniper sabina limit its application in therapeutic use. Most often, in folk medicine it is used as an external preparation. Ointment is rubed into the roots of hair in treatment of alopecia, applied to body areas affected by scabies, tincture is used against warts. Powder is sprinkled over pus ulcer. Essention from fresh branches with leaves is used in homeopathic treatment of diseases of the kidneys and the bladder, stranguria, gout, painful menstruations and impaired monthly cycle (Gubanov et al., 1976). The oils of this plant were dominated by α-pinene, sabinene, and cedrol with moderate amounts of limonene, terpinen-4-ol, and elemol (Adams et al., 1998). Alongside some well-known compounds from acidic fraction of the extract of n-Hexane from leaves of *J. sabina*, San Feliciano et al. (1991) isolated two new lignans, derivative of naphthalene called junaphtoic acid and 3-O-demethylyatein. While studying phylogeographic peculiarities of *J. sabina*, Guo et al. (2010) presumed absence of regional interpopulational differentiation.

These species of plants remain poorly studied in relation to antimicrobial activity and can have a significant potential in human and veterinary medicine and. The objective of the article was determining the antibacterial effect of ethanol extracts of *J. sabina*, *Ch. lawsoniana*, *P. menziesii* and *C. harringtonia* on some species of microorganisms of the families Enterobacteriaceae, Staphylococcaceae, Yersiniaceae, Bacillaceae, Listeriaceae, Corynebacteriaceae, Campylobacteraceae, Nocardiaceae, Pseudomonadaceae and Saccharomycetaceae *in vitro*.

Materials and methods

Leaves and shoots of four species of coniferous plants were collected in the territory of Botanical Garden of Oles Honchar Dnipro National University (Khromykh et al., 2018; Boyko & Brygadyrenko, 2019), dried at room temperature, cut, weighed and kept 24 h in 70% ethyl alcohol, filtrated. Antibacterial activity of the plant tinctures were identified using disk-diffusion in agar. Out of the 24 h culture of the ethanol strains of microorganisms of the family, a weighed amount was prepared according to the standard of turbidity of bacterial suspension equaling 0.5 units of density according to McFarland (McF) 1.5×10^8 CFU (colony-forming units), which was determined using a densitometer (Densimeter II). The obtained weighed amount was re-inoculated to Muller-Hinton agar (Himedia) followed by cultivation in a thermostat TSO-80/1 over 24 h at the temperature of 37 °C. On top of the re-inoculations, the disks saturated with tinctures of the extracted ethanol tinctures of the four species of plants were placed (Table 1).

Table 1
Used part of the four species of plants and the most important information on their antibacterial activity

Family	Species	Used part of plant	The most important literature sources on the effect of the plant on bacteria
Cupressaceae	*Juniperus sabina* L.	shoots	Elisoveckaya & Brindza (2018), Živić et al. (2019)
Cupressaceae	*Chamaecyparis lawsoniana* (A. Murray bis) Parl.	leaves	Smith et al. (2007), Kim et al. (2015), Palá-Paúl et al. (2015)
Pinaceae	*Pseudotsuga menziesii* (Mirb.) Franco	shoots	Takano et al. (2010), Dwivedi et al. (2015)
Taxaceae	*Cephalotaxus harringtonii* (Forbes) K. Koch	leaves	Watanabe & Fukao (2009)

As a positive control we used disks with 15.0 μg of azithromycin – macrolid antibiotic of broad spectrum. Discs with 15.0 μg amphotericinin were also used as a second control against *Candida albicans*. After 24 h growth of the culture was assessed using a multi-angle ruler for measuring growth inhibition zones in microorganisms (Antibiotic Zone Scale-C, model PW297, India) and the program TpsDig2 (2016, F. James Rohlf). The data in tables are presented as x ± SD (standard deviation).

Results

We observed inhibition of growth of separate strains of microorganisms exposed to ethanol extracts of the studied species of plants (Table 2). Extract from *J. sabina* moderately inhibited growth of *E. coli* (4.5 and 4.8 mm, hereafter average radius of the growth inhibition zone is indicated in millimeters), *E. faecalis* (4.3), poorly inhibited growth of *S. typhimurium* (3.4), *S. adobraco* (2.7), *P. vulgaris* (2.8), *P. mirabilis* (2.1), *Y. enterocolitica* (2.5), *S. marcescens* (1.2), *K. pneumoniae* (2.5). Ethanol extract of *Ch. lawsoniana* had more notable antibacterial action towards *E. coli* (8.8 and 9.3) and *P. vulgaris* (5.1), compared with *J. sabina*. Extract from *P. menziesii* was more efficient against *P. mirabilis* (10.5) compared with the three other plant preparations and the control. Ethanol extract of *C. harringtonia* had high inhibiting effect against strain of *E. coli* 055 (10.7), and also *P. vulgaris* (8.3) and *S. marcescens* (10.4). During the study on the effect of the preparations of plants against bacteria of the Staphylococcaceae family, we determined an intense antibacterial effect towards them demonstrated by ethanol extracts from *C. harringtonia* and *J. sabina*. We found significant inhibiting effect of the extracts from *J. sabina*, *Ch. lawsoniana* and *C. harringtonia* on the microorganisms of the Bacillaceae family: *B. cereus* – 14.2, 1.6 and 5.7, *B. subtilis* – 8.4, 10.6 and 0.0 mm, respectively. We determined high sensitivity of *L. ivanovi* and *L. monocytogenes* to *C. harringtonia* (9.3 and 8.1), *L. ivanovi* to *C. harringtonia* (9.3), *L. innocua* to *J. sabina* and *Ch. lawsoniana* (12.4 and 10.6, respectively). Against *C. xerosis*, we observed growth inhibition zone of 6.4 and 6.8 mm produced by alcohol extracts of *Ch. lawsoniana* and *P. menziesii* and 10.6 and 9.7 mm by *J. sabina* and *Ch. lawsoniana* (9.3 mm during impact of azithromycin in the control). Bacteria of *C. jejuni* were highly susceptible only to ethanol extract of *C. harringtonia* (11.4). High antibacterial effect of the extracts from *J. sabina*, *Ch. lawsoniana* and *P. menziesii* was exerted against *Rhodococcus equi* (27.5, 14.3 and 8.7) during moderate exposure to ethanol extract of *C. harringtonia* (4.4). Bacteria of *P. aeruginosa* were highly sensitive to ethanol extract from *J. sabina* (10.5), moderately sensitive to *Ch. lawsoniana* (6.4 mm). We should note antibiotic resistance of the studied strains of *L. monocytogenes*, *C. jejuni*, *P. vulgaris*, *P. mirabilis*, *P. aeruginosa*, *K. pneumoniae* and *S. marcescens* to azithromycin. Extract from *C. harringtonia* exerted high inhibiting action towards *C. albicans* (9.8). Compared to amphotericin, higher growth inhibition zones were also produced by ethanol extracts from *Ch. lawsoniana* and *P. menziesii* (3.7 and 3.4 mm, respectively).

Discussion

Research has been conducted on species of gymnosperm plants, which apart from antibacterial and antifungicidial activity are also charac-

terized by cytostatic properties against different types of the tumour tissues. Shokrzadeh et al. (2009), Janar et al. (2012) and Huyan et al. (2016) noted moderate inhibiting activity of diterpenoids of *J. sabina* towards five species of human tumour cells. Zhao et al. (2015) determined anti-inflammatory effect of shoots and leaves of *J. sabina* due to flavone glycoside isoscutellarein 7-O-β-D- rhamnopyranosyl -(1→3)-α-L- xylopyranoside. Analgesic and anti-inflammatory effects of general flavanoids from leaves of *J. sabina* have been proven on rodents (Zhao et al., 2018). Abdel-Kader et al. (2019) determined the hepatoprotective activity of *J. sabina*, which was assessed by biochemical parameters of blood serum of rats, such as aspartate-aminotransferase (AST), alanine aminotrasferase (ALT), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP) and total bilirubin. Khani et al. (2017) and Elisoveckaya & Brindza (2018) determined that essential oil from *J. sabina* has insecticidal, antifeedant, deterrent and repellent properties. Results of the studies by Živić et al. (2019) and Semerdjieva et al. (2019) allow one to state that ethanol extract of *J. communis* has a high antioxidant activity due to the content of total phenol compounds and total amount of flavonoids.

Table 2

Width of the growth inhibition zone (mm) demonstrated by the extracts from *Juniperus sabina*, *Chamaecyparis lawsoniana*, *Pseudotsuga menziesii* and *Cephalotaxus harringtonia* against 24 strains of microorganisms (n = 12)

Strains of microorganisms	*Juniperus sabina*	*Chamaecyparis lawsoniana*	*Pseudotsuga menziesii*	*Cephalotaxus harringtonia*	Control*
Enterococcus faecalis ATCC 19433	4.3 ± 0.65	0 ± 0	1.1 ± 0.23	0 ± 0	23.9 ± 2.45
Enterobacter aerogenes ATCC 10006	0 ± 0	0 ± 0	0 ± 0	0 ± 0	15.9 ± 1.67
Escherichia coli F50	4.8 ± 0.35	8.8 ± 0.67	4.3 ± 0.34	2.7 ± 0.21	17.8 ± 1.87
E. coli 055	4.5 ± 0.34	9.3 ± 0.56	3.7 ± 0.42	10.7 ± 1.45	15.6 ± 1.62
Staphylococcus aureus ATCC 25923	11.5 ± 0.64	4.4 ± 0.32	4.1 ± 0.33	21.2 ± 2.41	21.6 ± 2.45
S. epidermidis ATCC 14990	0 ± 0	0 ± 0	0 ± 0	13.4 ± 1.45	10.3 ± 1.34
Bacillus subtilis ATCC 6633	8.4 ± 1.12	10.6 ± 1.55	0 ± 0	0 ± 0	30.3 ± 3.05
B. cereus ATCC 10702	14.2 ± 1.87	1.6 ± 0.32	0 ± 0	5.7 ± 0.77	16.8 ± 1.86
Listeria ivanovi	2.3 ± 0.43	0 ± 0	0 ± 0	9.3 ± 0.76	14.7 ± 1.21
L. innocua ATCC 33090	12.4 ± 1.55	10.6 ± 1.18	0 ± 0	0 ± 0	25.1 ± 1.98
L. monocytogenes ATCC 19112	0 ± 0	0 ± 0	0 ± 0	8.1 ± 0.87	0 ± 0
Corynebacterium xerosis 1911	10.6 ± 0.56	6.4 ± 0.43	6.8 ± 0.57	9.7 ± 0.89	9.3 ± 1.34
Campylobacter jejuni ATCC 11322	0 ± 0	0 ± 0	0 ± 0	11.4 ± 1.12	0 ± 0
Rhodococcus equi ATCC 6939	27.5 ± 2.89	14.3 ± 1.54	8.7 ± 0.83	4.4 ± 0.42	19.1 ± 1.98
Proteus vulgaris ATCC 13315	2.8 ± 0.54	5.1 ± 0.43	0 ± 0	8.3 ± 0.87	0 ± 0
P. mirabilis ATCC 14153	2.1 ± 0.23	0 ± 0	10.5 ± 0.93	0 ± 0	0 ± 0
Salmonella typhimurium ATCC 14028	3.4 ± 0.21	2.2 ± 0.13	1.9 ± 0.13	0 ± 0	20.3 ± 1.54
S. adobraco 1	2.7 ± 0.19	2.7 ± 0.39	1.4 ± 0.12	0 ± 0	26.3 ± 2.76
Pseudomonas aeruginosa ATCC 2353	10.5 ± 0.98	0 ± 0	0 ± 0	0 ± 0	0 ± 0
P. aeruginosa ATCC 2799	0 ± 0	6.4 ± 0.78	0 ± 0	0 ± 0	0 ± 0
Klebsiella pneumoniae ATCC 13883	2.5 ± 0.21	1.7 ± 0.12	0 ± 0	0 ± 0	0 ± 0
Yersinia enterocolitica ATCC 9610	2.5 ± 0.22	0 ± 0	0 ± 0	0 ± 0	12.8 ± 1.27
Serratia marcescens ATCC 8100	1.2 ± 0.23	0 ± 0	2.8 ± 0.23	10.4 ± 0.94	0 ± 0
Candida albicans ATCC 2091	2.2 ± 0.41	3.7 ± 0.34	3.4 ± 0.31	9.8 ± 0.92	0 ± 0* 2.4 ± 0.21**

Note: * – discs with 15.0 μg of azithromycin were used for all bacteria as positive control; ** – discs with 15.0 μg amphotericinin were used as positive control for *Candida albicans* (Valle et al., 2015).

Palá-Paúl et al. (2015) researched *Ch. lawsoniana*. They showed that the essential oils from young shoots and leaves of *Ch. lawsoniana* exert high antibacterial and antifungicidal activity towards *Candida albicans*, *Bacillus subtilis*, *Staphylococcus aureus* and *Micrococcus luteus*: essential oil was more effective against gram-positive rather than gram-negative bacteria. Recently, interest has developed to the use of essential oils from shoots of *Ch. lawsoniana* in commercial products, such as preparations against pests and cosmetics (Duringer et al., 2015). To assess the relative toxicological risk from these oils for fresh-water and marine organisms, acute aquatic toxicity of these oils was assessed using the planktonic crustacean *Daphnia magna* Straus, rainbow trout *Oncorhynchus mykiss* (Walbaum) and microalga *Raphidocelis subcapitata* (Korshikov) Nygaard, Komárek, J. Kristiansen & O. M. Skulberg. For the oils from leaves of *Ch. lawsoniana*, toxicity against *D. magna* or *O. mykiss* was not observed even in 5.0 mg/L (the highest tested concentration and the threshold of solubility). Research on toxicity against *R. subcapitata* using density of cells of algae showed that the value of EC_{50} over 72 and 96 h equaled 1.7 mg/L, and the concentration without observed effect (NOEC) was 0.63 mg/L. Forty-eight h EC_{50} for *D. magna* equaled 1.9 mg/L; value of NOEC for density of the cells of algae equaled 1.25 (72 h) and 0.63 mg/L (96 h). The emergence of antibiotic-resistant bacteria has caused difficulties in the treatment of infectious diseases (Kim et al., 2015). Methicillin-resistant *Staphylococcus aureus* is one of the commonest recognized antibiotic-resistant bacteria. New antibiotics for the treatment of MRSA in humans are urgently needed. Raw materials obtained from natural sources can be used for the development of new antibiotics, for example *Chamaecyparis obtusa* (Siebold & Zucc.) Endl., which is traditionally used for treating asthma. Kim et al. (2015) studied antibacterial activity of essential oil from leaves of *Ch. obtusa* against MRSA. Growth of MRSA and production of acid during metabolism of glucose was inhibited in *Staphylococcus* by the concentration of 0.1 mg/mL of *Ch. obtusa*. Smith et al. (2007) showed that within the framework of a project aimed at characterizing the antibacterial or immune-modulating activity of compounds of immature conifer cones against multi-drug resistance (MDR) strains of *S. aureus*, eight compounds were extracted from *Ch. lawsoniana*. Active compounds are mostly diterpenes with minimum inhibitory concentrations equaling 4–128 μg/mL against strains of *S. aureus* which are resistant to methicillin (EMRSA). Out of *Ch. lawsoniana*, diterpene ferruginol, pisiferol and its epimer 5-epipisiferol, formazan oxide, transcommunic acid and torulosal, sesquiterpenic oplopanonil acetate and germacrane 4β-hydroxigermacra-1(10)-5-dien were obtained. Some of these compounds also exerted modulating activity in potentiation of antibiotic activity against efflux strains, and ferruginol used in sub-inhibiting concentration caused 80-fold increase in the activity of oxacillin against strain of EMRSA-15. Yang et al. (2007) determined that essential oil from leaves of *Ch. obtusa* has relatively strong antibacterial activity against gram-positive bacteria and some fungi.

Massicotte et al. (1992) determined that tubers on the roots of *P. menziesii* are present as ectomycorrhizal formations. Spectroscopy showed that the crystals found in the zone of loose hyphae of fungi perhaps contain calcium oxalate. Bacteria locate either in the hyphae of fungi in the bark of roots, or as colonies on the surface. These results allow us to assume that the roots of plants can be a valuable resource, because they contain a large amount of anti-fungi and allelopathc compounds (Massicotte et al., 1992).

Palá-Paúl et al. (2015) report new data on antibacterial and antifungal activity of essential oil from *Ch. lawsoniana*. Leaves of juniper are practically safe for aquatic organisms (Duringer et al., 2015). Cedar oil did not exhibit toxicity against *Oncorhynchus mykiss* (Walbaum) or

Selenastrum capricornutum Printz even in 0.5 mg/L (the highest tested concentration and threshold of solubility). Kim (2015) determined formation of biofilm of MRSA using a scanning microscopy and staining with c safranin. *Chamaecyparis obtusa* (Siebold & Zucc.) Endl. Inhibited the formation of biofilm of MRSA in concentrations of over 0.1 mg/mL. These data indicate that *Ch. obtusa* has antibacterial effect against MRSA, which could be associated with such components of the plant tissues as sabinene (19.1%), α-terpinyl acetate (17.0%), bornyl acetate (10.5%), limonene (8.5%), elemol (7.5%), myrcene (5.9%), γ-terpinene (4,0%) and hibaene (3.0%). Lee (2009) and Morikawa et al. (2012) studied the bioactivity of extracts of the pith of a branch of *Ch. obtusa* and compared it to the extracts from the pith of the trunk: antifungal activity was studied towards four species of fungi (*Trametes versicolor* (L.) Lloyd, *Fomitopsis palustris* (Berk. & M. A. Curtis) Gilb. & Ryvarden, *Trichoderma virens* (J. H. Mill. et al.) Arx and *Rhizopus oryzae* Went & H. C. Prinsen Geerligs) and antagonistic activity against aquatic crustaceans *Artemia salina* (L.).

Watanabe & Fukao (2009) determined that *C. harringtonia* exerted a broad anti-tumour activity in rodents and anti-leukemia effect in people. They discovered that the substances from the plants are metabolized to the acidic product HHT-acid (2'-hydroxy-2'-(α-acetic acid)-6'-hydroxy-6'-methylheptanoil cephalotaxine) during *in vitro* incubation with human or mice blood plasma. Concentration of HHT which inhibited by 50% growth of human leukemia cells HL-60 was 20 ng/mL, whereas for HHT-acid it equaled 14,500 ng/mL (acidic form was more than 700 times less cytotoxic than HHT). LD_{50} for HHT equaled 6.7 mg/kg, at the same time HHT-acid exhibited no notable toxic effects even in the doses of 280 mg/kg.

Extract from immature fruits of *C. harringtonia* is most efficient in inhibiting the growth of *Bacillus cereus* and *Leuconostoc mesenteroides* (Tsenkovskii) van Tieghem, its antibacterial activity was tested against 22 species of gram-positive, 7 species of gram-negative bacteria and 13 species of fungi. Extract from *C. harringtonia* exerted antibacterial activity towards gram-positive bacteria with minimum inhibiting concentrations (MIC) of 25–200 μg/mL in agar broth and 5–40 μg/mL in liquid broth, antimicrobial effect was higher in acidic and alkaline media than in neutral conditions (Watanabe & Fukao, 2009).

From leaves of *C. harringtonia* f. *fastigiata*, Morita et al. (2000, 2010) a new alkaloid cephastigiamide A was extracted and its structure was studied using two-dimensional NMR spectroscopy and chemical degradation. Harringtonin, desoxyharringtonin and homodesoxyharringtonin demonstrated notable antiplasmatic action towards *Plasmodium falciparum* Welch, but not towards *Leishmania major* Yakimoff et Schokhor. Six new alkaloids (cephalezomines A–F) were isolated together with ten already known alkaloids from leaves of *C. harringtonia* var. *nana*; cephalezomines A–F displayed potent cytotoxicity against tumour cells (Morita et al., 2010, 2012).

Takano et al. (2010) and Dwivedi et al. (2015) focused on infections associated with biomaterials. Microbial adhesion on implants caused formation of biofilm, which ultimately led to damage to the implants. Therefore, for effective treatment against biofilms of *S. aureus*, which are responsible for most infections associated with biomaterials, these authors propose using silver nanoparticles and extracts from leaves of *Pseudotsuga menziesii*.

From seeds of *C. harringtonia*, Politi et al. (2003) extracted six diterpenoids (8β-hydroxi-9(11),13- abietadien -12-one and 5,6-didehydroferruginol, ferruginol, sugiol, 6,12-dihydroxiabieta-5,8,11,13-tetraene-7-one and abieta-8,11,13-trien-7β-ol). They conducted *in vitro* studies on these compounds against clinically isolated bacteria and strains of *Candida*. Ferruginol and 6,12-dihydroxiabieta-5,8,11,13-tetraene-7-one exerted antimicrobial activity against several gram-positive bacteria. None of the six diterpenes was active against gram-negative organisms and tested species of yeasts.

Evanno et al. (2008) observed significant antifungal activity of harringtonolide – a complex polycyclic condensed norditerpene extracted from *C. harringtonia* var. *drupacea*. Ram & Kumari (2001) also confirmed anti-tumour activity of *C. harringtonia*.

Smith et al. (2008) extracted a new abietan diterpene, from the bark of the stem of *Prumnopitys andina* (Poepp. ex Endl.) de Laub. This new compound presented antibacterial activity in 8 μg/mL against two strains of *S. aureus*, but, it is interesting that it was not active in 128 μg/mL against a strain of wild-type and Methicillin-resistant *S. aureus*. Smith et al. (2008) note that ferruginol was active against these four strains of *S. aureus*. Presence of acetoxy group caused harmful effect on the antibacterial activity towards certain strains.

The antibacterial in vitro effect which we observed from *J. sabina* exceeded a 8 mm wide zone of inhibition of growth of 7 species of bacteria (*S. aureus*, *B subtilis*, *B. cereus*, *L. innocua*, *C. xerosis*, *Rh. equi* and *P. aeruginosa*), *Ch. lawsoniana* had the same effect against 5 species (*E. coli*, *B. subtilis*, *L. innocua* and *Rh. equi*), *P. menziesii* – only against 2 species (*Rh. equi* and *P. mirabilis*) and *C. harringtonia* – against 10 species of microorganisms (*E. coli*, *S. aureus*, *S. epidermidis*, *L. ivanovi*, *L. monocytogenes*, *C. xerosis*, *C. jejuni*, *P. vulgaris*, *S. marcescens* and *C. albicans*). Further detailed study of individual chemical substances present in those plants should be undertaken in both *in vitro* and *in vivo* experiments.

Conclusion

None of the four species of gymnosperms produced notable (growth inhibition zone of over 8 mm) effect towards *E. faecalis*, *E. aerogenes*, *S. typhimurium*, *S. adobraco*, *K. pneumoniae* and *Y. enterocolitica*. Negative effect (growth inhibition zone of over 8 mm) on *E. coli* was exerted by alcohol extracts of *Ch. lawsoniana* and *C. harringtonia*, extracts of *J. sabina* and *C. harringtonia* took effect on *S. aureus*, only *C. harringtonia* affected *S. epidermidis*, against *B. subtilis* – *J. sabina* and *Ch. lawsoniana* were effective, against *B. cereus* – only *J. sabina*, against *L. ivanovi* – only *C. harringtonia*, against *L. innocua* – *J. sabina* and *Ch. lawsoniana*, against *L. monocytogenes* – only *C. harringtonia*, against *C. xerosis* – *J. sabina* and *C. harringtonia*, against *C. jejuni* – only *C. harringtonia*, against *Rh. equi* – *J. sabina*, *Ch. lawsoniana* and *P. menziesii*, against *P. vulgaris* – only *C. harringtonia*, against *P. mirabilis* – only *P. menziesii*, against *P. aeruginosa* – only *J. sabina*, against *S. marcescens* – only *C. harringtonia*, and against *C. albicans* only alcohol extract of *C. harringtonia* had negative effect. We consider that it is practical to recommend ethanol extracts from *J. sabina*, *Ch. lawsoniana*, *P. menziesii* and *C. harringtonia*, or individual compounds present in them, to be used in further studies on treatment against poly-resistant strains of the abovementioned microorganisms.

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