DOI: 10.2428/ecea.2020.27(1-2)9 ECOL CHEM ENG A. 2020;27(1-2)

Bartosz RYŁ[1], Monika SPOREK[2]
and Teresa KRZYŚKO-ŁUPICKA[3*]

THE BACTERICIDAL EFFECT OF EXTRACTS FROM *Humulus lupulus* L MARYNKA VARIETY ON SELECTED BACTERIA

BAKTERIOBÓJCZE DZIAŁANIE EKSTRAKTÓW Z *Humulus lupulus* L ODMIANY MARYNKA NA WYBRANE BAKTERIE

Abstract: The aim of the research was to obtain extracts of *Humulus lupulus* L hop cones of the Marynka variety and to evaluate their antibacterial properties against three strains of gram-negative bacteria (*Escherichia coli* ATCC 10536, *E. coli* IPS and *Pseudomonas aeruginosa* ATCC 27853) and against two strains of gram-positive bacteria (*Enterococcus hirae* ATCC 10541 and *Bacillus cereus* ATCC 12826).

The research material consisted of dried Marynka variety hop cones, from which extracts in the form essential oil (EO), infusion and decoction were obtained. EO was obtained in the process of hydrodistillation. The antibacterial activity of the extracts was evaluated by the plate-cylinder diffusion method against the tested gram-negative and gram-positive bacteria.

Diverse antibacterial activity of water extracts of *H. lupulus* L hop cones of the Marynka variety (essential oil, infusion and decoction) on the tested bacteria was demonstrated, with higher sensitivity of gram-positive than gram-negative bacteria. The highest resistance to hop extracts was demonstrated by *P. aeruginosa* ATCC 27853. The best antibacterial properties were demonstrated by the essential oil at a concentration of 2.0 %. In the group of gram-positive bacteria, *E. hirae* ATCC 10541 was more sensitive to EO, and *E. coli* IPS in the group of gram-negative bacteria. In the case of *B. cereus* ATCC 12826, the sensitivity to infusions and decoctions of hop cones requires further investigation, as the obtained large zones of growth inhibition were unstable.

Keywords: hop (*Humulus lupulus*), Marynka variety, bactericidal properties, hydrodistillation of essential oil in Deryng apparatus, decoction, infusion

[1] Scientific Circle of Biotechnologists UO.

[2] Institute of Biology, Faculty of Natural and Technical Science, University of Opole, ul. kard. B. Kominka 6a, 45-035 Opole, Poland.

[3] Institute of Environmental Engineering and Biotechnology, Faculty of Natural and Technical Science, University of Opole, ul. kard. B. Kominka 6a, 45-035 Opole, Poland.

* Corresponding author: teresak@uni.opole.pl

Introduction

Research on application of plant extracts in fighting microorganisms has been carried out for years. Recently, more and more attention has been paid to this subject due to the increase of antibiotic resistance among microorganisms as well as the growing demand for "green chemicals" – cleaning agents, preservatives and biopesticides of natural origin.

Hop, a plant commonly used in brewing not only for its characteristic bitterness and aroma but also for increasing the shelf life of beer, has become the focus of our attention. Ungroundbeer beer quickly lost its freshness and as a result became sour and unfit for consumption, so it can be concluded that in the process of brewing the wort with hops, compounds with antimicrobial activity (acting as a biological preservative) were formed. However, not all hop varieties have such an effect. Out of three known hop species such as *Humulus lupulus* L., *H. japonicus* and *H. yunnanensis,* only extracts from hop cones of the species *H. lupulus* L. are characterized by a broad spectrum of biological activity, whereas the other two species do not have such properties due to the lack of lupulin glands.

Common hop *H. lupulus L*. is a perennial, dioecious climbing plant belonging to the family Cannabaceae and order Rosales, cultivated in all temperate regions of the world [1, 2]. This species occurs in different varieties e.g. Agnus, Ariana, Callista, Lubelski, Magnum, Marynka, Saaz and Vital [3–5]. From the point of view of the herbal and brewing industry, only the inflorescences of female plants, formed by green overlapping scales forming the hop cone, are of interest.

Scientific reports indicate that *H. lupulus* L. extracts *in vitro* can inhibit to varying degrees the growth of bacteria of the genera: *Staphylococcus, Streptococcus, Bacillus, Listeria, Propionibacterium, Helicobacter, Escherichia, Salmonella, Pseudomona*s, *Treponema* [3, 5–11]. However, the antimicrobial activity depends not only on the sensitivity of the bacterial species tested, but is also related to the chemical composition of hop extracts. Hop cones contain essential oils and bitter α- and β-acids, as well as – in smaller amounts – unique prenylflavonoids, mainly in the form of xanthohumol, 8-prenylnaringenin and ferulic acid [5, 12, 13].

Compounds with specific antimicrobial properties include: prenylflavonoids (xanthohumol and 6-prenylnaringenin), flavan-3-ols, flavonols, ferulic acid, tannins and α- and β-acids, sesquiterpenes – α-caryophyllene and β-humulene and monoterpenes – myrcene, limonene and β-pinene [5, 7, 12]. The chemical composition of Marynka and Lubelski variety hop cones is presented in Table 1.

The amount of chemical compounds in hop cones may vary due to the weather and soil conditions of crops, type of hops, drying method, time and storage parameters of plant material [4, 5], as well as due to extraction methods and pH of the environment in which the bactericidal activity of the obtained extracts is examinated [15]. *H. lupulus* hop, both fresh and dry, of different varieties, is used in the research in the form of aqueous, alcoholic or aqueous-alcoholic extracts obtained by extraction with supercritical carbon dioxide or extraction in a Soxhlet apparatus with hexane [5, 7, 10, 16, 17]. For the sake of environmental safety, it is necessary to focus on the extraction

Table 1

Chemical composition of *Humulus lupulus* L hop cones of Lubelski and Marynka hop varieties [14]

Component	Hop varieties	
	Lubelski	Marynka
α-acids [%]	4.0	11.1
Cohumulone in α-acids [% w/w]	31.8	25.3
Oil content [% v/w]	1.2	2.3
Myrcene [% v/w]	38.8	37.6
Kariofilen [% v/w]	7.6	8.0
Humulene [% v/w]	38.5	39.7
Linalool [% v/w]	0.58	0.36
α-Terpinol [% v/w]	0.27	0.5
Geraniol [% v/w]	0.19	0.12
Humulol + humulenol-2 [% v/w]	0.7	0.66
Caryophyllene and humulene oxides [% v/w]	2.44	0.98

of active compounds using economically attractive methods, as well as not requiring the use of organic solvents. Due to, it will be possible to implement preparations obtained in this way in the food industry, special purpose household chemicals, organic farming or animal husbandry. The use of aqueous extraction techniques also limits the potentially harmful effects of organic solvents on the environment and fits perfectly into the current trend of "clean", environmentally friendly technologies.

The selection of methods to assess the antibacterial properties of hop cone extracts is also important. In practice, many methods are used to assess the in vitro biocidal properties of chemical compounds, both on liquid and solid culture media, and one of the most popular is the diffusion method [5, 18, 19].

The aim of this study was to obtain aqueous extracts from the *Humulus lupulus* hops of the Marynka variety and to evaluate their antibacterial activity against gram-negative (*Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 10536 and *Escherichia coli* IPS) and gram-positive (*Bacillus cereus* ATCC 12826 and *Enterococcus hirae* ATCC 10541) bacteria.

Research methods and material

The research material consisted of dried *Humulus lupulus* hops of the Marynka variety, in vacuum-packed sachets, from the 2019 harvest. The essential oil (EO) as well as the infusion (HI) and decoction (HD) were obtained from hop cones by the hydro-distillation process in a Deryng apparatus.

The microbiological material consisted of five bacterial strains – two gram-positive bacteria (*Bacillus cereus* ATCC 12826 and *Enterococcus hirae* ATCC 10541) and three gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 27853, E*scherichia coli* ATCC 10536 and *Escherichia coli* IPS, which were isolated from the food processing equipment.

The following extracts were obtained from the *Humulus lupulus* L hop cones of the Marynka variety:

a. The essential oil (EO) obtained by hydro-distillation in a Deryng apparatus (Fig. 1). The distillation was carried out three times. Each time about 50 g of hop cones were placed in a flask and poured over with enough distilled water to cover the plant material (500 cm^3). The flask was assembled with the cap of the Deryng apparatus, placed in the heating mantle and the cold water circuit of the cooler was switched on. The entire hydro-distillation process was carried out for 1.5 hours after contents in the flask started boiling. From 100 g of dried Marynka variety hop cones, 1.20 cm^3 of oil was obtained (Fig. 2), which was stored in a chiller at 4 °C.

b. The decoction (HD) was obtained by pouring 200 cm^3 of tap water into 10 g of hop cones and boiling the mixture under cover. Then, under sterile conditions, the hop cones were separated from the resulting decoction which was allowed to cool.

c. An infusion (HI) was prepared by pouring 200 cm^3 of hot (90 °C) tap water onto 10 g of hop cones. The whole mixture was left covered for 15 minutes, then the plant material was separated from the infusion which was allowed to cool.

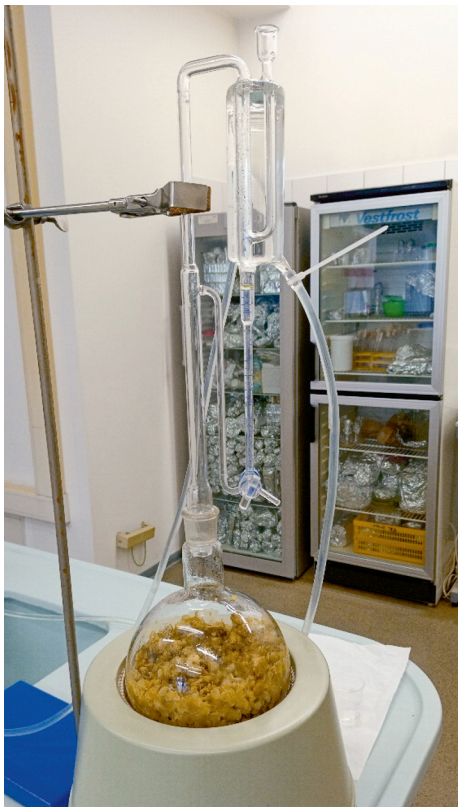

Fig. 1. Hydrodistillation on hop cones in the Deryng apparatus

Fig. 2. Hop cone essential oil in an Eppendorf tube

Evaluation of the antibacterial properties of the hop cone extracts by the plate-cylinder diffusion method [5, 18–21]

The evaluation of the antibacterial activity of hop cone essential oil solutions at concentrations of 0.5; 1.0 and 2.0 %, as well as infusion and decoction in an undiluted (U) and diluted (in boileg water) form (at ratios of 3:1 and 1:2) on the growth of the tested bacterial strains, was carried out in Nutrient Lab-Agar^{TM} culture medium using the plate-cylinder diffusion method. This method is based on the principle of diffusion of substances in agar and measurement of the inhibition zone of micro-organism growth around the formed wells after the incubation period. The culture media were inoculated with 1 cm^3 of a standardized bacterial suspension with an optical density of $\xi = 1$ at $\lambda = 460$ nm. The samples were incubated for 48 h at 35 ± 2 °C. The results were reported as the mean value of the growth inhibition diameter [mm ± *SD*] (*SD* – standard deviation). The *inhibition effect* was assumed to be the lack of growth around the wells, *stimulation* – stronger growth around the wells, and the *neutral* effect – to stop growth at the wells boundary. The control sample (C) was 0.05 % Tween 80. Each experiment was repeated 4 times.

Results and discussion

Common hop (*Humulus lupulus* L) extracts contain bioactive compounds, witch determinates, among others antimicrobial properties of both essential oil and hop cone infusions [22]. The degree of bacterial growth inhibition depends both on the variety of hops and on the concentration and chemical composition associated with the methods of obtaining extracts [5, 12, 13].

In our research, bioactive substances from dry hops of the Marynka variety (Fig. 3) were obtained in the form of infusions (HI), decoctions (HD) and essential oil (EO) by

Fig. 3. Dry hop cones of the Marynka variety

hydro-distillation in the Deryng apparatus (Fig. 1). This is a common extraction method to separate phyto-chemical compounds from plant material, although in recent years the usefulness of new methods, using microwaves and extraction in supercritical CO_2, has been tested [7, 23]. However, according to Kobus-Cisowska at al. [5], extraction with water at elevated temperature allows the release of active compounds with biocidal properties, occurring in hop cones. These are often associated with the presence of xanthohumol, the main prenylated flavonoid present in hops [24, 25], which may favour the transport of flavonoids into the cell or facilitate the enzymes blockade by binding to the active centre and ultimately lead to cell damage [5].

The obtained extracts with a citrus-resin scent, more intense in the case of EO, were used to evaluate the antibacterial properties against selected gram-positive bacteria (*Bacillus cereus* ATCC 12826 and *Enterococcus hirae* ATCC 10541) and gram--negative bacteria (*E. coli* ATCC 10536, *Pseudomonas aeruginosa* ATCC 27853 and *E. coli* IPS). As shown in this study, the application of the plate-cylinder diffusion method, also used by other researchers [5, 19–21], made it possible to demonstrate that, regardless of the species and origin of the tested bacteria, the degree of inhibition of their growth (except for *P. aeruginosa* ATCC 27853) depended on the form and concentration of the extract. The exception was *P. aeruginosa* ATCC 27853, the growth of which was not inhibited by any form of the extract, but growth stimulation (11.82 ± 0.75 mm) was recorded in the presence of undiluted decoction (Table 2). High resistance of the *Pseudomonas aeruginosa* species to extracts from hop cones was also pointed out by Rozalski et al. [26], although Bocquet et al. [11] showed a slight antibacterial activity of the aqueous-alcoholic extract from hop rhizomes (MIC value = 1500 μg/ml).

Table 2

Zones of inhibition of bacterial growth [mm]

Bacterial species/ Extract types	Gram-positive bacteria		Gram-negative bacteria		
	Bacillus cereus ATCC 12826	*Enterococcus hirae* ATCC 10541	*E. coli* ATTC 10536	*E. coli* IPS	*Pseudomonas aeruginosa* ATCC 27853
Essential oil [%]					
C	0	0	0	0	0
0.5	0	0	0	0	0
1	0	0	0	0	0
2	14.67 ± 1.51	20.33 ± 1.63	13.83 ± 0.75	15.17 ± 0.41	0
Infusion [dilution ratio]					
C	0	0	0	0	0
1:2	24.5 ± 0.84	0	0	0	0
3:1	28 ± 0.63	0	0	0	0
U	29 ± 1.26	0	0	15 ± 3.16	0

Table 2 contd.

Bacterial species/ Extract types	Gram-positive bacteria		Gram-negative bacteria		
	Bacillus cereus ATCC 12826	*Enterococcus hirae* ATCC 10541	*E. coli* ATTC 10536	*E. coli* IPS	*Pseudomonas aeruginosa* ATCC 27853
Decoction [dilution ratio]					
C	0	0	0	0	0
1:2	23.67 ± 0.52	0	0	0	0
3:1	26.83 ± 1.17	0	0	0	0
U	28.83 ± 1.33	12.67 ± 0.52	13.67 ± 0.52	19.17 ± 1.17	S*11.82 ± 0.75

± Standard deviation (*SD*).
* Stimulation of bacterial growth [mm].

In the group of gram-negative bacteria (with exception of the *P. aeruginosa* ATCC 27853), EO only at the highest tested concentration (2.0 %) inhibited the growth of the environmental isolate *E. coli* IPS to a greater extent than from the ATCC collection, and the zones of growth inhibition were 15.17 ± 0.41 mm and 13.83 ± 0.75 mm, respectively (Table 2, Fig. 4). The tested bacteria showed a different reaction only to undiluted infusion and decoction of hop cones. In this group of bacteria, only the environmental isolate *E. coli* IPS showed sensitivity to undiluted infusion, and the zone of growth inhibition was 15.00 ± 3.16 mm. A broader spectrum of bactericidal activity compared to the infusions was shown by the decoction, but only its undiluted form also

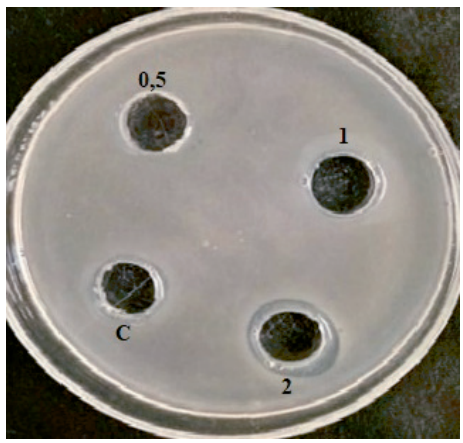

Fig. 4. Growth inhibition zones of *E. coli* ATCC 10536 against the essential oil (EO) of hop cones cv. Marynka;
C – control sample, 0.5 – EO concentration 0.5 %, 1 – EO concentration 1.0 %, 2 – EO concentration 2.0 %

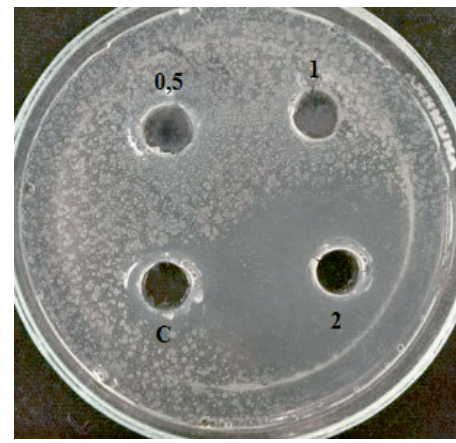

Fig. 5. Growth inhibition zones of *E. hirae* ATCC 10541 against the essential oil (EO) of hop cones cv. Marynka
C – control sample, 0.5 – EO concentration 0.5 %, 1 – EO concentration 1.0 %, 2 – EO concentration 2.0 %

inhibited the growth of *E. coli* IPS to a greater extent (19.17 ± 1.17 mm) than *E. coli* ATCC 10536 (13.67 ± 0.5 mm).

However, many researchers include gram-negative *E. coli* bacteria among the strains highly resistant to hop extracts and chemical compounds isolated from hop cones [7, 8], but one of the reasons for the discrepancy in the results may be the so-called strain sensitivity within the *E. coli* species, as well as the pH of the environment in which the antibacterial activity is tested. Extracts containing α- and β-acids showed no antibacterial activity against *E. coli* at pH = 7.2 ± 0.1 (MIC > 5000 ppm), while at pH 5.0 ± 0.1 regulated with citric acid, the MIC values were 1250 ppm for β-acid extract and 2500 ppm for α-acid extract. In contrast, at an environmental pH also equal to 5.0 ± 0.1, but regulated by lactic acid, MIC values were lower [15].

In the group of gram-positive bacteria, higher susceptibility to the tested extracts from hop cones was demonstrated by the *E. hirae* ATCC 1054 coccus, as permanent zones of growth inhibition were obtained in the presence of 2.0 % EO (20.33 ± 1.63 mm) (Fig. 5) and undiluted decoction (12.67 ± 0.52 mm). However, growth inhibition of this bacterium in the presence of infusion was not shown. Thus, as in the case of gram-negative *E. coli*, decoction was more effective. No studies have been found in the literature to determine the effect of hop cone extracts on *E. hirae*. However, various types of hop cone extracts were tested against other species of the genus Enterococcus – the clinical isolate of *E. faecalis* and *E. faecium* ATCC 27270 [5], but the obtained zones of growth inhibition differed from those determined in our research and in the presence of an aqueous extract of Marynka variety hop cones the inhibition zones of these isolates were 2.0 ± 0.0 mm and 1.0 ± 0.0 mm, respectively.

A permanent but smaller zone of growth inhibition was also noted for spore-forming *B. cereus* ATCC 12826 in the presence of 2.0 % EO (inhibition zone 14.67 ± 1.51 mm). On the other hand, in the presence of decoction and infusion, irrespective of the concentration, large inhibition zones were recorded, but after 48 hours their overgrowth was observed (Table 2, Fig. 6, Fig. 7). This phenomenon was not observed by Roj et al. [7] but they did not test aqueous extracts from hop cones, but extracts obtained by the Soxhlet method, by supercritical CO_2 extraction, by supercritical CO_2 extraction isomerised MgO or KOH, and an extract with up to 6.5 % xanthohumol obtained by supercritical CO_2 extraction and 96 % xanthohumol. Antibacterial activity tests of the obtained extracts carried out on strains *Listeria monocytogenes*, *E. coli, Bacillus cereus* environmental isolate, *B. cereus* isolate from animal skin, *Staphylococcus. aureus* MRSA ATCC 43300, *Staphylococcus aureus* ATCC 29213, *Lactobacillus ssp*. and *E. coli* showed that both *B. cereus* isolates were sensitive to each of the six hop extracts and showed the highest sensitivity among all tested bacteria. In addition, the environmental *B. cereus* strain exhibited greater resistance than the strain isolated from animal skin [7].

Summing up, it can be concluded that higher sensitivity to extracts from hop cones characterized the tested gram-positive bacteria than gram-negative ones (Table 2), which is also confirmed by other authors [7, 15].

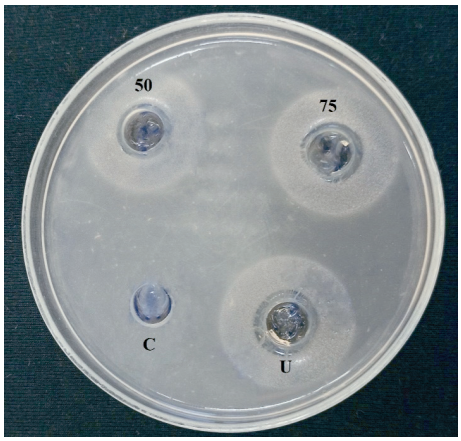

Fig. 6. Growth inhibition zones of *B. cereus* ATCC 12826 against the hop cones infusion (HI) of hops cv. Marynka
C – control sample, 50 – HI dilution ratio 1:2, 75 – HI dilution ratio 3:1, U – HI undiluted

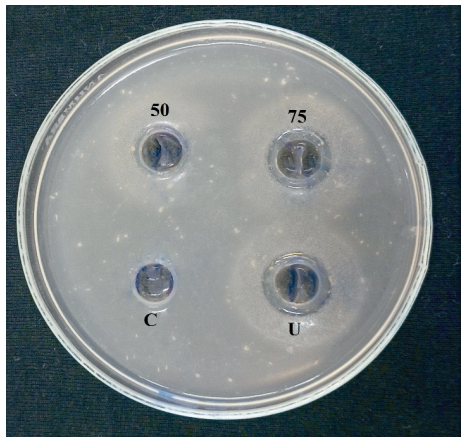

Fig. 7. Growth inhibition zones of *B. cereus* ATCC 12826 against the hop cones decoction (HD) of hops cv. Marynka
C – control sample, 50 – HD dilution ratio 1:2, 75 – HD dilution ratio 3:1, U – HD undiluted

The conducted *in vitro* studies, allow to indicate that extracts from Marynka variety hop cones in an appropriate form and concentration can be used as a potential antibacterial agent, which can help in the fight against the ongoing „antibiotic crisis".

Conclusion

1. Aqueous extracts of *Humulus Lupulus* L Marynka variety hop cones (essential oil, infusion and decoction) showed various antibacterial activity, but the tested gram--positive bacteria were more sensitive to the extracts than gram-negative ones.

2. The best antibacterial properties were shown by essential oil with a concentration of 2.0 %, obtained by hydro-distillation process of hop cones. In the group of gram--positive bacteria, *E. hirae* ATCC 10541 was more sensitive to EO, and in the group of gram-negative bacteria – *E. coli* IPS.

3. The highest resistance to hop extracts was shown by *P. aeruginosa* ATCC 27853.

4. In the case of *B. cereus* ATCC 12826, the sensitivity to infusions and decoctions of hop cones requires further investigation, as the obtained large zones of growth inhibition were unstable.

References

[1] Almaguer C, Schönberger C, Gastl M, Arendt EK, Becker T. Humulus lupulus – A story that begs to be told. A review. J Inst Brew. 2014;120:2"9-314. DOI: 10.1002/jib.160.

[2] Ocvirk M, Nečemer M, Košir IJ. The determination of the geographic origins of hops (Humulus lupulus L.) by multi-elemental fingerprinting. Food Chem. 2019;277:32-7. DOI: 10.1016/j.foodchem.2018.10.070.

[3] Cermak P, Palečková V, Houška M, Strohalm J, Novotná P, Mikyška A, et al. Inhibitory Effects of Fresh Hops on *Helicobacter pylori* strains. Czech J Food Sci. 2015;33:302-7. DOI: 10.17221/261/2014-CJFS.
[4] Gahr A, Forster A, Schuell FA. Comparison of the Hop Varieties Callista and Ariana in Years of Different Climate Conditions 2015 and 2016. Hopfen-Rundschau International 2018/2019. 2018;48-55. Available from: https://www.researchgate.net/publication/329092524.
[5] Kobus-Cisowska J, Szymanowska-Powałowska D, Szczepaniak O, Kmiecik D, Przeor M, Gramza--Michałowska A, et al. Composition and In Vitro Effects of Cultivars of *Humulus lupulus* L. Hops on Cholinesterase Activity and Microbial Growth. Nutrients. 2019;11(6):1377-90. DOI: 10.3390/nu11061377.
[6] Yamaguchi N, Satoh-Yamaguchi K, Ono M. In Vitro Evaluation of Antibacterial, Anticollagenase, and Antioxidant Activities of Hop Components (*Humulus lupulus*) Addressing Acne Vulgaris. Phytomedicine. 2009;16:369-76. DOI: 10.1016/j.phymed.2008.12.021.
[7] Rój E, Tadić VM, Mišić D, žižović I, Arsić I, Dobrzyńska-Inger A, et al. Supercritical Carbon Dioxide Hops Extracts with Antimicrobial Properties. Open Chem. 2015;13:1157-71. DOI: 10.1515/chem-2015-0131.
[8] Stompor M, Żarowska B. Antimicrobial Activity of Xanthohumol and Its Selected Structural Analogues. Molecules. 2016;21(5):608-17. DOI: 10.3390/molecules21050608.
[9] Cermak P, Olšovská J, Mikyška A, Dušek M, Kadleckova Z, Vanicek J, et al. Strong Antimicrobial Activity of Xanthohumol and Other Derivatives from Hops (*Humulus lupulus* L.) on Gut Anaerobic Bacteria. APMIS. 2017;125(11):1033-8. DOI: 10.1111/apm.12747.
[10] Weber N, Biehler K, Schwabe K, Haarhaus B, Quirin KW, Frank U, et al. Hop Extract Acts as an Antioxidant with Antimicrobial Effects against *Propionibacterium Acnes* and *Staphylococcus Aureus*. Molecules. 2019;24(2):223-35. DOI: 10.3390/molecules24020223.
[11] Bocquet L, Sahpaz S, Bonneau N, Beaufay C, Mahieux S, Samaillie J, et al. Phenolic Compounds from *Humulus lupulus* as Natural Antimicrobial Products: New Weapons in the Fight against Methicillin Resistant *Staphylococcus aureus*, *Leishmania mexicana* and *Trypanosoma brucei* Strains. Molecules. 2019;24(6):1024-49. DOI: 10.3390/molecules24061024.
[12] Karabin M, Hudcova T, Jelínek L, Dostálek P. Biologically Active Compounds from Hops and Prospects for Their Use. CRFSFS. 2016;15(3):542-67. DOI: 10.1111/1541-4337.12201.
[13] Knez Hrnčič M, Španinger E, Košir IJ, Knez ž, Bren U. Hop Compounds: Extraction Techniques, Chemical Analyses, Antioxidative, Antimicrobial, and Anticarcinogenic Effects. Nutrients. 2019;11(2):257-93. DOI: 10.3390/nu11020257.
[14] Brudzynki A, Baranowski K. Laboratory and Industrial Scale Brewing Trials with Lubelski, Marynka, Oktawia and Other Polish Hop Varieties. J Inst Brew. 2003;109(2):154-6. DOI: 10.1002/j.2050-0416.2003.tb00146.x.
[15] Kramer B, Thielmann J, Hickisch A, Muranyi P, Wunderich J, Hauser C. Antimicrobial Activity of Hop Extracts Against Foodborne Pathogens for Meat Applications. J Appl Microbiol. 2015;118(3):648-57. DOI: 10.1111/jam.12717.
[16] He GQ, Xiong HP, Chen QH, Ruan H, Wang ZY, Traoré L. Optimization of Conditions for Supercritical Fluid Extraction of Flavonoids from Hops (*Humulus lupulus* L.). J Zhejiang Univ Sci B. 2005;6(10):999-1004. DOI: 10.1631/jzus.2005.B0999.
[17] Zekovic Z, Petrović L, Pfap-Sovijanski I, Grujic O. Supercritical fluid extraction of hops. J Serb Chem Soc. 2007;72(1):81-7. DOI: 10.2298/JSC0701081Z.
[18] Johnson OO, Ayoola GA, Adenipekun T. Antimicrobial Activity and the Chemical Composition of the Volatile Oil Blend from *Allium sativum* (Garlic Clove) and *Citrus reticulata* (Tangerine Fruit). Int J Pharm Sci Drug Res. 2013;5(4):187-93. Available from: https://ijpsdr.com/index.php/ijpsdr/article/view/284
[19] Białoń M, Krzyśko-Łupicka T, Nowakowska-Bogdan E, Wieczorek P. Chemical Composition of Two Dierent Lavender Essential Oils and Their Effect on Facial Skin Microbiota. Molecules. 2019;24(18):3270-86. DOI: 10.3390/molecules24183270.
[20] Krzyśko-Łupicka T, Mysłek M, Błaszczyk K. Sensitivity to the essential oils of environmental, resistant to drugs strains of *Escherichia coli.* Proc ECOpole. 2015;9(5):633-9. DOI: 10.2429/proc.2015.9(2)074.
[21] Di Lododvico S, Menghini L, Ferrante C, Recchia E, Castro-Amorim J, Gameiro P, et al. Hop Extract: An Efficacious Antimicrobial and Anti-biofilm Agent Against Multidrug-Resistant Staphylococci Strains and *Cutibacterium acnes*. Front Microbiol. 2020;11:1852-63. DOI: 10.3389/fmicb.2020.01852.

[22] Craig WJ. The Therapeutic Use and Safety of Common Herbal Beverages BT. In: Wilson, T, Temple NJ, editors. Beverages in Nutrition and Health. Totowa, NJ, USA: Humana Press; 2004. ISBN: 978-1-59259-415-3.

[23] Périno-Issartier S, Zill-e-Huma, Abert-Vian M, Chemat F. Solvent Free Microwave-Assisted Extraction of Antioxidants from Sea Buckthorn (*Hippophae rhamnoides*) Food By-Products. Food Bioprocess Technol. 2011;4:1020-8. DOI: 10.1007/s11947-010-0438-x.

[24] Stevens JF, Taylor AW, Nickerson GB, Ivancic M, Henning J, Haunold A, et al. Prenylflavonoid variation in *Humulus lupulus*: Distribution and taxonomic significance of xanthogalenol and 4'-O-methylxanthohumol. Phytochemistry. 2000;53:759-75. DOI: 10.1016/s0031-9422(00)00005-4.

[25] Stevens JF, Page JE. Xanthohumol and related prenylflavonoids from hops and beer: To your good health! Phytochemistry. 2004;65:1317-30. DOI: 10.1016/j.phytochem.2004.04.025.

[26] Rozalski M, Micota B, Sadowska B, Stochmal A, Jedrejek D, Wieckowska-Szakiel M, et al. Antiadherent and Antibiofilm Activity of *Humulus lupulus* L. Derived Products: New Pharmacological Properties. BioMed Research International. 2013. ID: 101089. DOI: 10.1155/2013/101089.