BIOLOGICALLY ACTIVE PROPERTIES OF THE ETHANOL AND AQUEOUS EXTRACTS FROM THE NEEDLES OF *JUNIPERUS COMMUNIS*

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Abstract. The authors have tested different methods for preparing ethanol and aqueous extracts from the needles of the natural forms of the common juniper and researched some of their biologically active properties. They found 70% ethanol to be the best extractor of bioactive substances from the needles of the common juniper in contrast to 50% and 30% ethanol. They prove that the optimal way to obtain alcohol extracts is crushing the needles of the common juniper to linear sizes: 0.5-2 mm and to infuse in 70 % of ethanol for at least 20 days. Alcohol extracts obtained in this way (10%, g/g) contain polyphenols in the mass concentration of 0.40±0.02 mg/g biomass, ascorbic acid – 1.66±0.1 μg/g of mass and have weak antibacterial properties in relation to the microbiological test culture *Escherihia coli*, but not in relation to *Streptococcus epidermis*. A simple and at the same time optimal method to obtain aqueous extracts (10%, g/g) is boiling the uncrushed pieces of *J. communis* in water for 5 minutes. The aqueous extracts obtained by boiling of both crushed and uncrushed raw materials do not have an antibacterial action on the microbial test cultures.

Keywords: Juniperus communis, needles, extracts, polyphenols.

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Introduction

Juniperus communis L. belongs to the licorice plants used in pharmacology and folk medicine of Ukraine and some European countries. Its cones, needles, bark and roots of the *Juniperus* genus are described to have many bio-active substances (BAS), such as: α-pinene, sabinene, apigenin, β-sitosterol, amphophlavone, campsterol, kupesflavon, limonen, and others that have a sedative, anti-anaphylactic, antifungal, antimicrobial, dispersing and immunomodulatory effects *(Angioni et al., 2003; Bais et al., 2017; Cavalerio et al., 2006; Malyk et al., 2010; Taviano et al., 2013; Voloshanska et al., 2014)*. Extracts from different parts of *J. communis* are used in some pharmaceutical and technical preparations, cosmetics and as nutritional supplements *(Malyk et al., 2010; Stark et al., 2010)*. In traditional medicine, the cones of *J. communis* are used to treat skin diseases. This therapeutic effect is due to the ability of the flavonoid (7-O-β-D-xylopyranoside glylatetin) extracted from fruits of

J. communis to inhibit tyrosinase cells, which leads to slowing down of the synthesis of melanin *(Jegal et al., 2016).* Aqueous extracts of the related species *J. sabina* L. in the Chinese folk medicine are used to treat inflammatory diseases, infections, tumors, etc. *(Huyan et al., 2016).* This property of the Aqueous extracts of *J. sabina (Huyan, et al., 2016)* is due to their ability to inhibit onkogenes of cancer cell, reduce their viability, induce apoptosis by amplifying the expression associated with the apoptosis of FasL caspase 3 and caspase 9 genes.

The extracts of *J. oxycedrus* L. *subsp. oxycedrus* and *J. oxycedrus* L. *subsp. Macrocarpa* are known to have a high content of polyphenols, antibacterial and high antioxidant properties *(Karaman et al., 2003; Taviano et al., 2013).* A high cytotoxic effect of methanol extracts from needles and berries of *J. oxycedrus subsp. oxycedrus* against two breast cancer cell lines (MDA-MB-468 and MCF-7), with no cytotoxicity toward normal cells (PBMCs) *(Ben Mrid, et al., 2019).* Researchers suggest that the possible cause of observed cytotoxic effects is the generation of active forms of oxygen *(Ben Mrid et al., 2019)*. It is well-known that the antibacterial activity (in vitro) of essential oils of *Juniperus excels* has an effective against strains of *Mycobacterium tuberculosis* (including MDR), *Mycobacterium kansasii* and *Mycobacterium gordonae (El Omari et al., 2019).*

J. communis L. is an evergreen bush of 1-3 m high or occasionally a tree of 8-12 m high, belonging to the class *Coniferales* of the family *Cupressacea* Monogane or tribal plants. According to the heuristic classification, woody plants have a high embolism to tolerate dryness in arid habitats *(Wei et al., 2019). J. communis* grows in Volyn, Ivano-Frankivsk and Lviv regions of Ukraine, originating from Europe, South Asia and North America *(Bais et al., 2017). J. communis* grows on different types of soils, most often on dry and poor sand and podzolic soils, which, at moderate humidity, are most favorable for its growth; It also occurs on overly flowing-wet, slightly marshy soils *(Voloshanska et al., 2014). J. communis*, being widespread in a large area with a variety of environmental conditions, is quite variable. There exists a description of a series of subspecies, varieties and forms have been described *(Adams, 2003; Adams et al., 2004).* The sexual dimorphism in *J. communis* and the usual procedure for cutting off shoots have little effect on the synthetic ability by plants secondary bioactive metabolites, in particular, polyphenols and terpenoids *(Stark, Martz, 2018).*

The fruits of *J. communis*, are of the main medicinal value, whereas juniper needles are used to a lesser extent. The latter is the depot of some vitamins, most notably – ascorbic acid (246 mg%), contains polyphenols and carotene in large quantities. The fir-needle, along with the cones and the bark of J.communis, is a source of essential oil used for the production of immersion oil and refreshing essences. The literature states that essential oil of needles has strong disinfectant properties *(Malyk et al., 2010; Karaman et al., 2003).* It has been shown that the ethyl acetate fraction of the J. communis needles possesses high antioxidant and hepatoprotective properties without any signs of cytotoxicity and can be included in the nutraceuticals of prophylactic and therapeutic effect to improve the health of humans or animals *(Ved et al., 2017).*

The literature on traditional medicine gives a significant number of recipes for the preparation of infusions, decoctions, extracts from the needles *J. communis*. This prompted us to carry out an analysis of the methods of preparation of extracts from the needles of *J. communis* and identify the best of them according to the bioactive properties of the extracts obtained.

The purpose of the work is to study the methods of obtaining biologically active extracts from the needles of natural forms of *J. communis* plants, which grow on the territory of Lviv region.

Materials and methods

The material for research was the needles of natural populations *J. communis* plants growing on the territory of Sambir Hills, at an altitude of 300-400 m. The gathering of needles took place at the end of September 2018 in dry weather. Drying of needles was at a temperature of +20-22^{0}C in dark conditions and good ventilation. The material was stored at a temperature of +3-5 ^{0}C for no more than six months.

Preparation of alcohol extracts of *J. communis* needles (10%, m / v). Sample weights of air-dry needles weighing 10 g were crushed in a laboratory mill to linear sizes of 0.5-2 mm (II method), to 0.5-1 mm (III method) and with scissors to 0.5-1.5 mm (IV method). Ethanol of different concentrations (70%, 50% or 30%) was added to the whole (I method) and the crushed needles and kept for 30 days at 20±2°C in the dark place.

Preparation of aquatic extracts of *J. communis* needles (10%, m/v). Sample weights of similarly chopped (see above) needles 10 g were poured with 100 ml of boiling water, then boiled for 5, 10 and 15 minutes, infused for 2 hours at room temperature. The extract was filtered; the residue was pressed and the volume of water was increased to 100 ml.

We determined the content of polyphenolic compounds in the aqueous and alcohol extracts of the *J. communis* needles using the Folin–Ciocalteu phenolic reagent *(Singleton et al., 1999).*

The content of ascorbic acid (AA) in the aqueous and alcohol extracts of the *J. communis* needles was determined by the Murri method using the Tylmans reagent (2.6-dichlorophenolindophenol) *(Latimer, 1990).*

Antibacterial activity of the aquatic and alcohol extracts of *J. communis* needles was determined by a decrease in the total number of colony forming units (CSF) of microbial test cultures of *Escherihia coli* and *Staphylococcus epidermidis* on Petri dish using the serial dilution in agar method compared to control *(Klepach et al., 2017).*

Statistical analysis of experimental data. Researches were carried out in 5 repetitions. Arithmetical mean (M), standard error of mean value (m), Student test (t) and adequacy (p) were determined for each selection of indices. Statistical significant effects are indicated by $p \leq 0.05$.

Results and Discussion

The literature describes various methods for obtaining extracts from the plants' needles, depending on the goal *(Klepach et al., 2017).* For this research, we chose methods that include such typical stages as grinding and infusing raw materials for 30 days in 70%, 50% or 30% of ethanol (for alcohol extracts) and boiling and infusing in water (for obtaining aqueous extracts). The evaluation of the biologically active properties of the extracts was carried out on the content of polyphenolic compounds (PP) and ascorbic acid (AA), as well as their bactericidal action against microbial test cultures of *E. coli* and *St. epidermidis*.

The measurement of the content of AA and polyphenols in ethanol extracts was carried out during 30 days. Analyzing the kinetics of AA extraction with 70%, 50% and 30% ethanol (see table 1), we found that the *J. communis* needle extracts of different preparation methods

had the highest content of AA on the 10th day pf infusion; longer extraction leads to a decrease in its content: for 20-29 days – 1.5-2 times. We should note that 70% alcohol extracts obtained by infusion (in a dark place at room temperature) of the whole (not crushed) *J. communis* needles (I method) on the 10th day contain a higher mass concentration of AA (2.76±0.2 μg/g of mass or 0.27±0.02 μg/ml of extract) compared to extracts obtained by infusion of crushed raw material (II-IV methods).

Table 1

Content of ascorbic acid (μ/g biomass) in ethanol extracts of needles *J. communis* by different methods of obtaining

Method of	Day of exstraction								
	6	9	10	13	15	17	20	25	30
70 % ethanol extraction									
I	1,30±0,06	1,54±0,07	2,76±0,11	2,20±0,10	2,00±0,09	1,88±0,07	1,81±0,08	1,75±0,07	1,61±0,07
II	1,20±0,05	1,23±0,07	2,06±0,09	2,00±0,09	1,98±0,08	1,74±0,07	1,73±0,07	1,62±0,06	1,53±0,06
III	1,10±0,05	1,12±0,05	1,86±0,08	1,80±0,07	1,71±0,07	1,67±0,07	1,57±0,06	1,42±0,05	1,40±0,05
IV	1,09±0,04	1,10±0,05	1,76±0,07	1,80±0,08	1,68±0,07	1,62±0,07	1,50±0,06	1,38±0,05	1,35±0,05
50 % ethanol extraction									
I	1,39±0,06	1,64±0,07	2,61±0,12	2,10±0,09	1,89±0,08	1,82±0,05	1,75±0,07	1,71±0,07	1,61±0,07
II	1,40±0,06	1,70±0,07	2,53±0,10	1,98±0,09	1,77±0,08	1,71±0,07	1,66±0,07	1,65±0,06	1,57±0,06
III	1,41±0,06	1,76±0,07	2,42±0,10	1,96±0,09	1,85±0,08	1,77±0,07	1,72±0,07	1,68±0,07	1,59±0,06
IV	1,60±0,06	2,23±0,11	2,55±0,12	2,12±0,11	1,91±0,09	1,88±0,08	1,84±0,08	1,79±0,08	1,68±0,07
30 % ethanol extraction									
I	1,39±0,06	2,45±0,11	2,54±0,11	1,92±0,09	1,73±0,08	1,65±0,07	1,57±0,07	1,52±0,07	1,50±0,06
II	1,40±0,06	2,15±0,09	2,43±0,10	1,879±0,08	1,73±0,08	1,65±0,07	1,53±0,07	1,42±0,06	1,39±0,05
III	1,41±0,06	2,36±0,10	2,44±0,10	1,92±0,08	1,73±0,08	1,69±0,07	1,60±0,05	1,54±0,05	1,50±0,05
IV	1,60±0,06	2,21±0,09	2,48±0,10	2,05±0,09	1,88±0,09	1,66±0,07	1,59±0,07	1,53±0,07	1,49±0,06

Studying the kinetics of extraction of PP from the *J. communis* needles (varying degrees of grinding) by 70%, 50% and 30% ethanol (see table 2), we found that extracts obtained by different methods had the highest PP content on the 20th-23rd day; longer extraction leads to a decrease in their content in extracts, in particular, on the 27th day – in 1.1-1.3 times. It should be noted that the extract obtained by infusing of the crushed *J. communis* needles (to linear sizes of 0.5-2 mm) in the 70% ethanol contains a significantly higher content of PP (0.40±0.02 mg/g biomass or 0.040±0.002 mg/ml of extract) compared to extracts of other preparation methods.

Table 2

Content of polyphenols (mg/g biomass) in ethanol extracts of *J. communis* needles by different methods of obtaining

Method of	Day of exstraction								
	6	9	10	13	15	17	20	25	30
70 % ethanol extraction									
I	0,16±0,01	0,18±0,01	0,22±0,01	0,24±0,10	0,32±0,02	0,36±0,02	0,37±0,02	0,35±0,02	0,30±0,01
II	0,18±0,01	0,25±0,01	0,26±0,01	0,26±0,01	0,38±0,02	0,38±0,02	0,40±0,02	0,33±0,01	0,32±0,01
III	0,14±0,01	0,16±0,01	0,20±0,01	0,22±0,10	0,30±0,02	0,34±0,02	0,35±0,02	0,33±0,02	0,28±0,01
IV	0,17±0,01	0,19±0,01	0,23±0,01	0,24±0,10	0,31±0,02	0,35±0,02	0,36±0,02	0,34±0,02	0,31±0,01
50 % ethanol extraction									
I	0,20±0,01	0,23±0,01	0,24±0,01	0,24±0,01	0,24±0,01	0,24±0,01	0,32±0,02	0,30±0,02	0,28±0,02
II	0,26±0,01	0,26±0,01	0,26±0,01	0,27±0,02	0,28±0,02	0,28±0,01	0,35±0,02	0,25±0,02	0,21±0,02
III	0,21±0,01	0,23±0,01	0,24±0,01	0,25±0,01	0,27±0,02	0,29±0,02	0,34±0,02	0,30±0,02	0,28±0,02
IV	0,24±0,01	0,24±0,01	0,24±0,01	0,24±0,01	0,33±0,02	0,34±0,01	0,36±0,02	0,31±0,02	0,31±0,02
30 % ethanol extraction									

I	0,20±0,01	0,23±0,01	0,24±0,01	0,24±0,01	0,24±0,01	0,25±0,01	0,32±0,02	0,30±0,02	0,28±0,02
II	0,24±0,01	0,26±0,02	0,26±0,02	0,27±0,02	0,27±0,02	0,28±0,02	0,35±0,02	0,31±0,02	0,29±0,02
III	0,22±0,01	0,23±0,01	0,24±0,01	0,25±0,01	0,27±0,02	0,28±0,02	0,34±0,02	0,32±0,02	0,28±0,02
IV	0,24±0,01	0,26±0,02	0,27±0,02	0,28±0,02	0,33±0,02	0,35±0,02	0,36±0,02	0,32±0,02	0,29±0,02

The water extracts were obtained from the whole and crushed (to different linear sizes) needles of *J. communis* (see above). Analyzing the content of AA in aqueous extracts (see fig. 1), it was found that on the second day (when stored at 5°C) its content in extracts increased from 1.76±0.15 μg/g of mass (day I) to 2.18±0.15 μg /g (day 2). Such an artefact – an increase in the content of AA on the second day – can be explained by the interfering effects of the reducing compounds *(Pavlishko et al., 2005)* extracted from the needles on the reaction of the AA remediation by the Thilmans reagent (in the Murri method), which results in a decrease of its content in aqueous extracts. On Day 2, the effect of reducing compounds on the reaction of determination of AA in the extracts is reduced, probably, due to the loss of activity and/or co-precipitation. It should be noted that there is no significant difference in the content of AA in aqueous extracts of different preparation methods (on the second day of storage) ($t<0.896$; $p=0$). As can be seen from fig. 1 on the 3rd day of storage, the content of AA in all extracts is reduced; besides, there is turbidity and an unpleasant smell caused by microbial contamination. Therefore, water extracts of the *J. communis* needles are suitable for use during two days.

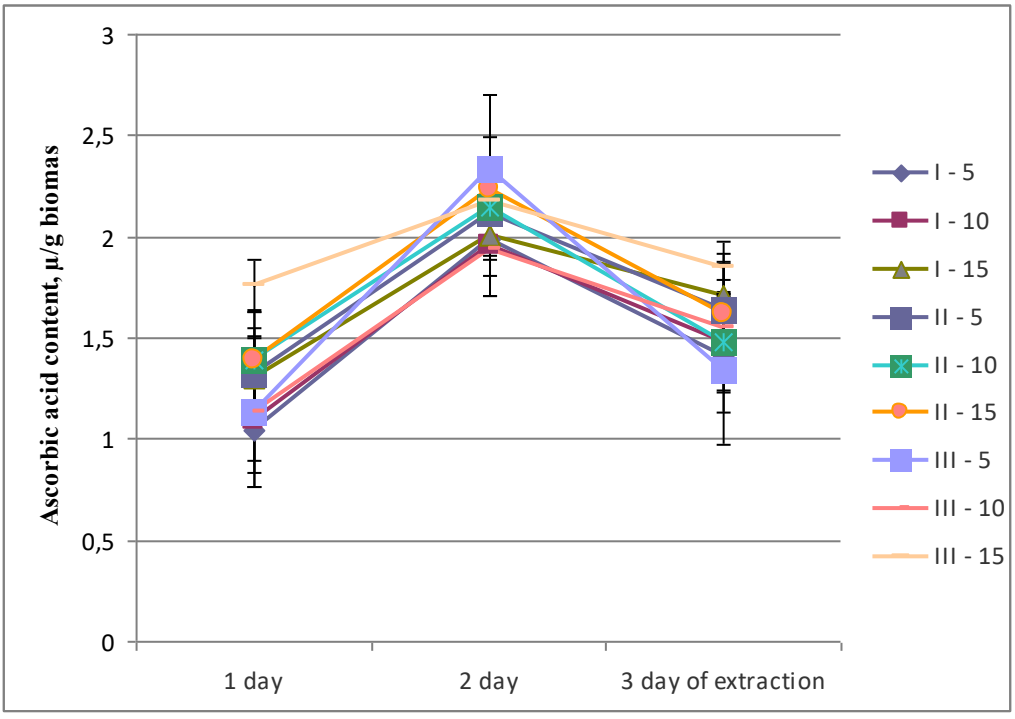

Fig. 1. The content of ascorbic acid in aqueous extracts of the *J. communis* needles, obtained in different ways: method I (without crushing) and its variants: 1-5 – boiling for 5 minutes; 1-10 –10 min; 1-15 –15 min; II method (crushing to linear sizes of 0.5-

2 mm) and its variants: II-5 – boiling for 5 minutes; II-10 – 10 min; II-15– 15 min; III method (crushing to linear sizes 0.5-1 mm) and its variants: III-5 – boiling for 5 minutes; III-10 – 10 minutes; III-15 – 15 minutes.

According to literature, *J. communis* coniferous water has septic properties *(Malyk et al., 2010).* Therefore, we researched the antibacterial properties of water extracts of the *J. communis* needles in relation to microbial test cultures of *E. coli* and *St. epidermidis*. Applying the method of serial dilutions, we found that aqueous extracts of the *J. communis* needles (10%) of different preparation methods for different volumetric ratios (v/v, ml – 1:99, 2:98, 5:95 and 10:90) in a nutritious agar medium do not cause any bactericidal or bacteriostatic action in terms of *E. coli* and *St. epidermidis* (see table 3). We did not find any significant difference (t=0.29-0.83) in the number of CFU of test cultures and their linear sizes (diameter) on experimental and control cups.

Based on the obtained results, we can recommend of the most economical methods for the preparation of aqueous bioactive extract, which includes the following steps: hot water pouring of the whole needle, its boiling for 5 minutes and infusion for 2 hours at room temperatures.

Table 3

Antibacterial action of aqueous extracts (10%) of *J. communis* needles of various obtaining methods regarding microbial test cultures

Microbial test culture	Number of CFU (M ± m) and survival (in %) of test culture cells for different volumetric contents of aqueous extract					
	K*	Method of obtaining	Volume content of the extract in the medium (v:v)			
			1:99	2:98	5:95	10:90
E. coli	153±14	I	148±13 (100%)	145±6 (100%)	149±13 (100%)	143±13 (100%)
		II	150±14 (100%)	146±13 (100%)	147±13 (100%)	144±13 (100%)
		III	147±13 (100%)	150±13 (100%)	145±13 (100%)	147±13 (100%)
		IV	151±13 (100%)	149±12 (100%)	148±13 (100%)	150±13 (100%)
St. epidermidis	137±12	I	135±12 (100%)	133±12 (100%)	135±12 (100%)	134±12 (100%)
		II	138±12 (100%)	135±12 (100%)	139±12 (100%)	135±12 (100%)
		III	133±11 (100%)	139±12 (100%)	134±12 (100%)	131±12 (100%)
		IV	134±11 (100%)	136±12 (100%)	138±12 (100%)	139±12 (100%)

Note: *К – control.

We studied antibacterial properties on the example of 70% ethanol extract of the *J. communis* needles, as the most bioactive in the content of PP, obtained by the II method. As we see (see table 4), in volume ratios at 1:99, 2:98 and 5:95 of the said alcohol extract in the nutrient agar (NA) medium, the number of microorganisms of microbial test cultures in the experimental cups is significantly closer to the control ones, their survival is 100 %. At a volume ratio of 10:90 of the extract in the NA-medium, there is a decrease in the number of CFU *E. coli* by 15 % – the survival of the test culture cells is 85 % compared to the control. Thus, 70 % of ethanol extracts of the *J. communis* needles have a weak antibacterial action on the *E. coli* test culture. In the case of *S. epidermidis*, the antibacterial action of 70 % of the ethanol extract of *J. communis* on the studied volume proportions in the experimental cups is

not observed – the number of CFU *E. coli* in the control and experiment are close to each other.

Table 4

Antibacterial action of 70 % of ethanol extract of *J. communis* needles in relation to microbial test cultures

Microbial test culture	The number of CFU (M±m) and survival (in %) of test cultures cells in experimental cups (E) for different contents of the extract								
	Volume content of the extract in the medium (v:v)								
	K*	1:99		2:98		5:95		10:90	
		KE**	E***	KE**	E***	KE**	E***	KE**	E***
E. coli	153±14	145±13	143±13 100%	141±13	137±12 100%	130±12	127±12 110%	120±11	102±10 85%
St. epidermidis	137±13	140±12	138±12 100 %	133±12	130±12 100 %	117±11	110±10 100 %	73±6	75±6 100 %

Note: *K – control (without extract and 70 % ethanol); **KE – control with 70 % ethanol equivalent volume; ***E is an experiment with 70% ethanol extract.

Conclusions

It has been established that the best extractant of some bioactive substances from the needles of *J. communis* is 70 % ethanol in contrast to 50 % and 30 % ethanol. It is proved that the optimal method of obtaining alcohol biologically-active extracts from the needles of *J. communis* enriched with PP is to: crush the raw material to linear sizes of *0.5-2* mm and insert 70 % ethanol for at least 20 days (in the dark). Alcohol extracts obtained in this way (10 %, g/g) contain polyphenols in the mass concentration – 0.40±0.02 mg/g biomass or 0.040±0.002 mg/ml of extract, AA – 1.66±0.1 μ/g of mass, or 0.16 ± 0.01 μ/ml of extract. The extracts obtained in this way have weak antibacterial properties in relation to the microbial test culture *E. coli* and have no antibacterial action in relation to the test culture *St. epidermis* whatsoever.

It was found that a simple, economical and reasonably optimal method of obtaining aqueous biologically active-extracts is the boiling of the uncrushed pine needles of *J. communis* in water during 5 minutes. The biologically active extracts obtained in this way (10 %, g / g) contain AA: 1.76–2.18±0.15 μ/g mass. However, water-borne aqueous extracts obtained by boiling (5 or 10 or 15 minutes) of both crushed and uncrushed raw materials (10 %, g/g) do not have an antibacterial action on *E. coli* and *St. epidermis* test cultures.

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References

Adams, R. P. (2004). Junipers of the World: The genus Juniperus. Victoria: Trafford. [in English].

Adams, R. P., Pandey, R. N., Leverenz, J. W., Dignard, N., Hoegh, K., & Thorfinnsson, T. (2003). Pan-Arctic variation in Juniperus communis: Historical Biogeography based on DNA fingerprinting. Biochemical *Systematics and* Ecology, *31, 181-192. [in English].*

Angioni, A., Barra, A., Russo, M. T., Coroneo, V., Dessi, S., & Cabras, P. (2003). Chemical composition of the essential oils of Juniperus from ripe and unripe berries and leaves and their antimicrobial activity. Journal of Agricultural and Food Chemistry, 51 (10), 3073–3078. DOI: 10.1021/jf026203j. [in English].

Bais S., Abrol, N., Prashar, Y., & Kumari, R. (2017). Modulatory effect of standardised amentoflavone isolated from Juniperus communis L. agianst Freund's adjuvant induced arthritis in rats (histopathological and X Ray anaysis). Biomed Pharmacother, 86, 381-392. DOI: 10.1016/j.biopha.2016.12.027. [in English].

Ben, M. R., Bouchmaa, N., Bouargalne, Y., Ramdan, B., Karrouchi, K., Kabach, I., El Karbane, M., Idir, A., Zyad, A., & Nhiri, M. (2019). Phytochemical Characterization, Antioxidant and In Vitro Cytotoxic Activity Evaluation of Juniperus oxycedrus subsp. oxycedrus Needles and Berries. Molecules, 24(3). DOI: 10.3390/molecules24030502. [in English].

Cavalerio, C., Pinto, E., Gonçalves, M. J., & Salguero L. (2006). Antifungal activity of Juniperus essential oils against dermatophyte, Aspergillus and Candida strains. Journal of Applied Microbiology, vol. 100 (6), 1333-1338. DOI:10.1111/j.1365-2672.2006.02862.x. [in English].

El Omari, K., Hamze, M., Alwan, S, Osman, M., Jama, C., & Chihib, N. E. (2019). In-vitro evaluation of the antibacterial activity of the essential oils of Micromeria barbata, Eucalyptus globulus and Juniperus excelsa against strains of Mycobacterium tuberculosis (including MDR), Mycobacterium kansasii and Mycobacterium gordonae. Journal of infection and public health. doi: 10.1016/j.jiph.2019.01.058. [in English].

Huyan, T., Li, Q, Wang, Y.L, Li, J., Zhang, J.Y, Liu, Y.X, Shahid, M.R., Yang, H., & Li, H.Q. (2016). Anti-tumor effect of hot aqueous extracts from Sonchus oleraceus (L.) L. and Juniperus sabina L - two traditional medicinal plants in China. Journal of Ethnopharmacology*, 185, 289-99. DOI: 10.1016/j.jep.2016.03.044. [in English].*

Jegal, J., Park, S. A, Chung, K., Chung, H. Y, Lee, J., Jeong, E. J., Kim, K. H., & Yang, M. H. (2016). Tyrosinase inhibitory flavonoid from Juniperus communis fruits. Bioscience, Biotechnology, *and* Biochemistry, *80 (12), 2311-2317. DOI: 10.1080/ 09168451.2016.1217146. [in English].*

Karaman, I., Sahin, F., Gulluce, M., Sengul, M., & Adiguzel, A. (2003). Antimicrobial activity of aqueous and methanol extracts of Juniperus oxycedrus L. Ethnopharmacol, 85, 231-235. [in English].

Klepach, H. M., & Monastyrska, S. S. (2017). Methods of obtaining extracts from some medicinal plants and assessing their antibacterial and insecticidal action. Promoting healthy lifestyle: health and environment: monographic series. Drohobych: Publication Department at Drohobych Ivan Franko State Pedagogical University, 2, 197-210. [in Ukraine].

Latimer, G. W. (1990). Official Methods of Analysis of the Association of Official Analytical Chemistry. AOAC: Arlington, 2, p. 1058. [in English].

Malyk, O. G., Voloshans'ka, S. Y., & Kravtsiv, M. M. (2010). Biologically active substances and medicinal products made from Juniper ordinary. The Animal Biology, 12(1), 37-43. [in Ukraine].

Pavlishko, H. M., Ryabinina, O. V., Zhilyakova, T. A., Sakharov I., Yu., Gerzhikova, V. G., & Gonchar, M. V. (2005). Oxidase-Peroxidase Method of Ethanol Assay in Fermented Musts and Wine Products. Applied Biochemistry *and* Microbiology, *41(6), 604-609. [in English].*

Singleton, V. L., Orthofer, R., Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidations substractes and antioxidans by means of Folin-Ciocalteu reagent. Methods in enzymology, 299, 152-178. DOI: 10.1016/S0076-6879(99)99017-1. [in English].

Stark, S., Martz, F. (2018). Gender Dimorphism Does Not Affect Secondary Compound Composition in Juniperus communis After Shoot Cutting in Northern Boreal Forests, 9,1910. DOI: 10.3389/fpls.2018.01910. [in English].

Stark, S., Niva, A., Martz, F., & Vuorela, E. (2010). Sustainable Gathering of Juniper and Bilberry Shoots for Natural Product Industry (In Finnish, English abstract). Jokioinen: MTT Raportti 3. [in English].

Taviano, M. F, Marino, A., Trovato, A., Bellinghieri, V., Melchini, A., Dugo, P., Cacciola, F., Donato, P., Mondello, L., Güvenç A, De Pasquale R, & Miceli N. (2013). Juniperus oxycedrus L. subsp. oxycedrus and Juniperus oxycedrus L. subsp. macrocarpa (Sibth. & Sm.) Ball. "berries" from Turkey: comparative evaluation of phenolic profile, antioxidant, cytotoxic and antimicrobial activities. Food *and* Chemical Toxicology *, 58, 22-29. DOI: 10.1016/j.fct.2013.03.049. [in English].*

Ved, A., Gupta, A., Rawat A.K. (2017). Antioxidant and Hepatoprotective Potential of Phenol-Rich Fraction of Juniperus communis Linn. Leaves. Pharmacognosy Magazine, 13 (49), 108-113. DOI: 10.4103/0973-1296.197648. [in English].

Voloshanska, S. Ya., Kossak, G. M., Skrobach, T. B., & Harachko, T. I. (2014). Pharmacological Properties of Juniperus communis L. and Peculiarities of its Extension within Drohobych Region. The Scientific Bulletin of UNFU, 24 (4), 179-184. [in Ukraine].

Wei, L., Xu, C., Jansen, S., Zhou, H., Christoffersen, B. O., Pockman, W. T., Middleton, R. S., Marshall, J. D., & McDowell, N. G. (2019). A heuristic classification of woody plants based on contrasting shade and drought strategies. Tree Physiology*, tpy146. DOI: 10.1093/treephys/tpy146. [in English].*