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*Original scientific paper*

# Antibacterial activity of essential oil of *Juniperus communis* L.

Vesna Kalaba[1], Željka Marjanović-Balaban[2], Dragana Kalaba[3], Dijana Lazić[4], Vesna Gojković Cvjetković[5]

*[1]Veterinary Institute of the Republic of Srpska "Dr Vaso Butozan" Banja Luka, Republic of Srpska, Bosnia and Herzegovina, vesna.kalaba@yahoo.com*

*[2]University of Banja Luka, Faculty of Forestry, Republic of Srpska, Bosnia and Herzegovina*

*[3]Faculty of Medicine-Department of Pharmacy, Banja Luka, Republic of Srpska, Bosnia and Herzegovina*

*[4]Public Enterprise "Forests of the Republic of Srpska", Center for Seed and Seed Production, Doboj, Republic of Srpska, Bosnia and Herzegovina*

*[5]Faculty of Technology, University of East Sarajevo, Republic of Srpska, Bosnia and Herzegovina*

**Abstract**: Many herbal raw materials have been used for centuries as a means of treating various types of diseases. Herbal preparations in pharmaceutical forms for oral and external use show a wide range of effects, among which one of the most important antimicrobials. The effect of such herbal preparations on various microorganisms is the subject of many scientific research today, which is mainly fueled by the fact that the high use of antibiotics in more than half a century has led to resistance to microorganisms. Enriched with new results and knowledge of chemistry and the activity of active ingredients derived from plants, today we are experiencing a growing use of herbs in various industries. The Republic of Srpska has a great potential of woody greenery as a possible raw material for the production of essential oils. Therefore, the aim of this study is to define antibacterial effect of juniper berries (*Juniperus communis* L.) essential oil, obtained by the hydrodestilation process in industrial production conditions. A boiler with capacity of 2000 liters has been used in the hydrodistillation process (hydromodule1:3), the temperature was 102°C, pressure of 0.5 bar, and the time of hydrodistillation process was 2 hours. The yield of essential oil was 1.3 %.The antibacterial effect of the essential oil of the juniper berries was tested using the agar diffusion method on the reference cultures of *Salmonella enterica* WDCM 00030, *Pseudomonas aeruginosa* WDCM 00024, *Escherichia coli* WDCM 00013, *Staphylococcus aureus* WDCM 00032, *Bacilus cereus* WDCM 00151- (CEST- Coleccion Espanola De CultivosTipo, Espana), as well as clinical isolates of *Providenciastuarti*, coagulase positive staphylococci, streptococcus group D and *Salmonella* spp. from the collection of microbiological laboratory of the Veterinary Institute "Dr Vaso Butozan" Banja Luka.

The juniper berries essential oil in a concentration of 20 µl showed antibacterial activity only on the clinical isolate of coagulase-positive staphylococci with an inhibition zone of 12.00 mm, while at a concentration of 100 µl it was shown antibacterial activity on all investigated pathogens (with an inhibition zone of 8.33 mm to 18.00 mm), except on *P.aeruginosa* and *E. coli*. The research results indicate to better effect of essential oils compared to reference antibiotics.

**Keywords**: Essential oil, *Juniperus communis* L., Antibacterial activity.

## INTRODUCTION

Essential oils are volatile, natural, complex substances of strong scent and represent secondary metabolites of aromatic plants.

They are usually obtained by steam distillation. Famous for its antiseptic, i.e. bactericidal, viral, fungicidal properties, and their fragrance, are used for balm, food preservation, as well as antimicrobials, analgesics, sedatives, anti-inflammatory drugs, spasmolytics and local anesthetics. Essential oils are isolated from various aromatic plants, from all plant organs, i.e. buds, flowers, leaves, seeds, fruits, branches, roots, bark or wood, are found in secretory cells, cavities, channels, epidermal cells or in glandular trichomes. The discovery, development and use of antimicrobial drugs and their introduction into wide clinical practice during the 20th century significantly changed the treatment of infectious diseases. As a consequence of widespread and unreasonable use of antibacterial drugs, there is a worrying appearance of pathogens that are resistant to antibacterial drugs, resulting in a major threat to the health of the world's population, especially with the emergence of multiple resistant bacteria [Fabricant and Farnsworth, 2001; Morris et al., 1976; Durić, 2016]. The problem of resistance has directed the scientific community in search of antibacterial agents that would be effective in combating pathogenic bacteria resistant to current antibacterial drugs.

One of the largest studies of antimicrobial effects of essential oils was done by Morris et al. [Morris et al., 1976] who examined 521 essential oils and their components and found that 44% of the investigated oils inhibited the growth of at least one tested microorganism. Good antimicrobial activity was shown by terpenes - citral, eugenol, menthol, carvacol and geraniol [Durić, 2016]. The most common component of essential oils are phenolic compounds and they generally show the highest antimicrobial activity [Stanojević et al., 2019]. The exact mechanism of action of essential oils is still not well known, although it is assumed that essential oils due to their lipophilic nature are well dissolved in the lipid bilayer of the bacterial membrane and in this way lead to increased permeability of the membrane and death of the cell. There is also evidence that substances that are present in smaller amounts in essential oils play a critical role in antibacterial activity, most likely by causing a synergistic effect with other ingredients [Burt, 2004; Bakkali et al., 2008; Stojković et al., 2013]. The difference in the chemical composition of essential oils is responsible for the different antibacterial activity of the same [Burt, 2004].

In many studies [Durić, 2016; Feizi et al., 2013], it has been proven that some of the active ingredients present in essential oil (carvacol) can inhibit the spread of *Salmonella* spp. in the meat as well as to reduce the number of aerobic bacteria in meat and meat products and to act antibacterial on *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumonia*, *Mycoplasma gallisepticum*, *Clostridium perfringens* and *Salmonella enterica* [Feizi et al., 2013].

Essential oils show remarkable antibacterial potential, but their use is partly limited due to intense scent and taste. With the advancement of technology, it is possible to successfully combine the sensory quality and microbiological stability of food [Solorzano-Santos et al., 2012].

Juniper (*Juniperus communis*) is a shrubby plant from which the berry is very tasty, but clearly refreshing, spicy, balsamico-sweet, slightly woody, a scent that resembles the scent of pine needles [Solorzano-Santos et al., 2012].

Juniper essential oil is bactericidal, immunostimulant, analgesic, hypertensive, diuretic, antipsychotic, coughing, sweating, anti-inflammatory, antireumatic, brain stimulation, digestion and soothing. It is used in chronic diseases of the respiratory tract, functional disorders of the digestive system, inflammatory diseases of the urogenital system and kidneys, atherosclerosis, intoxication (including alcohol), decreased sexual activity, frigidity, impotence, arthritis, rheumatism, lumbago, sciatica, metabolic disorders, hemorrhoids, wounds, perspirical skin diseases (eczema, psoriasis, acne, dermatitis) and Parkinson's disease [Ved et al., 2017;Al-Snafi, 2015].

The aim of this study is to determine the antibacterial activity of the juniper berry essential oil from the plant material from the Republic of Srpska to the reference cultures of *S. enteric*, *P. aeruginosa*, *E. coli*, *S. aureus*, *B. cereus*, as well as the bacterial culture of *Providenciastuarti*, coagulase positive staphylococci, streptococcus D group and *Salmonella* spp., isolated from the clinical material and to determine the type of its action. In this study, the antibacterial action of essential oil with reference antibacterial drugs was compared, the type of action was determined, the results were statistically processed, all in order to investigate the possible application of the obtained essential oil of juniper berries in the industry as a possible natural preservative.

## EXPERIMENTAL PART

### Plant material

As a raw material for the production of essential oil, the fruits of juniper (*Juniperus communis*), were harvested in October 2018 at the Sokolac location, Republika Srpska, Bosnia and Herzegovina (Lat. 43.938, Lng. 18.801).

### Essential oil isolation

The essential oil of the juniper berry was produced in industrial conditions by the hydrodestilation process in a 2000 liter distiller, in cooperation with the PEF "Forest of Republika Srpska", a.d.Sokolac. For the purpose of faster and better distillation, the juniper berry were passed through the crusher before the production process of the essential oil. The hydrodestilation process was carried out at temperatures of 102 °C, a pressure of 0.5 bar and a duration of 2 hours.

### Test microorganisms

To test the antibacterial activity of essential oil of the juniper berry, the reference cultures were used *Salmonella enterica* WDCM 00030, *Pseudomonas aeruginosa* WDCM 00024, *Escherichia coli* WDCM 00013, *Staphylococcus aureus* WDCM 00032, *Bacilus cereus* WDCM 00151 (CEST- Coleccion Espanola De CultivosTipo, Espana) and bacterial cultures isolated from the clinical material *Providenciastuarti* (vaginal swab), coagulase positive staphylococci (ice cream), streptococcus group D (throat swab),ß-hemolytic E. coli (urine) and *Salmonella* spp.,(meat) (from the collection of microbiological laboratory of the Veterinary Institute "Dr Vaso Butozan" Banja Luka). The cultures were sifted in a nutrient substrate and incubated at 18h at 37°C. Suspensions were made in saline from the grown colonies corresponding to the density of 0.5 McFarland standard. Petri plates with the appropriate substrate (Müeller - Hinton agar – MHA, (HiMedia, Indija)were seeded with 0.1ml of a bacterial suspension with a concentration of $10^5$cfuml$^{-1}$.

### Test method

The antibacterial activity of the essential oil was tested at two different volumes (20 and 100 µl) of essential oil. An agar diffusion method was used [Bauer et al., 1966] by placing 9 mm diameter cylinders on a solid sterile base (MHA) in which different qvantity of essential oil were added. As a control, 20µl (100µl) of 96% alcohol was charged in the cylinders. In order to accelerate the diffusion of the essential oil into agar, the plates were incubated at 4°C for 1h and then incubated for 18 hours under aerobic conditions at 37°C ± 4°C. The inhibition zone diameter is measured and recorded in at least 5 reps. Analysis of variance

(ANOVA) was carried out over all the variables in the general linear model. A statistically significant difference between the mean values obtained in the experimental data is calculated using *t* - test with statistical significance of $p \leq 0.05$.

### Antibacterial drugs

The sensitivity of the selected bacterial species to the reference antibacterial substances was performed using a standard disk-diffusion method using the Müeller-Hinton agar. The tablet antibiogram ("Conda", Proanalysis) ampicillin (AM) 2µg, cefuroxime (CXM) 30µg and sulphamethoxazole (SMZ) 50µg were used. After incubation from 18-24h to 37ºC, the obtained results were interpreted according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) from 2014. To control the performance of this method, reference strain *S. aureus* WDCM 00034 was used.

### Type of action

To see if the essential oil of juniper berry was bactericidal or bacteriostatic, a small piece of agar was taken from the inhibition zone and added to the nutrient substrate, that is, the type of action was determined. Incubation was carried out 24h at 37ºC. If, after incubation, there is blur in the substrate, the effect of the oil is bacteriostatic, that is, if the substrate remains clear the effect of the oil is bactericidal.

## RESULTS AND DISCUSSION

A light yellow essential oil, a pleasant smell, yield of 1.3% was obtained.

The antibacterial activity of the juniper berry essential oil of the antibacterial agent was made by disc diffusion method at two different volumes. Test results are shown in Tables 1.

**Table 1.** Antibacterial activity of juniper berry essential oil

| Microorganism | Juniper berry essential oil Inhibition zones in mm | |
|---|---|---|
| | 20µl | 100 µl |
| *S. enterica* WDCM00030 | 0.00±0.00 | 16.66±0.15 |
| *P. aeruginosa* WDCM00024 | 0.00±0.00 | 0.00±0.00 |
| *E.coli* WDCM00090 | 0.00±0.00 | 0.00±0.00 |
| *S. aureus* WDCM00032 | 0.00±0.05 | 13.33±0.15 |
| *B. cereus* WDCM00001 | 0.00±0.00 | 15.00±0.10 |
| E. faecalis | 0.00±0.05 | 10.00±0.00 |
| Coagulase pos. staphylococci | 12.00±0.00 | 12.33±0.15 |
| Streptococcus D group | 0.00±0.00 | 18.00±0.00 |
| *Salmonella* spp. | 0.00±0.00 | 11.66±0.15 |
| ß-hemolytic *E. col*i | 0.00±0.05 | 8.33±0.15 |

Nine out of ten tested pathogens showed resistance to the effect of the juniper berry essential oil in a volume of 20µl. Coagulase-positive staphylococci, isolated from clinical material, exhibited a certain sensitivity to the effect of essential oil at a volume of 20µl with an inhibition zone of 12.0 mm. The volume of 100µl of essential oil gave a moderate antibacterial effect with inhibition zones from 8.3mm to 18.0mm. The juniper berry essential oil in an amount of 100 µl did not appear antibacterial on the *P. aeruginosa* and *E. coli* reference strain, while the almost identical antibacterial activity exhibited a coagulase positive staphylococci as well as a volume of 20 µl. An increase in the concentration of essential oil led to an increase in the inhibition zone, which was expected. This is also confirmed by the values of the *t*-test for *S. enterica*, *S. aureus*, *B. cereus*, *E. faecalis*, streptococ group D, *Salmonella* spp. and ß-haemolytic *E. coli*, which are above *t* (2 and 0.05)=4.30, which indicates a significant statistical difference when the concentration of the bran essential oil of the bran pulp is concerned.

Similar results on the antibacterial action of juniper berry have also been published by other authors [Snafi, 2015; Haziri et al., 2013; Asili et al., 2008; Al-Snafi, 2017; Sela et al., 2013; Pepeljnak et al., 2005].

Examination of essential oils is hampered by their lipophilic character, volatility and complexity. The largest number of antibacterial activity studies of essential oils are performed in vitro, and the most commonly used methods are diffusion and dilution (micro and macro) methods. The results greatly influenced by the following factors: methods of isolation of essential oils, volume of inoculum, growth stages of microorganisms, culture used, pH of the substrate, incubation time and temperatures [Casadevall and Fang, 2010].

Many studies have shown that the chemical composition of essential oils has a direct effect on their antibacterial activity, as some compounds with a particular chemical structure exhibit a stronger, and the other a weaker effect. Oils rich in phenolic compounds (carvacol, thymol and eugenol) have the strongest antibacterial activity, followed by alcohols and alcohol terpenoids [Soković et al., 2010].

A large number of studies indicate that the cell membrane is the site of the action of essential oils, or their constituents. Hydrophobicity is a very important feature of both oils and their components, since it increases the permeability of the cell membrane to microorganisms and allows their easier passage through its lipid layer. This is important because the change in the permeability of the cell membrane is usually followed by the loss of osmotic control of the cell, which is considered the basic principle of antibacterial effects of essential oils [Bakkali et al., 2008].

It is known that the hydrophobicity of thymol and carvacrol damage the membrane of gram-negative bacterial cells rich in lipopolysaccharides, which ensures smooth passage of oil and their components into the cell's interior [Soković et al., 2010; Čančarević et al., 2013].

Inhibitory activity of juniper berry essential oil is found to be very effective in comparison with reference ampicillin. Such results open up a space for further exploration of juniper berry essential oil originating from the territory of the Republic of Srpska, all with the goal of possible application in different branches of industry, especially with reference to the increasingly frequent occurrence of multiresistant strains.

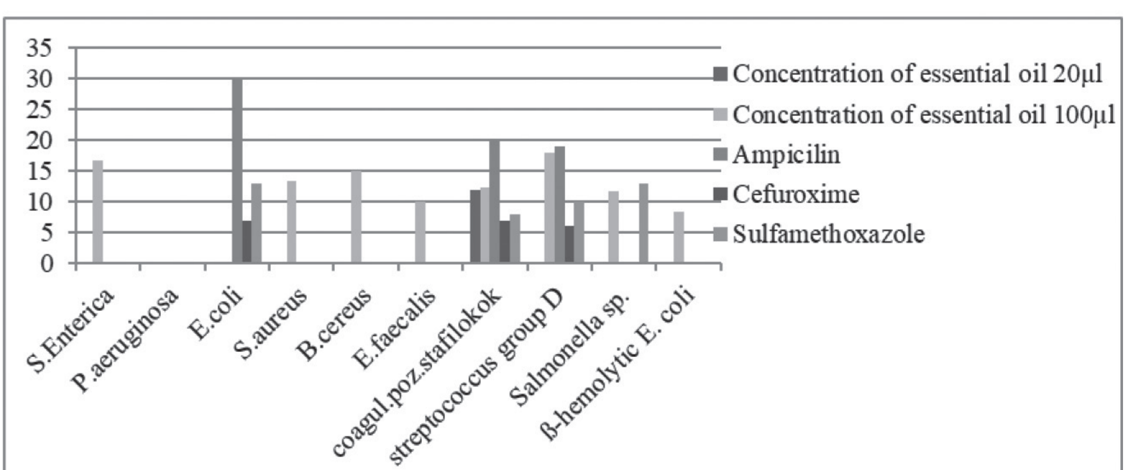


**Chart 1.** Antibacterial activity of juniper berry essential oil and reference antibiotics

For comparison, Chart 1 shows the results of the antibacterial effect of juniper berry essential oil and reference antibiotics.

In order to determine the type of action of juniper berry essential oil, from the inhibition zone, a small piece of agar was taken and added to the substrate. The results of the bactericidal and bacteriostatic effects of juniper berry essential oil on the examined pathogens are shown in Chart 2.

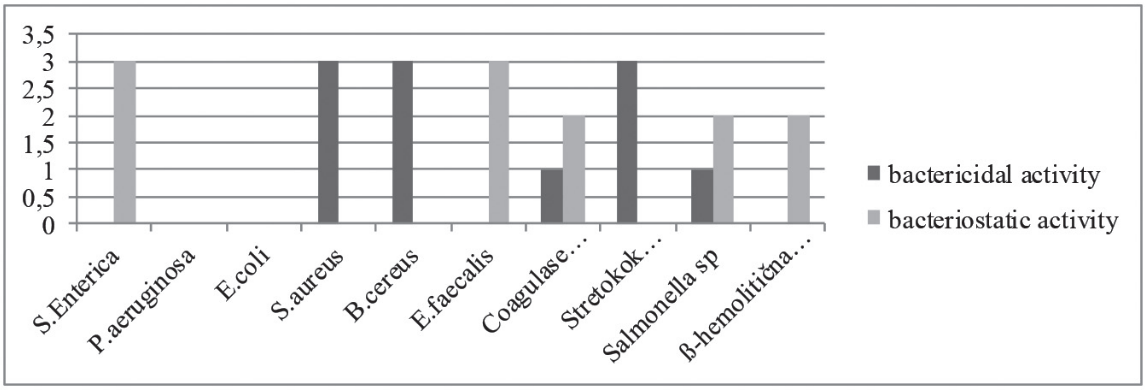


**Chart 2.** Bactericidal and bacteriostatic activity of juniper berryessential oil

Best bactericidal activity was obtained from *S. aureus*, *B. cereus* and streptococcus D group, while the best bacteriostatic activity was observed in the *S. enterica* and *E. facealis*.

## CONCLUSION

The results of the study of antibacterial activity of juniper berry essential oil from the region Republic of Srpska indicate that concentration of essential oil of 20µl acts as inhibitory to the clinical coagulase isolation of carotid staphylococci. 100µl of essential oil concentration is antibacterial to eight out of ten tested pathogens. The reference strains of *P. aeruginosa* and *E. coli* are absolutely resistant to the effect of juniper berry essential oil in both tested concentrations. The inhibitory activity of the examined essential oil proved to be very effective compared to the antibacterial drugs used.

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