Original Article

Evaluation of the antibacterial effect of *Artemisia lerchiana* compared with various medicines

Avaliação do efeito antibacteriano da *Artemisia lerchiana* comparado a outros medicamentos

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Abstract

Many antimicrobial substances, mainly of chemical origin, are not effective and reliable. Many of them tend to accumulate in organs and tissues and have allergic and toxicological effects and side effects. Therefore, the purpose of our research was to conduct a comparative analysis of the antibacterial effect of Artemisia lerchiana against other herbal preparations. A. lerchiana was used in the form of an infusion and essential oil extract to fulfill the set goal and objectives. For comparative analysis, tinctures of A. absinthium, Calendula officinalis L., and Chamomilla recutita L., as well as preparations based on Abies Sibirica, Eucalyptus, Limon, Bergamottae, Melaleuca alternifolia, and A. absinthium essential oils were used. As a test standard for comparing antibacterial properties, we used the Septarius chemical preparation manufactured at a biofactory. The experiment was carried out in vitro using test microbes of gram-positive Staphylococcus aureus and Streptococcus and gram-negative Escherichia coli. The results of the study showed that A. lerchiana had a high antibacterial effect against gram-positive test microbes (Staphylococcus, Streptococcus). The most effective form was A. lerchiana in the form of essential oil, which had an antibacterial effect even in low concentrations. A comparative assessment of A. lerchiana with the Septarius test antiseptic showed that A. lerchiana had a similar effect in general. In the form of essential oil, it even surpassed the effect of Septarius. It was found that A. lerchiana surpassed medicinal plants used in the experiment in terms of antibacterial effect. The obtained results of the study will be useful for scientists researching various medicinal products of plant origin and for veterinary specialists looking for therapeutic and preventive measures.

Keywords: Artemisia lerchiana, microorganisms, antimicrobial effect, essential oil, infusion.

Resumo

Muitas substâncias antimicrobianas, principalmente de origem química, não são eficazes e confiáveis. Muitos deles tendem a se acumular em órgãos e tecidos e apresentam efeitos alérgicos e toxicológicos e efeitos colaterais. Portanto, o objetivo desta pesquisa foi realizar uma análise comparativa do efeito antibacteriano da Artemisia lerchiana contra outras preparações fitoterápicas. A. lerchiana foi utilizada na forma de infusão e extrato de óleo essencial para cumprir a meta e os objetivos traçados. Para análise comparativa, foram utilizadas tinturas de A. absinthium, Calendula officinalis L. e Chamomilla recutita L., bem como preparações à base de óleos essenciais de Abies Sibirica, Eucalyptus, Limon, Bergamottae, Melaleuca alternifolia, e A. absinthium. Como padrão de teste para comparação de propriedades antibacterianas, utilizamos a preparação química Septarius fabricada em uma Biofábrica. O experimento foi realizado in vitro utilizando micróbios de teste de Staphylococcus aureus e Streptococcus gram-positivos e Escherichia coli gram-negativa. Os resultados do estudo mostraram que A. lerchiana teve um alto efeito antibacteriano contra micróbios de teste gram-positivos (Staphylococcus, Streptococcus). A forma mais eficaz foi a A. lerchiana na forma de óleo essencial, que teve efeito antibacteriano mesmo em baixas concentrações. Uma avaliação comparativa de A. lerchiana com o antisséptico Septarius test mostrou que A. lerchiana teve um efeito semelhante, em geral. Na forma de óleo essencial, superou até o efeito do Septário. Verificou-se que A. lerchiana superou as plantas medicinais utilizadas no experimento em relação ao efeito antibacteriano. Os resultados obtidos do estudo serão úteis para cientistas que pesquisam diversos medicamentos de origem vegetal e para especialistas veterinários que buscam medidas terapêuticas e preventivas.

Palavras-chave: Artemisia lerchiana, microrganismos, efeito antimicrobiano, óleo essencial, infusão.

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1. Introduction

Many antimicrobial substances, mainly of chemical origin, are not effective and reliable. Some are used as disinfectants or antiseptics for the skin and others for the prevention of infectious diseases in animals. In addition, many of them tend to accumulate in organs and tissues and have allergic and toxicological effects and side effects (Turzhigitova et al., 2022).

In recent years, scientific studies have confirmed the growth of microorganism strains resistant to all classes of chemical compounds (Temreshev et al., 2022). The absence of a unified scientific concept of microorganism control based on the laws of biology, physics, and chemistry does not make it possible to achieve any significant success in the sanitary and epidemiological protection of the population and animals (Bakhir et al., 2003; Fraise et al., 2013; Nosik et al., 2004). The allergic tendencies arising in the animal's body to existing antimicrobial substances and the formation of resistance of microorganisms can be called one of the most important problems.

To date, the search for new highly effective herbal preparations without side effects is becoming relevant in solving the main issues of veterinary medicine (Boranbayeva et al., 2023; Gayirbegov and Engurazov, 2023; Korotkiy et al., 2023; Smirnova et al., 2023). To do this, substances with an antimicrobial effect must meet several following requirements:

- they must have a wide spectrum of antimicrobial action, that is, effectively destroy bacteria, mycobacteria, viruses, fungi, and spores, regardless of the duration and frequency of use, ensuring that these preparations have properties that prevent the development of resistance in microorganisms;
- the preparations should not harm humans and animals, both during their preparation and application and after the end of their purposeful use, that is, during the period of degenerative and destructive changes caused by environmental factors or biodegradation processes in the human body. In other words, antimicrobial substances and products of their natural or artificial degradation should not contain xenobiotic substances;
- the antimicrobial substance should have a washing capacity with the least damaging and corrosive activity concerning various materials and be as easy to use and relatively cheap (Gunar et al., 2022; Lee et al., 2023).

Given the large number of commercially available herbal and natural medicines, selecting the most effective ones becomes difficult. The medicinal plant *Artemisia lerchiana* is of the greatest interest. In recent years, studies of antibacterial, viral, anti-inflammatory, nematocidal, and fungicidal properties of essential oils and extracts of medicinal plants have been of undoubted interest (Ćavar et al., 2012; Mamatova, 2018; Nibret and Wink, 2010; Verma et al., 2011). Among the class of metabolites of the *Artemisia* family, terpenoids are the most studied substances. A promising field in the treatment of inflammatory diseases is the local use of plant essential oils.

Recently, medicinal plants have been of the greatest interest, as they can be not only a basis for the development of agents with antimicrobial effects but also a source of compounds with the necessary transforming activity concerning the resistance of pathogens (Badea and Delian, 2014; Ghendov et al., 2018; Lenardis et al., 2011). Recent studies have shown that the formation of biofilm by microorganisms for resistance to antibiotics, phagocytosis, and other components of the body's defense system has led to the discovery and isolation of many compounds, such as monoterpenoids, sesquiterpenoids, flavonoids, and coumarins, as well as aliphatic and lipid compounds (Bajpai et al., 2014; Naili et al., 2010; Utkina et al., 2012).

A priority field is the study of the basic properties of essential oils and extracts of *Artemisia* growing in the Republic of Kazakhstan and the development of new medicines based on them. Kazakhstan has unique reserves of medicinal plant raw materials. 81 *Artemisia* species grow in Kazakhstan, and only a small part of them have been studied: *A. armeniaca Lam., A. atomentella, A. annua, A. pontica, A. tournefortiana, A. laciniata, A. semiarida, A. albida, A. marschalliana*), etc. (Mamatova, 2018).

Analyzing these data, we can state the prospect of studying *Artemisia* essential oils as active and auxiliary substances in the creation of new medicines for the treatment of infectious and inflammatory diseases associated with stable microflora. The antimicrobial effect of various *Artemisia* types also depends on the place of growth and extraction technology. Among the medicinal plants growing on the territory of Kazakhstan, only some *Artemisia* species have been studied. The *Artemisia* species are considered endemic species growing in the West Kazakhstan region. The determination of the antimicrobial action of biologically active substances contained in *A. lerchiana* emphasizes the importance of the study.

Therefore, the purpose of our research work was to conduct a comparative analysis of the antibacterial effect of *A. lerchiana* against other herbal preparations.

The objectives of the research included studying the antibacterial effect of *A. lerchiana* against gram-positive and gram-negative test microbes, as well as conducting a comparative assessment with other medicinal plants with antibacterial properties. The evaluation of the antibacterial effect was carried out in comparison with the Septarius chemical antiseptic.

2. Materials and Methods

2.1. Place and conditions of the study

To reach the set goals and objectives, we carried out experiments in the research laboratory of microbiology of the Zhangir Khan West Kazakhstan Agrarian and Technical University in equal conditions under a certain microclimate (t: 20°C, relative humidity: 55%, air velocity: 0.2 m/s).

2.2. Comparative analysis

The test preparation *A. lerchiana* was used in the form of an infusion and essential oil extract, which had been made by generally accepted methods of pharmacology. For comparative analysis, we used the infusions of *A. absinthium, Calendula officinalis L.*, and *Chamomilla recutita L.*, as well as preparations based on essential oils of Abies Sibirica, Eucalyptus, Limon, Bergamottae, Melaleuca alternifolia, and A. absinthium.

As a test standard for comparing antibacterial properties, we used the Septarius chemical preparation manufactured at a biofactory.

The experiment was carried out *in vitro* using test microbes of gram-positive *Staphylococcus aureus* and *Streptococcus* and gram-negative *Escherichia coli*.

2.3. Study of antibacterial properties

To study the antibacterial properties of infusions made of various plants, we used the antibacterial effect determination method for the preparation against test microbes.

To do this, we used meat-peptone broth (MPB) in test tubes (5 ml), to which 0.3 ml of the test microbe was added (using doses of 500 million, 1 billion, and 2 billion colony-forming units (CFU)) and the test preparations in doses of 0.5, 1, and 2 ml. Subsequently, the test tubes were placed in a thermostat for 24 hours. The results of the experiments were evaluated by the growth of microbes according to the turbidity standard with calibration in percentages and cross-calibration (Table 1). The control evaluation was carried out by counting microbes under a microscope, where colonies of microorganisms were counted in four fields per 1 cm². The calculation was carried out as a percentage relative to the turbidity standard of the test microbe.

In the control group, 1 ml of distilled water was used instead of the test preparation.

To study the antibacterial properties of essential oils of various plants, we used the disk diffuse method in the MPB. For this purpose, the MPB was used in Petri dishes, where one test microbe (*S. aureus, Streptococcus, E. coli*) was added. Subsequently, disks soaked in essential oils of various test plants or a Septarius solution were placed on the MPB.

After that, the Petri dishes were placed in a thermostat for 24 hours.

The results of the experiments were evaluated by determining the lysis zone in mm.

2.4. Stages of the study

The experiment was conducted in two stages. At the first stage, the antibacterial effect of *A. lerchiana* in various dilutions was determined (100%, 50%, 25%, 12.5%, 6.25%). In the second stage, the antibacterial effect of various plant essential oils was determined.

A comparative analysis was carried out concerning the antibacterial effect of preparations from various plants and Septarius. For reliability, all experiments were carried out in a fivefold repetition.

3. Results

Studies of the antibacterial efficacy of *A. lerchiana* against *Staphylococcus* (Table 2) showed its high effect at a 500 million CFU dose of microbes, regardless of the volume of the preparation. At a 1 billion CFU dose of microbes, the effect of the preparations remained at high volumes (1 and 2 ml), whereas in 0.5 ml of the preparation, there was a noticeable decrease in effect by 1 point (25%).

With a billion CFU dose of *S. aureus* microbes, the effect of the test preparation is defined as 50% at 1 and 2 ml and only 25% at 0.5 ml.

Studies of the antibacterial effect of other plant infusions have shown 50% effectiveness at 500 million and 1 billion CFU, while at 2 billion CFU no antibacterial effect was observed. The worst indicators of antibacterial effect were observed in the calendula infusion.

Studies of the antibacterial effect of Septarius showed an identical result with the preparation *of A. lerchiana*.

Studies of the antibacterial effect of *A. lerchiana* against *Streptococcus* (Table 3) showed a high effect at 500 million and 1 billion CFU doses of microbes in 1 and 2 ml.

A decrease in the effect of *A. lerchiana* (50%) was observed at a 2 billion CFU dose of microbes in 1 and 2 ml. In a volume of 0.5 ml, the preparation had almost no effect at a 2 billion CFU dose of microbes.

The antibacterial effect of *A. lerchiana* was higher than that of Septarius at a 2 billion CFU dose of microbes.

We noted the low antibacterial effect of *A. absinthum* and *C. recutita L.* infusion at 500 million and 1 billion CFU doses of microbes. The antibacterial effect was not observed in the tested preparations at a 2 billion CFU dose of microbes.

The infusion of *C. Officinalis L.* proved ineffective against microbes at doses of 1 billion and 2 billion CFU and showed a 50% effect at a 500 million CFU dose of microbes.

Studies of the antibacterial effect of *A. lerchiana* against *E. coli* (Table 4) showed a high effect at a 500 million CFU dose of microbes in 1 and 2 ml, whereas a 50% effect was observed in 0.5 ml and at a 1 billion CFU dose of microbes. In 1 and 2 ml, the antibacterial effect of *A. lerchiana* was not observed against *E. coli* microbes at a dose of 2 billion CFU.

Table 1.	Test microbe growth evaluation scale.	
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	Microflora growth rate									
Test microbe dosage in CFU	1-25%	26-50%	51-75%	76-100%						
	(low)	(average)	(substantial)	(high)						
500 million	+	++	+++	++++						
1 billion	+	++	+++	++++						
2 billion	+	++	+++	++++						

Table 2. Antibacterial effect of medicinal plant infusion against S. aureus.

	Volume of the preparations under study (ml)											
Infusion name	0.5	1.0	2.0	С	0.5	1.0	2.0	С	0.5	1.0	2.0	С
	Growth of S. aureus											
		500 mil	lion CFL	J	1 billion CFU				2 billion CFU			
Infusion of A. absinthum	++	+	+	++++	+++	++	++	++++	+++	+++	+++	++++
Infusion of C. officinalis L.	+++	++	++	++++	+++	+++	+++	++++	+++	+++	+++	++++
Infusion of C. recutita L.	+++	++	++	++++	+++	++	++	++++	+++	+++	+++	++++
Infusion of A. lerchiana	+	+	+	++++	++	+	+	++++	+++	++	++	++++
Septarius	+	+	+	++++	++	+	+	++++	++++	++	++	++++

C is the control group; CFU is the colony-forming units.

Table 3. Antibacterial effect of medicinal plant infusion against Streptococcus.

	Volume of the preparations under study (ml)											
Infusion name	0.5	1.0	2.0	С	0.5	1.0	2.0	С	0.5	1.0	2.0	С
infusion name	Growth of <i>Streptococcus</i>											
	500 million CFU				1 billion CFU				2 billion CFU			
Infusion of A. absinthum	++	+	+	++++	+++	++	++	++++	+++	+++	+++	++++
Infusion of C. officinalis	+++	++	++	++++	+++	+++	+++	++++	+++	+++	+++	++++
Infusion of C. recutita	+++	++	++	++++	+++	++	++	++++	+++	+++	+++	++++
Infusion of A. lerchiana	+	+	+	++++	++	+	+	++++	+++	++	++	++++
Septarius	+	+	+	++++	++	+	+	++++	+++	++	++	++++

C is the control group; CFU is the colony-forming units.

Table 4. Antibacterial effect of medicinal plant infusion against E. coli.

Volume of the preparations under study (ml)												
Infusion name	0.5	1.0	2.0	С	0.5	1.0	2.0	С	0.5	1.0	2.0	С
infusion name	Growth of E. coli											
		500 mil	lion CFU	I	1 billion CFU				2 billion CFU			
Infusion of A. absinthum	+++	+++	++	++++	++	+++	++	++++	+++	++++	++++	++++
Infusion of C. officinalis	+++	+++	+++	++++	+++	+++	+++	++++	+++	++++	++++	++++
Infusion of C. recutita	++	++	++	++++	+++	+++	+++	++++	+++	++++	++++	++++
Infusion of A. lerchiana	++	+	+	++++	++	++	++	++++	+++	+++	+++	++++
Septarius	+	+	+	++++	++	+	+	++++	++	++	++	++++

C is the control group; CFU is the colony-forming units.

Septarius showed a high antibacterial effect at a dose of microbes of 500 million and 1 billion CFU in 1 and 2 ml and a 50% effect in other studies.

The infusion of *C. recutita L.* showed a 50% antibacterial effect at a 500 million CFU dose of microbes while in other cases, this preparation had no effect.

Besides, infusions of A. Sibirica and C. Officinalis L. also showed no effect against Escherichia coli.

In all experiments in the control group, an intensive growth of microbes was observed, which proves the high activity of test microbes.

Thus, the results of experiments show the effectiveness of *A. lerchiana* infusion against *S. aureus, Streptococcus,* and *E. coli* at a 500 million CFU dose of microbes. A comparison of this preparation with Septarius, as an antiseptic standard, shows the identity of the result concerning *S. aureus* and *Streptococcus. A. lerchiana* was less effective against *E. coli* compared to Septarius.

Comparative analysis of various herbal infusions with *A. lerchiana* infusion shows a high antibacterial effect of the test preparation against both gram-positive (*S. aureus, Streptococcus*) and gram-negative (*E. coli*) test microbes. The infusion of *C. Officinalis L.* showed a very low antibacterial effect, while the infusions of *A. Sibirica and C. recutita L.* were 50% effective.

The results of the study allow us to conclude that there is a direct correlation between the volume of the preparation for an antibacterial effect and an inverse correlation between the dose of microbes for an antibacterial effect.

In the following experiment, we conducted studies to determine the antibacterial effect of the essential oils of the test preparation *A. lerchiana* and other herbal preparations widely used in veterinary medicine. Septarius was used as a test standard.

To determine the antibacterial effect *in vitro*, the method of disk diffusion in MPB was used.

Cultures of *S. aureus*, *Streptococcus*, and *E. coli* in doses of 500 million and 2 billion CFU were used as test microbes.

In the first part of the experiment, we studied the antibacterial effect of *A. lerchiana* in various doses (100%, 50%, 25%, 12.5%, 6.25%) against *S. aureus, Streptococcus,* and *E. coli* at a dose of 500 million CFU (Figure 1).

The results of the experiment showed that the high concentration of *A. lerchiana* had a strong effect on all microbe cultures, especially on *S. aureus*.

Reducing the concentration to 50 and 25% reduced the antibacterial effect from 20 to 13-17 mm (by 35-50%) for *Staphylococcus* and *Streptococcus*. At concentrations of 12.5 and 6.25%, the antibacterial effect was determined as 30-40% against *Staphylococcus* and *Streptococcus*.

A decrease in the concentration of *A. lerchiana* essential oil significantly reduced the antibacterial effect against *E. coli* (at 50% concentration to 45%, at 25% concentration to 30%), while at a concentration of 12.5% and below, no antibacterial effect was observed.

Thus, the antibacterial effect of *A. lerchiana* against *Staphylococcus* and *Streptococcus* was noticeable even with a decrease in its concentration. Whereas the effect of *A. lerchiana on E. coli* was lower. We noted a direct correlation between its antibacterial effect against all microbe cultures.

In the second part of the experiment, we conducted a comparative analysis of the antibacterial effect of *A. lerchiana* compared to other plant-based preparations against the three types of microbial cultures. Septarius was used as a test antiseptic (Figure 2).

According to the results of the study, we observed a high antibacterial effect of *A. lerchiana* against *Staphylococcus* (24 mm at 500 million CFU, 20 mm at 1 billion CFU). Septarius showed an effect against *Staphylococcus* equaling 17 mm at 500 million CFU and 14 mm at 1 billion CFU. The remaining preparations showed an effect level of less than 13 mm.

Studies of the effect of essential oils on *Streptococcus* showed that *A. lerchiana* has the highest effect (19 mm at 500 million CFU and 15 mm at 1 billion CFU). Septarius has a relatively high effect (16 mm at 500 million CFU

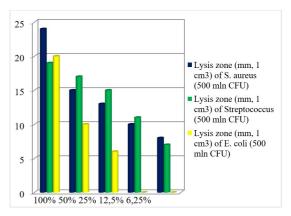


Figure 1. Diagram of the antibacterial effect of A. lerchiana in various doses against the test microbes.

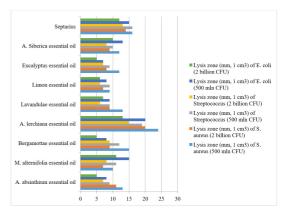


Figure 2. Diagram of the antibacterial effect of essential oils obtained from various plants against the test microbes in diffuse agar.

and 13 mm at 1 billion CFU). The remaining preparations were below the level of 12 mm.

A low antibacterial effect was observed in all preparations against *E. coli. A. lerchiana* ranged from 20 to 13 mm, and *M. alternifolia* and Septarius from 15 mm to 11 mm. The remaining preparations were below the level of 3 mm.

Thus, according to the results of experiments, we have noted the high antibacterial effect of *A. lerchiana* against *Staphylococcus* and *Streptococcus*, while the essential oils of other medicinal plants showed a low antibacterial effect. As for E. coli, the antibacterial effect of *A. lerchiana*, *A. Sibirica*, *M. alternifolia* essential oils and Septarius was higher than that of other medicinal plants (above 11 mm).

4. Discussion

The antimicrobial effect of different *Artemisia* species varies depending on the microorganisms (*S. aureus, S. epidermidis, E. coli, C. albicans*) and their effect on the ability of microorganisms to form biofilms. According to the study data, a bactericidal effect was detected in

14 Artemisia species used in the studies. Nine Artemisia species demonstrated bactericidal properties against S. epidermidis, six Artemisia species against S. aureus, and 11 Artemisia species against E. coli (Kartashova et al., 2009). The results of our study showed that A. lerchiana had a high antibacterial effect against gram-positive microbes (Staphylococcus and Streptococcus). A. lerchiana was also highly effective against gram-negative E. coli but at a low dose of the test microbe (500 million CFU). With an increase in the dose of microbes, the growth rate of the microbes will increase to a high (76-100%), which is associated with a low antibacterial effect. After evaluating the antibacterial effect of A. lerchiana in comparison with Septarius, we note that it is not less effective than the test antiseptic against gram-positive microbes (Staphylococcus and Streptococcus). The effect is high for small doses of test microbes and average for large doses of test microbes (2 billion CFU).

A comparative analysis of *A. lerchiana* infusion with other infusions of the studied medicinal plants showed its superiority by 25% concerning *Staphylococcus, Streptococcus,* and *E. coli.* Only *A. absinthium* corresponded to the effect level of *A. lerchiana* at low doses of the test microbe (500 million CFU). Considering that the concentration of antibacterial active substances is higher in essential oils of plants than in their infusions, we note a more pronounced effect of the studied preparations on test microbes in the form of essential oils. Tsedenova et al. (1999) in their studies also show that some components of essential oil (1,8-cineol, borneol, α -thujone, bornyl acetate, α -thujone) obtained from *A. lerchiana* protect the body from pathogens and have an active fungicidal and bactericidal effect.

Studies of the antibacterial effect of *A. lerchiana* essential oil, depending on its concentration, have shown its effectiveness against *Staphylococcus* and *Streptococcus* even in low concentrations. However, the antibacterial effect against *E. coli* has been observed only at high concentrations. At low concentrations, there is no lysis zone. The results of our study are confirmed by the studies of other scientists from different countries and different climatic zones (Salimov et al., 2019).

Comparative analysis of *A. lerchiana* with other medicinal plants showed its high antibacterial efficacy against all test microbes. The preparations closest to the effect level of *A. lerchiana* were *A. Sibirica* and *M. alternifolia* against *Staphylococcus* and *Streptococcus*, while the rest of the tested preparations were ineffective against all test microbes. In the evaluation of *A. lerchiana*, its antibacterial effects can be considered high, while the rest of the preparations had an average evaluation result. In other studies, an extract of *A. lerchiana* based on methanol and acetone also showed a bacteriostatic effect against *Russula aeruginosa* 1390, as well as against two other strains (*E. coli* K16, *Bacillus subtilis* 168) (Sotirova et al., 2022).

We observed a direct dependence of the antibacterial effect of the preparation on its volume and the dose of test microbes. That is, the higher the volume of the preparation, the more effective the action, and the higher the dose of the test microbe, the more the antibacterial effect decreases. Even in small volumes and doses, *A. lerchiana* has a good antibacterial effect.

The obtained results of the study will be useful for scientists researching various medicinal products of plant origin and for veterinary specialists looking for therapeutic and preventive measures.

5. Conclusion

The results of the study show that *A. lerchiana* has a high antibacterial effect against gram-positive test microbes (*Staphylococcus* and *Streptococcus*). The most effective preparation is *A. lerchiana* in the form of essential oil when even its low concentration has an antibacterial effect.

A comparative assessment of *A. lerchiana* with Septarius shows that *A. lerchiana* has a similar effect in general and in the form of essential oil even surpasses the effect of Septarius. *A. lerchiana* surpasses medicinal plants used in the experiment in terms of antibacterial effect.

Thus, the results of the study allow us to recommend *A. lerchiana* to be used in production as an effective antibacterial agent. The study was limited to the comparative analysis of antibacterial effect of *Artemisia lerchiana*. Further research should be directed to the practical study of the effect of *Artemisia lerchiana* in the medical and veterinary activities.

Acknowledgments

This research was funded by the Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan (Grant N° AP15473422 «Determination of efficacy of Artemisia lerchiana based medication in the treatment of surgical injuries in animals»).

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