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Comprehensive Evaluation of Natural Antiseptic Compounds for Veterinary Use

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ABSTRACT

In modern practical disinfection science, it is asserted that ideal chemical agents, besides having high antimicrobial activity and certain other properties, should also be environmentally safe, have minimal toxic effects on humans and animals, and be easy to dispose of after use. Our research aimed to evaluate the antimicrobial, cumulative, toxicological, and allergenic properties of anolyte, ozone, Shungite, propolis, and their complexes shozan, prozan, and shuprosan. To study the antimicrobial properties of the substances above and their complexes *in vitro*, we used the method of microbial culture growth inhibition in meat-peptone broth (MPB) and the disk diffusion method on meat-peptone agar (MPA) with test microbes - gram-positive cocci *Staphylococcus aureus* and gram-negative rods *Escherichia coli*. The results of the experiments were evaluated based on turbidity standards and the determination of the lysis zone. The toxicological properties of the substances were determined in white mice by intraperitoneal injection of the said substances. Our study's results indicate that ozone, anolyte, Shungite infusion, and propolis infusion while demonstrating the antimicrobial activity of classical antiseptics, their complexes - shodan, frozen, and shuprosan - exhibit antimicrobial activity comparable to classical antiseptics such as ethanol and hydrogen peroxide against *S. aureus* and *E. coli*. Importantly, these complexes of natural origin antiseptics have been found to be safe, not showing toxicological, cumulative, or allergenic effects on laboratory animals, and meeting the required standards. This provides a strong basis for their potential use as antiseptic agents in medicine and veterinary practice.

Key words: Anolyte, Ozone, Shungite, Propolis, Antimicrobial activity, Cumulative

INTRODUCTION

The current complex epidemiological and epizootic situation justifies increased attention to infectious disease prevention and heightened requirements for the quality of disinfection measures aimed at eliminating pathogens in environmental objects that act as transmission factors (Vetkina et al. 2005).

Modern disinfectants are typically composed of a balanced formula that includes one or more active substances in ratios that achieve maximum synergy or potentiation of effects against the most resistant microorganisms, along with functional additives that purposefully alter their properties.

Currently, the market offers a large number of disinfectants. These products are developed and held to reliability, effectiveness, and safety standards. In modern practical disinfection science, it is asserted that ideal chemical agents, besides having high disinfecting activity and certain other properties, should also be environmentally safe, have minimal toxic effects on humans and animals, and be easy to dispose of after use (Asanova et al. 2015).

It is known that a disinfectant should meet several requirements:

1. It should have a broad spectrum of antimicrobial action, i.e., effectively kill bacteria, mycobacteria, viruses, fungi, and spores regardless of the duration and frequency of use, implying properties that prevent microorganisms from developing resistance.
2. It should be safe for humans and animals during its preparation and use, as well as after its intended use, i.e., during the period of degradation and destructive changes under the influence of environmental factors

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or due to biodegradation processes in the human body. In other words, the antimicrobial agent and its natural or artificial degradation products should not contain xenobiotic substances.

3. The antimicrobial agent should have universal action, i.e., possess antimicrobial properties and cleaning capabilities with minimal damaging and corrosive activity towards various materials. It should also be easy to use and relatively inexpensive (Reuter 1988; Votavá et al. 2005; Röhm-Rodowald et al. 2008; Miller et al. 2012; Bocian et al. 2014; Nametov et al. 2014; Ahmed et al. 2024; Cai et al. 2024; Večerková et al. 2024; Xue et al. 2024).

Analysis of the practical use of all disinfectants has revealed their non-compliance with modern requirements for specific effectiveness and hygienic safety. To address this problem, natural, non-chemical antiseptics are of great importance. Among the substances that meet the above requirements are:

- Anolyte ANK has an antimicrobial spectrum of action at a very low concentration of active substances (500mg/L); it is safe for humans and the environment; its active substances and degradation products do not accumulate in the external environment. The active substances are a mixture of highly active metastable (electrochemically activated) chlorine-oxygen and hydroperoxide compounds (oxidants). This combination of active substances ensures the absence of microorganism adaptation to the biocidal action of anolyte ANK, and the low total concentration of active oxygen and chlorine compounds guarantees complete safety for humans and the environment with prolonged use (Bakhir 1998; Aliyev et al. 2018; Prokopenko et al. 2019; Kuklin et al. 2022; Buyanov et al. 2023; Styazhkina et al. 2023).
- Ozone (O3), a strong oxidizer, is used to disinfect and sterilize food products and raw materials to prevent spoilage and ensure maximum shelf life. It is a gaseous chemical agent capable of oxidizing various organic and inorganic compounds through interaction. Continuous rearrangement of various molecules causes the natural decomposition of ozone into oxygen, preventing accumulation. Because ozone converts to oxygen and leaves no trace except for its reaction with organic compounds and the formation of safe by-products, it is of great interest as a sterilization method. These properties allow ozone to be used as an additional agent to other conventional disinfectants to reduce side effects (Bakhchevnikov and Braginets 2021; Kaavya et al. 2021; Maryam et al. 2021; Donato et al. 2024).

Shungite carbon is a petrified fullerene-containing substance from highly carbonized organic bottom sediments. Shungite stone, with its rich mineral composition and pronounced sorption, bactericidal, and catalytic properties, has been successfully used for many years in drinking water purification and activation systems. Additionally, water infused with Shungite, which is a molecular-colloidal solution of hydrated fullerenes, has anti-inflammatory, bactericidal, antiseptic, analgesic, antiallergenic, and immunostimulatory effects on the human and animal bodies (Tremasova and Beletsky 2012; Khromushkin et al. 2014; Jurgelane and Locs 2021; Ignatov et al. 2022).

Propolis is a product bees process from resinous substances collected from plants. It is non-toxic even after prolonged use, does not suppress the normal microflora of the gastrointestinal tract, does not lead to dysbiosis or a reduction in natural immunity, and enhances the body's defenses. The antibacterial and immunomodulating properties of propolis are used in medicine and veterinary practice for disease prevention in animals (Karomatov 2018; Saidova et al. 2021; Betancur-D'Ambrosio et al. 2024; Talib Al-Sudani et al. 2024).

In comparison, conventional, classical antiseptics, both organic (ethanol) and inorganic (hydrogen peroxide), have toxicological and allergenic effects. According to the literature, ethanol irritates skin receptors, causes dehydration, and leads to protein coagulation in cells. Hydrogen peroxide, when administered parenterally, has toxic effects and irritates skin receptors (Selvam et al. 2023; González-Penagos et al. 2024; Gordon and Nadolski 2024; Li et al. 2024).

Among the numerous natural substances with antiseptic properties, anolyte, ozone, Shungite, and propolis are of the greatest interest. However, studies on the antiseptic properties of these substances have been fragmented and non-comprehensive. The breadth of their action has not been fully explored, and their antiseptic properties and effects on micro- and macroorganisms need deeper investigation. The pharmacokinetics and pharmacodynamics of these substances, as well as their side and undesirable effects on animals, have not been studied. Moreover, there have been no studies on the antiseptic effectiveness of the complexes of anolyte, ozone, Shungite, and propolis, or their interactions—whether synergistic or antagonistic. This research, therefore, presents a novel and comprehensive approach to understanding the properties of these substances and their complexes.

Therefore, the aim of our research was to evaluate the antimicrobial, toxicological, cumulative, and allergenic properties of natural substances such as anolyte, ozone, Shungite, and propolis, as well as their complexes. This research is crucial in providing a comprehensive understanding of the potential benefits and risks associated with these substances, thereby contributing to the development of safer and more effective antiseptic preparations.

MATERIALS AND METHODS

This work was carried out within the framework of the project funded by the grant from the Committee of Science of the ministry of Science and Higher Education of the Republic of Kazakhstan, AP19679451 - Development of an environmentally safe antiseptic preparation based on natural substances and compounds for use in veterinary medicine and medicine.

Neutral Anolyte ANK was meticulously and precisely produced using the STEL-10N-120-01 mod. 120 IIP installation through the electrochemical treatment of a 0.9g/L sodium chloride solution in distilled water. The resulting product is a colorless, clear liquid with a chlorine smell, containing highly active chlorine-oxygen compounds. The primary active substances of this metastable solution are chlorinated oxygen and hydroperoxide oxidants. The total content of dissolved substances in Anolyte ANK at an oxidant concentration of 500mg/L is no more than 5.0g/L. Anolyte ANK was stored in laboratory conditions at room temperature in tightly closed glass bottles, protected from direct sunlight, and used without prior preparation or dilution.

Aqueous ozone was produced using a DICHO ozonator (model TQ-Z08) by placing the device's nozzle in a container with distilled water for 10-20min. This resulted in an ozonated solution with a saturation concentration of 1.5mg/L.

To prepare Shungite water infusion, a 0.5kg sample was repeatedly washed with tap water. The Shungite was placed in a sterile five-liter glass bottle filled with distilled water, tightly sealed with a plastic lid, and infused at room temperature for three days. The resulting infusion was stored in laboratory conditions at room temperature in tightly closed glass bottles, protected from direct sunlight, and used without prior preparation or dilution.

To prepare propolis water infusion, a 50-gram sample was frozen in a household freezer and grated. The grated propolis was then added to a container with 500mL of distilled water and infused in a water bath for 1 hour, resulting in a 10% propolis infusion. The infusion was cooled to room temperature, filtered through sterile gauze, and stored in tightly closed glass bottles in a refrigerator at +4°C. It was used without prior preparation or dilution.

The Shungite+Ozone+Anolyte complex ("Shozan") was obtained by meticulously mixing equal parts of Shungite infusion and ozonated anolyte. The Propolis+Ozone+Anolyte complex ("Prozan") was prepared with the same meticulousness by mixing equal parts of propolis infusion and ozonated anolyte. The Shungite+Propolis+Ozone+Anolyte complex ("Shuprozan") was prepared by meticulously mixing equal Shungite and propolis infusions with ozonated anolyte.

Study of antimicrobial properties

In vitro studies of the antimicrobial properties of the substances above and their complexes were carried out using the microbial growth inhibition method on MPB (meat-peptone broth) and the disk diffusion method on MPA (meat-peptone agar).

To determine the antimicrobial activity of the substances and their complexes by the microbial growth inhibition method on MPB, a comprehensive and thorough approach was taken. Test microbes—gram-positive *Staphylococcus aureus* and gram-negative *Escherichia coli*—were used. Cultures of *S. aureus* (strain 209P) and *E. coli* (strain ATCC 25922) were inoculated in test tubes on slant MPA and incubated in a thermostat at 37°C for 24 hours. The prepared substances and their complexes were incubated for 24 hours at room temperature, after which they, along with classical antiseptics (70% ethanol solution and 3% hydrogen peroxide solution), were added to MPB at concentrations of 0.5, 1.0, and 2.0mL per 5.0mL of nutrient medium. The same medium was then inoculated with suspensions of staphylococcus and *E. coli* prepared according to the McFarland standard (McFarland 1907), with microbial counts of 500 million, 1 billion, and 2 billion CFU/mL. Control tubes did not contain antiseptic solutions. The tubes were incubated in a thermostat at 37°C for 24 hours and the growth of the cultures was monitored. Studies were conducted on a photoelectric colorimeter (PEC) with a red filter at a wavelength of 490nm using cuvettes with a working length of 10mm to determine the optical density of the contents of the control and experimental tubes. The results were assessed based on the number of CFUs calibrated against the McFarland standard. Quantitative microbial indicators were converted into percentages relative to the turbidity standard. The antimicrobial activity was evaluated based on microbial growth intensity in percentages: high – 0-25%, medium – 26-50%, below average – 51-75%, low – 76-100%. According to the methodology, 24-hour exposure in the control yielded microbial growth equal to the 100% turbidity standard.

The results of the study on the antimicrobial activity of the substances and their complexes by the disk diffusion method are of significant importance. Test microbes *S. aureus* and *E. coli* were inoculated on MPA. MPA was poured into sterile Petri dishes to a thickness of 4±0.5mm. Bacterial suspensions from 24-hour cultures of microorganisms grown on MPA were prepared according to the McFarland standard (500 million, 1 billion, and 2 billion CFU/cm^3). Several uniform, clearly isolated colonies grown on MPA were selected, and a loop was used to transfer a small amount of material from the tops of the colonies into a tube with a sterile physiological solution, adjusting the suspension density according to the McFarland standard. The suspension was used within 15min of preparation. Petri dishes with MPA were inoculated with the test microbe suspensions (*S. aureus* and *E. coli*). Subsequently, disks impregnated with ozone, anolyte, propolis, Shungite, their complexes (Shozan, Prozan, Shuprozan), and baseline antiseptics (70% ethanol solution and 3% hydrogen peroxide solution) were placed on the MPA. No more than four disks with different test substances were placed on one 100mm diameter dish. The Petri dishes were then incubated at 37°C for 24 hours. Antimicrobial activity was evaluated based on the lysis zone of colony growth in millimeters: high – 12-10mm, medium – 9.5-7mm, below average – 6.5-5mm, low – 3-0mm. All experiments were performed in triplicate. Control disks were placed without substances. To determine the cumulative coefficient, a method based on monitoring animal deaths upon repeated substance administration was used - a subchronic toxicity test per the "Guidance for Experimental (Preclinical) Study of New Pharmacological Substances." The formula determined the cumulative coefficient (K_cum):

K_cum = LD_{50}(n) : LD_{50}

Where:

LD_{50}(n) - median lethal dose upon \(n \)-time administration;

LD_{50} - median lethal dose upon single administration.

The research was conducted in the scientific laboratory of the veterinary clinic at Zhangir Khan WKATU. Animals were kept in separate cages according to sanitary regulations and on a standard diet by established norms. For the experiment, 40 mixed-breed white mice were selected and divided into test and control groups of 10 animals each. Comprehensive preparations "Shozan," "Prozan," and "Shuprozan" were orally administered to the test groups of mice for the first four days at a dose of 2mL, then the dose was increased by 1.5 times every subsequent four days. "Shozan" was administered to the first group of animals, "Prozan" to the second group, and "Shuprozan" to the third group. The fourth control group of mice was administered 2mL of water. The experiment lasted for 28 days; throughout the experiment, observations were made on the animals, considering their condition and level of activity. Additionally, the overall condition (excitement, depression), character and degree of activity and coordination of movements, reaction to pain stimuli, presence of tremors, convulsions, paresis, paralysis, discharge from eyes, nose, urinary tract, changes in color of skin coverings, changes in body weight, and appetite were evaluated (Weisbrod et al. 2009; Zashchepkina 2019; Kornilov et al. 2020; Kutsova et al. 2020).

To determine the toxicological properties of the preparations, their complexes, and classical antiseptics (20% ethanol and 1% hydrogen peroxide), 100 sexually mature, mixed-breed white mice weighing 20-27 grams were used, which were divided into 10 groups of 10 animals each. The animals were housed in a vivarium by sanitary rules and on a standard diet according to established norms. The first group of animals was administered ozone water, the second - anolyte, the third - Shungite infusion, the fourth - propolis infusion, the fifth - shozan, the sixth - prozan, the seventh - shuprozan, the eighth - 20% ethanol solution, the ninth - 1% hydrogen peroxide solution. The tenth group of animals was administered distilled water as a control. All preparations were administered once, intraperitoneally, at a dose of 1.0mL, observing aseptic and antiseptic rules. Clinical observations were conducted for three days; throughout the experiment, observations were made on the animals, considering their condition, level of activity, evaluating the overall condition (excitement, depression), coordination of movements, reaction to pain stimuli, presence of tremors, convulsions, paresis, paralysis, discharge from eyes, nose, urinary tract, changes in skin color, changes in body weight, and appetite. The mice of each group were kept in separate cages. At the end of the experiment, pathological-anatomical autopsies were performed on the white mice. It is important to note that the conditions of handling and housing the animals used in the experiment complied with generally accepted ethical norms and standards, ensuring their welfare and minimizing any potential harm (Medved et al. 1968; Baran et al. 2018).

To study the allergic properties of the test preparations, their complexes, and classical antiseptics - ethanol and hydrogen peroxide solutions, we used chinchilla breed rabbits. The animals were housed in a vivarium by sanitary rules and on a standard diet according to established norms. The allergic properties were determined by applying the test preparations to the rabbit's mucous membranes (conjunctival reaction) and rubbing them into the animal's skin (skin-resorptive reaction). To determine the conjunctival allergic reaction, 100 rabbits were divided into 10 groups of 10 animals each. The first group of animals was administered one drop of ozone water into the conjunctival sac on the right side and one drop of distilled water on the left. The second group received anolyte on the right side, the third - Shungite infusion on the right side, the fourth - propolis infusion on the right side, the fifth - shozan on the right side, the sixth - prozan on the right side, the seventh - shuprozan on the right side, the eighth - 20% ethanol solution on the right side, the ninth - 1% hydrogen peroxide solution on the right side, and distilled water was applied on the left side in all groups. The tenth group of animals received distilled water into both eyes, serving as a control. The reaction of the conjunctival mucosa and cornea and clinical changes in the animals' condition were recorded after 5, 15min, 1, and 3 hours. These findings are crucial in understanding the potential allergic reactions to these preparations and antiseptics.

To determine the skin-resorptive reaction, 100 rabbits were divided into 10 groups of 10 animals each. In the first group, rabbits had their hair trimmed and shaved on both sides of the scapula, in an area of 5 x 5cm, then the skin was dried with a sterile swab, and ozone water was rubbed into the skin on the right side, while distilled water was rubbed on the left side. In the second group, anolyte was applied on the right side, in the third - Shungite infusion, in the fourth - propolis infusion, in the fifth - shozan, in the sixth - prozan, in the seventh - shuprozan, in the eighth - 70% ethanol solution, in the ninth - 3% hydrogen peroxide solution, and distilled water was applied on the left side in all groups. The tenth group of animals received distilled water rubbed into the skin on both sides, serving as a control. The skin reaction, as well as clinical changes in the animals' condition, was observed after 30min, one, three, and four hours. Importantly, the conditions of handling and housing the animals used in the experiment were in strict compliance with generally accepted ethical norms and standards, ensuring the welfare of the animals throughout the study (McFarland 1907).

RESULTS AND DISCUSSION

In the study of the antimicrobial activity of ozone, anolyte, propolis, and Shungite by the method of growth inhibition of microbial culture on Mueller-Hinton agar (MHA), it was determined that regarding *Staphylococcus aureus* at a microbial dose of 500 million CFU/cm^3 with a volume of preparations of 0.5; 1.0; 2.0mL on MHA, they showed a decrease in the growth of *S. aureus*. At a microbial dose of 1 billion CFU/cm^3 with a volume of preparations of 0.5; 1.0; 2.0mL on MHA, they exhibited below-average antimicrobial activity, which is determined by the good growth of *S. aureus*. At a microbial dose of 2 billion CFU/cm^3 with a preparation volume of 0.5; 1.0; 2.0mL, the preparations did not show antimicrobial activity, which is determined by saturated growth of *S. aureus*. In the control, saturated growth of *S. aureus* was noted.

In the study of the antimicrobial activity of complex preparations anolyte+ozone+Shungite (shozan), anolyte+ozone+propolis (prozan), and anolyte+ozone+Shungite+propolis (shuprozan) by the method of growth inhibition of microbial culture on MHA, it was determined that regarding *S. aureus* at a microbial dose of 500 million CFU/cm^3 and 1 billion CFU/cm^3 with a volume of preparations of 0.5, 1.0, and 2.0mL on MHA, they showed moderate antimicrobial activity, which is determined by satisfactory growth of *S. aureus*. At a microbial dose of 2 billion CFU/cm^3 with a volume of preparations of 0.5, 1.0, and 2.0mL, the bacteriostatic effect slightly decreased but remained sufficiently effective, especially at a dose of 2.0mL.

In the study of the antimicrobial activity of classical antiseptics (70% ethanol and 3% hydrogen peroxide) by the method of growth inhibition of microbial culture on MHA, it was determined that regarding *S. aureus* at a microbial dose of 500 million CFU/cm^3 with a volume of preparations of 0.5; 1.0; 2.0mL on MHA, they showed high antimicrobial activity, which is determined by weak growth of *S. aureus*. At a microbial dose of 1 billion CFU/cm^3 with a volume of preparations of 0.5; 1.0; 2.0mL on MHA, these preparations showed moderate antimicrobial activity, which is determined by satisfactory growth of *S. aureus*. At a microbial dose of 2 billion CFU/cm^3 with a volume of preparations of 0.5; 1.0; 2.0mL on MHA, classical antiseptics showed below-average antimicrobial activity, which is determined by good growth of *S. aureus*. In the control, saturated growth of *S. aureus* was noted. Similar, a recent study by Abdelshafy et al. (2024) showed that hydrogen peroxide's efficacy diminishes significantly at higher microbial concentrations, consistent with our findings of below-average antimicrobial activity at 2 billion CFU/mL.

Thus, when studying the antimicrobial activity of ozone, anolyte, propolis, Shungite, their complexes shozan, prozan and shuprozan, as well as classical antiseptics (ethanol 70% and hydrogen peroxide 3%) by the method of growth retardation of microbial culture on BCH against *S. aureus* (Naliukhina et al. 2024), it was determined that the activity of natural antiseptics individually was lower, than their complex preparations. The antimicrobial activity of the basic antiseptics was no higher than the complex preparations. That is, with the combined, complex use of natural antiseptics, their antimicrobial activity increases, and synergy is observed (Table 1).

Our study on the antimicrobial activity of ozone, anolyte, propolis, and Shungite, using the method of inhibiting microbial growth in nutrient broth, a widely accepted method in the field, revealed that these substances, when tested against *Escherichia coli*, demonstrated moderate antimicrobial activity (Buienbayeva et al. 2024). This was indicated by the satisfactory growth of *E. coli*. However, when the bacterial dose was increased to 1 billion CFU/cm³, the substances showed below-average antimicrobial activity, as evidenced by the excellent growth of *E. coli*. At a bacterial dose of 2 billion CFU/cm³, the substances did not exhibit any antimicrobial activity, indicated by dense growth of *E. coli*. The control group also showed dense growth of *E. coli*.

In the study of the antimicrobial activity of the complex preparations anolyte + ozone + Shungite (Shozan), anolyte + ozone + propolis (Prozan), and anolyte + ozone + Shungite + propolis (Shuprosan) using the method of inhibiting microbial growth in NB, it was found that against *E. coli*, with a bacterial dose of 500 million and 1 billion CFU/cm^3 and volumes of 0.5, 1.0, and 2.0mL of the tested substances in NB, they demonstrated moderate antimicrobial activity. Specifically, the complex preparations showed satisfactory growth of *E. coli*. At a bacterial dose of 2 billion CFU/cm^3 with the same volumes of substances in NB, the complex preparations showed below-average antimicrobial activity, as evidenced by good growth of *E. coli*. In the control group, dense growth of *E. coli* was also observed.

In the study of the antimicrobial activity of classic antiseptics (70% ethanol and 3% hydrogen peroxide) using the method of inhibiting microbial growth in NB, it was found that against *E. coli*, with a bacterial dose of 500 million CFU/cm^3 and volumes of 0.5, 1.0, and 2.0mL of the tested substances in NB, they demonstrated high antimicrobial activity, as indicated by weak growth of *E. coli*. At a bacterial dose of 1 billion CFU/cm^3 with the same volumes of substances in NB, these substances showed moderate antimicrobial activity, as evidenced by satisfactory growth of *E. coli*. At a bacterial dose of 2 billion CFU/cm³ with the same volumes of substances in Broth, the classic antiseptics showed below-average antimicrobial activity, as evidenced by good growth of *E. coli*. In the control group, dense growth of *E. coli* was also observed.

Our study on the antimicrobial activity of ozone, anolyte, propolis, Shungite, and their complex preparations Shozan, Prozan, and Shuprosan, as well as classic antiseptics (70% ethanol and 3% hydrogen peroxide) using the method of inhibiting microbial growth in Broth against *E. coli*, revealed a promising potential. The individual activity of natural antiseptics was low, but the complex preparations of natural antiseptics demonstrated more pronounced antimicrobial properties. This finding highlights the potential of combined, complex application of natural antiseptics to enhance their antimicrobial activity, showcasing a synergistic effect (Table 2).

Table 1: Results of the determination of the antibacterial activity of drugs in relation to *Staphylococcus aureus* by the method of growth retardation of microbial culture on broth

No.	Drugs	The volume of the investigated drugs (mL)											
		0.5	1.0	2.0	K	0,5	1,0	2,0	K	0,5	1,0	2,0	K
		Presence of *Staphylococcus aureus* growth according to Fermentative Blood Volume (FBV) readings											
		500 million CFU				1 billion CFU (colony-forming units)				2 billion CFU (colony-forming units)			
1.	Ozone	58	28	20	100	81	64	66	100	95	91	89	100
2.	Anolyte	55	32	23	100	88	74	70	100	98	96	92	100
3.	Propolis	56	38	22	100	87	62	61	100	94	88	82	100
4.	Shungite	59	33	28	100	85	64	63	100	92	88	84	100
5.	Shozan	54	31	19	100	58	57	41	100	73	68	59	100
6.	Prozan	50	37	23	100	59	52	48	100	79	82	66	100
7.	Shuprozan	48	32	18	100	59	51	45	100	75	71	69	100
8.	Ethanol	22	17	15	100	52	41	35	100	73	67	65	100
9.	Peroxide	24	18	12	100	59	38	31	100	75	68	66	100

Table 2: Results of determining antibacterial activity of substances against *Escherichia coli* using the method of inhibiting microbial growth in broth

No.	Drugs	The volume of the investigated drugs (mL)											
		0.5	1.0	2.0	K	0.5	1.0	2.0	K	0.5	1.0	2.0	K
		Presence of *Staphylococcus aureus* growth according to Fermentative Blood Volume (FBV) readings											
		500 million CFU				1 billion CFU (colony-forming units)				2 billion CFU (colony-forming units)			
1.	Ozone	67	35	23	100	77	61	51	100	88	76	73	100
2.	Anolyte	58	36	24	100	78	60	52	100	83	74	71	100
3.	Propolis	56	23	21	100	76	62	53	100	85	78	73	100
4.	Shungite	58	25	22	100	79	61	52	100	84	75	64	100
5.	Shozan	55	23	20	100	62	53	46	100	74	63	60	100
6.	Prozan	56	23	21	100	61	58	44	100	75	72	65	100
7.	Shuprozan	58	25	22	100	63	57	46	100	77	73	62	100
8.	Ethanol	28	25	24	100	51	48	29	100	76	69	62	100
9.	Peroxide	29	26	23	100	54	40	30	100	74	68	63	100

In the study of the antimicrobial efficacy of ozone, anolyte, propolis, and Shungite, as well as complex preparations of anolyte+ozone+Shungite (Shozan), anolyte+ozone+propolis (Prozan), and anolyte+ozone+Shungite+propolis (Shuprozan) and also classical antiseptics (70% ethanol and 3% hydrogen peroxide) against *S. aureus* using the disk diffusion method on MPA, it was determined that at a microbial dose of 500 million CFU/cm^3, all the preparations showed high antimicrobial activity, indicated by a lysis zone of 10 to 11mm. At a microbial dose of 1 billion CFU/cm³, aqueous ozone, anolyte, propolis, and Shungite showed below-average antimicrobial activity, indicated by a lysis zone of 5 to 6mm. At a microbial dose of 2 billion CFU/cm³, these preparations showed low antimicrobial activity, indicated by a 0.5 to 0.8mm lysis zone.

Complex preparations Shozan, Prozan, and Shuprozan at a microbial dose of 1 billion CFU/cm³ showed average antimicrobial activity, indicated by a lysis zone of 8 to 9mm. At a microbial dose of 2 billion CFU/cm³, the antimicrobial activity of these preparations was below average, indicated by a lysis zone of 5.5 to 6mm.

Classical antiseptics (70% ethanol and 3% hydrogen peroxide) at a microbial dose of 1 billion CFU/cm³ showed average antimicrobial activity, indicated by a lysis zone of 9 mm. At a microbial dose of 2 billion CFU/cm³, the antimicrobial activity of these preparations was below average, indicated by a lysis zone of 5.5 to 6mm. In the control, the lysis zone for *S. aureus* was 0mm.

Thus, in the study of the antimicrobial activity of aqueous ozone, anolyte, propolis, Shungite, their complexes Shozan, Prozan, and Shuprozan, as well as classical antiseptics (70% ethanol and 3% hydrogen peroxide) on MPA using the disk diffusion method against *S. aureus* (Mussayeva et al. 2021), it was determined that the activity of individual natural antiseptics was lower than that of their complex preparations. The antimicrobial activity of basic antiseptics was not higher than that of complex preparations. That is, when natural antiseptics are used together in complex form, their antimicrobial activity is enhanced, demonstrating synergism (Fig. 1).

In the study of the antimicrobial efficacy of ozone, anolyte, propolis, and Shungite, as well as complex preparations of anolyte+ozone+Shungite (Shozan), anolyte+ozone+propolis (Prozan), and anolyte+ozone+Shungite+propolis (Shuprozan), and also classical antiseptics (70% ethanol and 3% hydrogen peroxide) against *E. coli* using the disk diffusion method on MPA, it was determined that at a microbial dose of 500 million CFU/cm^3, all the preparations showed high antimicrobial activity, indicated by a lysis zone of 11 to 11.5 mm. At a microbial dose of 1 billion CFU/cm^3, aqueous ozone, anolyte, propolis, and Shungite showed below-average antimicrobial activity, indicated by a lysis zone of 6 to 6.5mm. At a microbial dose of 2 billion CFU/cm^3, these preparations showed low antimicrobial activity, indicated by a lysis zone of 0.7 to 1mm. Contrary, Lenart-Boroń et al. (2024) reported that ozonated solutions could maintain antimicrobial activity against *E. coli* even at higher concentrations, which contrasts with the lack of activity observed in our study at 2 billion CFU. This discrepancy might be due to differences in experimental conditions, such as the medium used or the specific strains of *E. coli* tested.

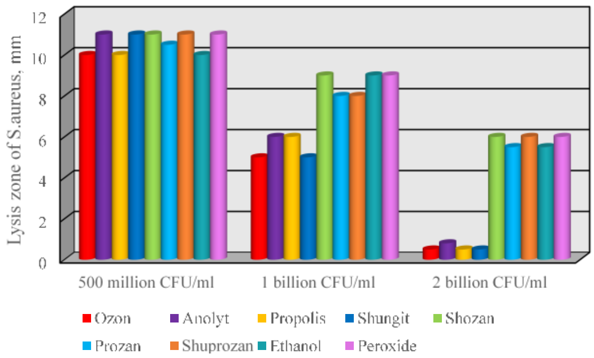

Fig. 1: Antimicrobial activity of preparations against *Staphylococcus aureus* on MPA using the disk diffusion method.

Complex preparations Shozan, Prozan, and Shuprozan at a microbial dose of 1 billion CFU/cm³ showed average antimicrobial activity, indicated by a 9 to 9.5mm lysis zone. At a microbial dose of 2 billion CFU/cm³, the antimicrobial activity of these preparations was below average, indicated by a lysis zone of 6 to 6.5mm.

Classical antiseptics (70% ethanol and 3% hydrogen peroxide) at a microbial dose of 1 billion CFU/cm³ showed average antimicrobial activity, indicated by a 9 to 9.5mm lysis zone. At a microbial dose of 2 billion CFU/cm³, the antimicrobial activity of these preparations was below average, indicated by a lysis zone of 6 to 6.5mm. In the control, the lysis zone for *E. coli* was 0mm.

Thus, in the study of the antimicrobial activity of ozone, anolyte, propolis, Shungite, their complexes Shozan, Prozan, and Shuprozan, as well as classical antiseptics (70% ethanol and 3% hydrogen peroxide) on meat pepton agar broth (MPA) using the disk diffusion method against *E. coli*, it was determined that the activity of individual natural antiseptics was lower than that of their complex preparations (Shevchenko et al. 2023). The antimicrobial activity of classical antiseptics was not higher than that of the complex preparations. When natural antiseptics are used together in complex forms, their antimicrobial activity is enhanced, demonstrating synergism (Fig. 2).

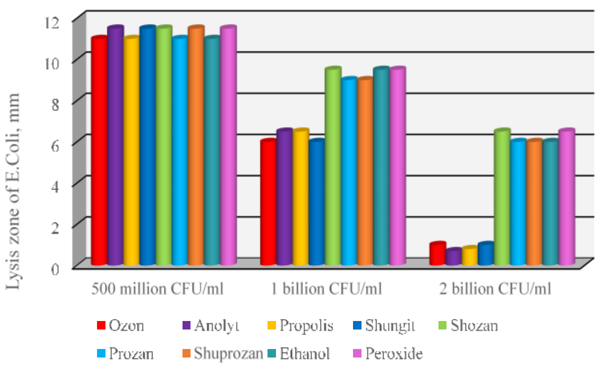

Fig. 2: Antimicrobial activity of preparations against *Escherichia coli* on MPA using the disk diffusion method.

Studying Shozan's cumulative properties, it was found that by the 28th day of the experiment, the total dose of the preparation administered over this period was 15,546.4 mg/kg. According to the accepted classification, this result categorizes Shozan as a substance with weakly expressed cumulative properties. The findings of this study are noteworthy, especially when compared with similar studies on the cumulative properties of other natural and synthetic compounds. Nusair et al. (2023) investigated the cumulative toxicity of a herbal formulation similar to Shozan. They found that despite high-dose administration over a prolonged period, the formulation exhibited minimal cumulative effects, supporting the findings in our study that Shozan has a low potential for accumulation and toxicity.

In contrast, Piskláková et al. (2024) found that synthetic antiseptics, such as quaternary ammonium compounds, tend to exhibit higher cumulative toxicity over extended use compared to natural antiseptics like Shozan. This suggests that natural compounds may offer safer alternatives for long-term use.

The cumulative coefficient (Kcum) of Shozan was 12.9, placing it in a specific category. According to the accepted classification, Shozan is classified as a substance with weakly expressed cumulative properties, as substances with a Kcum value > 5.5 are typically classified in the IV toxicity group. This classification underscores the unique nature of Shozan's cumulative properties (Table 3).

Studying Prozan's cumulative properties, it can be concluded that by the 28th day of the experiment, the total dose of the administered drug for this period amounted to 7957.4 mg/kg.

The cumulative coefficient (Kcum) of Prozan was 12.8. According to the accepted classification, Prozan belongs to the group of substances with weakly pronounced cumulative properties, as substances with a Kcum value > 5.5 belong to the IV toxicity group. The degree of Prozan accumulation is weakly pronounced (Table 4).

Upon studying the cumulative properties of Shuprozan, it was determined that by the 28th day of the experiment, the cumulative dose of the administered drug for this period amounted to 6795.2 mg/kg.

The cumulative coefficient (Kcum) of Shuprozan was 12.8. According to the accepted classification, Shuprozan belongs to the group of substances with weakly expressed cumulative properties, as substances with a Kcum value > 5.5 belong to the IV toxicity group, and the degree of cumulation of Anolyte is weakly expressed (Table 5). Thus, complex preparations Shozan, Prozan, and Shuprozan do not possess cumulative effects.

In the study of the toxicological properties of naturally derived antiseptics and their complexes, it was determined that in groups 1-7 and 10 of white mice, to which aqueous ozone, anolyte, Shungite extract, propolis extract, Shozan, Prozan, Shuprozan, and control were respectively administered, no clinical deviations were recorded within three days. The natural antiseptics and their complexes used in this study were composed of specific composition. Pathoanatomical examination results of laboratory animals showed no pathological changes in the organs, tissues, or anatomical arrangement in groups 1-7 and 10 of white mice (Mussayeva et al. 2021). However, clinical deviations were observed in the eighth and ninth groups, to which a 20% ethanol solution and a 1% hydrogen peroxide solution were administered, respectively. On the first day, excitement, followed by depression, was recorded immediately after the administration of the preparations. Animals exhibited impaired coordination, unsteady gait, and loss of appetite. Their reaction to pain stimuli was decreased. On the second day, tremors, convulsions, paresis, and paralysis of limbs were noted. Cyanosis of mucous membranes and discoloration of skin were observed. On the third day, discharge from the eyes and nose, involuntary urination, and defecation were recorded. The death of all mice in this group occurred due to paralysis of respiratory muscles and cardiac arrest. Pathoanatomical examination revealed intestinal and gastric meteorism, changes in the abdominal cavity's shape, mucous membranes' cyanosis, hemorrhages in the liver, gallbladder, and lungs, overfilled urinary bladder, and icterus of serous membranes (Gavrilova et al. 2023). Based on the pathological changes in the organs and tissues of white mice, the diagnosis of animal poisoning was made, indicating the toxic properties of a 20% ethanol solution and a 1% hydrogen peroxide solution. These results are consistent with other studies, such as Anggraeni et al. (2024), which similarly documented the toxic effects of these solutions in laboratory settings.

Therefore, it can be confidently stated that natural antiseptics and their complexes, when administered intraperitoneally at a dose of 1mL, do not exert a toxic effect on the organism of laboratory mice, ensuring their safety in such applications.

In the study of allergic reactions of the mucous membrane of the conjunctiva and cornea of the eye, it was determined that in groups 1-7 and 10 of rabbits, to which aqueous ozone, anolyte, Shungite extract, propolis extract, Shozan, Prozan, Shuprozan, and control were respectively administered into the right conjunctival sac, no clinical deviations in the reaction of the mucous conjunctiva and cornea of the eye, as well as clinical changes in the

Table 3: Assessment of the cumulative properties of Shozan

Days of Study	Daily Dose, mg/kg	Total Dose, mg/kg	Fallen / Survived		Mortality Rate, %	
			Experiment	Control	Experiment	Control
1-4	120.8	483.2	0/10	0/10	0	0
5-8	181.2	724.8	0/10	0/10	0	0
9-12	271.8	1087.2	0/10	0/10	0	0
13-16	407.7	1630.8	0/10	0/10	0	0
17-20	611.6	2446.4	0/10	0/10	0	0
21-24	917.4	3669.6	0/10	0/10	0	0
25-28	1376.1	5504.4	0/10	0/10	0	0
Totally for the 28-day period	3886.6	15546.4	KCum=15546.4:1208=12.9			

Table 4: Assessment of the cumulative properties of Prozan

Days of research	Daily dose (mg/kg)	Cumulative dose (mg/kg)	Fallen / Survived		Mortality Rate, %	
			Experiment	Control	Experiment	Control
1-4	61.8	247.2	0/10	0/10	0	0
5-8	92.7	370.8	0/10	0/10	0	0
9-12	139.1	556.4	0/10	0/10	0	0
13-16	208.7	834.8	0/10	0/10	0	0
17-20	313.1	1252.2	0/10	0/10	0	0
21-24	469.6	1878.4	0/10	0/10	0	0
25-28	704.4	2817.6	1/9	0/10	1	0
Totally for the 28-day period	1989.3	7957.4	KCum =7957.4:617.9=12.8			

Table 5: Evaluation of cumulative properties of Shuprozan

Daily studies	Daily dose (mg/kg)	Total dose (mg/kg)	Fallen / Survived		Mortality Rate, %	
			Experiment	Control	Experiment	Control
1-4	52.8	211.2	0/10	0/10	0	0
5-8	79.2	316.8	0/10	0/10	0	0
9-12	118.8	475.2	0/10	0/10	0	0
13-16	178.2	712.8	0/10	0/10	0	0
17-20	267.3	1069.2	0/10	0/10	0	0
21-24	401.0	1604.0	0/10	0/10	0	0
25-28	601.5	2406.0	0/10	0/10	0	0
Totally for the 28-day period	1698.8	6795.2	KCum = 6795.2:528 = 12.8			

condition of animals after 5min; 15min; 1 hour and 3hours were observed. No clinical deviations were observed in the left eye, where distilled water was administered. Clinical deviations were observed in the eighth and ninth groups of animals, to which a 20% ethanol solution and a 1% hydrogen peroxide solution were administered into the right conjunctival sac. In the first minute after the administration of the preparation, rabbits exhibited restlessness and increased respiratory and heart rates. They experienced profuse tearing and frequent blinking. After 5min, conjunctival hyperemia and enhanced corneal reflex were observed. Redness and irritation of the conjunctiva persisted after an hour, and signs of irritation diminished after 2 hours, ceasing entirely by the third hour. The animals calmed down.

Importantly, the study of allergic reactions on the mucous membranes of rabbits revealed that natural antiseptics and their complexes do not induce any such reactions, providing a sense of relief about their safety. This result is consistent with previous studies highlighting the generally low allergenic potential of natural antiseptics (Ramazanova et al. 2021). For example, studies by Ruiz-Hurtado et al. (2021) and Rivera-Yañez et al. (2023) have documented the minimal allergenic effects of propolis, one of the components of the antiseptic complexes studied here. Similarly, Shevchenko et al. (2023) found that Shungite, another component, does not provoke allergic responses, further supporting the safety profile observed in this study.

In the study of cutaneous-resorptive reactions, it was found that in groups 1-7 and 10 of rabbits, to which aqueous ozone, anolyte, Shungite extract, propolis extract, Shozan, Prozan, Shuprozan, and control were respectively rubbed into the skin in the area of the right scapula, no clinical deviations in the skin reaction, as well as clinical changes in the condition of animals after 30min, one hour, three hours, and four hours were observed. No clinical deviations were observed on the skin in the left scapula area, where distilled water was rubbed (Cortez and Guedes 2023). Clinical deviations were observed in the eighth and ninth groups of rabbits; a 70% ethanol solution and a 3% hydrogen peroxide solution were rubbed into the skin in the right scapula area. In the first 30min after application of the preparation, rabbits exhibited skin irritation and hyperemia, increased tactile sensitivity, agitation, and restlessness. After one hour, skin irritation and hyperemia decreased, and by the third to fourth hour, the changes ceased, and the animals calmed down.

Thus, natural antiseptics and their complexes do not induce allergic reactions on skin covers when determining cutaneous-resorptive reactions.

In the study of antimicrobial activity of ozone, anolyte, propolis, and Shungite, their complexes Shozan, Prozan, and Shuprozan, as well as classical antiseptics (70% ethanol and 3% hydrogen peroxide) by the method of inhibiting the growth of microbial culture on PCA about aerobic *E. coli* and facultatively anaerobic *S. aureus*, it was determined that at a dose of 500 million CFU/cm^3, all preparations showed high antimicrobial activity. With an increase in the dose of microorganisms from 1 billion to 2 billion CFU/cm³, ozone, anolyte, propolis, and Shungite did not exhibit high antimicrobial activity. In contrast, the effectiveness of their complexes Shozan, Prozan, and Shuprozan was on par with classical antiseptics (70% ethanol and 3% hydrogen peroxide). They possessed high bactericidal activity against both gram-negative and gram-positive bacteria. This means that complexes of natural antiseptics, in terms of antimicrobial activity, are equal to classical antiseptics, both against cocci and rods (Qosimah et al. 2023).

In the study of the antimicrobial activity of the mentioned preparations by the disc-diffusion method on Mueller-Hinton agar (MHA), with the determination of the lysis zone against *S. aureus cocci* and *E. coli* rods, similar results were obtained. The antimicrobial activity of all preparations at a microbial dose of 500 million CFU/cm^3 was high. With an increase in the microbial dose from 1 billion to 2 billion CFU/cm^3, the individual antimicrobial activity of naturally derived antiseptics was low. In contrast, that of their complexes and classical antiseptics remained at the same level. These results confirm the data from previous experiments.

Thus, the results of our studies show that at a microbial dose of 500 million CFU/cm^3, the antimicrobial activity of ozone, anolyte, propolis, and Shungite individually is on par with their complexes. In other words, they possess antimicrobial activity. However, their activity decreases with an increase in the microbial dose from 1 billion to 2 billion CFU/cm^3. Conversely, classical antiseptics (70% ethanol and 3% hydrogen peroxide) showed good antimicrobial activity at a microbial dose of up to 1 billion CFU/cm^3. Their activity decreased with a microbial dose of up to 2 billion CFU/cm^3. Complexes of natural antiseptics Shozan, Prozan, and Shuprozan demonstrated high antimicrobial efficiency at minimal doses against *S. aureus* cocci and *E. coli* rods. Increasing the microbial dose reduced their effectiveness but remained at the level of classical antiseptics. Therefore, the synergistic effect of natural antiseptic complexes enhances their antimicrobial activity when used together.

As mentioned earlier, antiseptic preparations should minimize cumulative, toxicological, and allergic effects on animals.

According to the accepted classification, when determining the cumulative coefficient (Kcum) of complex preparations, Shozan, Prozan, and Shuprozan belong to toxicity group IV - substances with weakly pronounced cumulative properties, as their Kcum > 5.5. Accordingly, complex preparations of Shozan, Prozan, and Shuprozan do not exhibit cumulative effects (Boranbayeva et al. 2023).

In the study of the toxicological properties of naturally derived antiseptics and their complexes, it was found that experimental mice showed no clinical deviations within three days after administering the preparations. Pathoanatomical examination of the test animals revealed no pathological changes in organs, tissues, or anatomical arrangement. Thus, natural antiseptics and their complexes do not exert toxicological effects on laboratory mice. Meanwhile, in the study of the toxicological properties of classical antiseptics (70% ethanol and 3% hydrogen peroxide), clinical deviations and pathological anatomical changes were observed in white mice.

Conclusion

In conclusion, the research has determined that natural antiseptics possess antibacterial properties, but only at low microbial doses up to 500 million CFU/cm^3. Their activity decreases with an increase in microbial dose. Complex preparations of natural antiseptics Shozan, Prozan, and Shuprozan demonstrate antibacterial properties with an increase in microbial dose from 1 billion to 2 billion CFU/cm^3. The antibacterial activity of complex antiseptics is comparable to that of classical antiseptics (70% ethanol and 3% hydrogen peroxide). In the study of the toxicological and allergic properties of the investigated preparations, it was found that classical antiseptics exert toxicological and allergic effects on laboratory animals. In contrast, complex preparations of natural antiseptics Shozan, Prozan, and Shuprozan do not possess toxicological, cumulative, or allergic properties.

The novelty of the work lies in the fact that complex preparations of natural antiseptics exhibit antibacterial activity comparable to classical antiseptics, meet modern disinfection standards, and can be used as antiseptic agents in medicine and veterinary practice.

Authors contributions

A.M. Nametov: conceptualization, research design, and data collection. R.S. Karmaliyev: data analysis, statistical work, or methodological development. L.Zh. Dushayeva: literature review, manuscript writing, interpretation of results. K.E. Murzabayev: data acquisition, technical support. B.T. Kadraliyeva: funding acquisition, project administration, supervision. K.A. Orynkhanov: data interpretation, critical revisions, overall study supervision. A.I. Karagulov: provided technical support, participated in data analysis, and assisted with manuscript preparation. M.B. Marat: compiling data, the methodological aspects. Zh. Bazar: reviewing the manuscript, editing, and providing key resources and materials.

REFERENCES

Abdelshafy AM, Hu Q, Luo Z, Ban Z and Li L, 2024. Hydrogen peroxide from traditional sanitizer to promising disinfection agent in food industry. Food Reviews International 40(2): 658-690. http://dx.doi.org/10.1080/87559129.2023.2191690

Ahmed WI, Mohammed AN and Sleim AA, 2024. Efficacy evaluation of hydrogen peroxide disinfectant based zinc oxide nanoparticles against diarrhea causing Escherichia coli in ruminant animals and broiler chickens. Scientifc Reports 14(1): 9159. https://doi.org/10.1038/s41598-024-59280-4

Aliyev AA, Shapiev BI, Shapieva KB, Atalaev MM, Ismailov IA and Kanbulatova ZSH, 2018. Bactericidal effectiveness of neutral anolyte in combination with potassium permanganate. Ecological Medicine 1: 81-86.

Anggraeni S, Triesayuningtyas DC, Luthfidyaningrum H, Hendaria MP, Widia Y, Endaryanto A and Prakoeswa CRS, 2024. Severity assessment and IL-5 evaluation after immunotherapy in atopic dermatitis mice. Advances in Life Sciences 11(2): 309-314.

Asanova BM, Fakhradiev IR, Muratova KA and Zhuldyzbayeva AYA, 2015. Comparative analysis of the effectiveness of the antiseptic "Kodan tincture forte colored". Bulletin of the Kazakh National Medical University named after S.D. Asfendiyarov 3: 207-210.

Bakhchevnikov ON and Braginets AV, 2021. Ozonation for feed raw materials disinfection (review). Taurida Herald of the Agrarian Sciences 2(26): 41-61.

Bakhir VM, 1998. Mediko-tekhnicheskiye sistemy i tekhnologii dlya sinteza elektrokhimicheski aktivirovannykh rastvorov [Medico-technical systems and technologies for the synthesis of electrochemically activated solutions]. VNIIIMT, Moscow, Russia.

Baran A, Köktürk M, Atamanalp M and Ceyhun SB, 2018. Determination of developmental toxicity of zebrafish exposed to propyl gallate dosed lower than ADI (Acceptable Daily Intake). Regulatory Toxicology and Pharmacology 94: 16-21. https://doi.org/10.1016/j.yrtph.2017.12.027

Betancur-D'Ambrosio MC, Pérez-Cervera CE, Barrera-Martinez C and Andrade-Pizarro R, 2024. Antimicrobial activity, mechanical and thermal properties of cassava starch films incorporated with beeswax and propolis. Journal of Food Science and Technology 61(4): 782-789. https://doi.org/10.1007/s13197-023-05878-x

Bocian E, Grzybowska W and Tyski S, 2014. Evaluation of mycobactericidal activity of selected chemical disinfectants and antiseptics according to European standards. Medical Science Monitor 20: 666-673. https://doi.org/10.12659/msm.890175

Boranbayeva KE, Zamanbekov NA, Sattarova RS, Spiridonov GN, Zhylgeldiyeva AA and Turzhigitova SHB, 2023. Comparative pharmacotherapeutic effectiveness of therapeutic ointments in infectious keratoconjunctivitis in cattle. Research Journal of Pharmacy and Technology 16(1): 46-4. https://doi.org/10.52711/0974-360X.2023.00009

Buienbayeva ZK, Latypova ZA, Issakulova BZH, Bakiyeva FA, Namet AM and Valdovska A, 2024. Development of veterinary and sanitary measures for the prevention of pasteurellosis infection in cattle: The case of the Republic of Kazakhstan. International Journal of Veterinary Science 13(2): 164-171.

Buyanov VV, Nikolskaya VP, Kanishchev VV, Lakomov VP, Mitusov NV, Suprun IP, Pudova OB and Titova KV, 2023. Disinfection products for eliminating the consequences of biological contamination at various ambient temperatures. Institute of Problems of Chemical Physics of the Russian Academy of Sciences, Chernogolovka, Russia, pp: 277.

Cai D, Ding J, Li F, Zhuang G, Li M and Guo LH, 2024. Sulfonamide disinfection byproducts exhibited severe toxicity to human commensal bacteria. Water Research 256(1): 121551. https://doi.org/10.1016/j.watres.2024.121551

Cortez BRS and Guedes RMC, 2023. A review on the evolution of methods for intestinal in vitro organ culture and its application in veterinary science. Veterinary World 16(2): 347–356.

Donato GG, Nebbia P, Stella MC, Scalas D, Necchi D, Bertero A, Romano G, Bergamini L, PolettomL, Peano A, Robino P and Nervo T, 2024. In vitro effects of different ozone preparations on microorganisms responsible for endometritis in the mare. Theriogenology 219: 132-137. https://doi.org/10.1016/j.theriogenology.2024.02.011

Gavrilova NN, Ratnikova IA, Sadanov AK, Orazymbet SE, Shorabaev EZH, Baimakhanova BB and Protasiuk LE, 2023. Determination of the toxicity and sensitizing effect of a therapeutic probiotic agent against intestinal and concomitant human infections. Research Journal of Pharmacy and Technology 16(9): 4161-1. https://doi.org/10.52711/0974-360X.2023.00682

González-Penagos CE, Zamora-Briseño JA, Améndola-Pimenta M, Cruz-Quintana Y, Santana-Piñeros AM, Torres-García JR, Cañizares-Martínez MA, Pérez-Vega JA, Peñuela-Mendoza AC and Rodríguez-Canul R, 2024. Sargassum spp. ethanolic extract elicits toxic responses and malformations in Zebrafish (Danio rerio) embryos. Environmental Toxicology and Chemistry 43(5): 1075-1089. https://doi.org/10.1002/etc.5840

Gordon SB and Nadolski AC, 2024. Medical management of pneumoperitoneum, gastric pneumatosis, and hepatic venous gas secondary to 3% hydrogen peroxide toxicity in a dog. Journal of Veterinary Emergency and Critical Care 34(2): 179-185. https://doi.org/10.1111/vec.13363

Ignatov I, Popova TP, Petrova T and Ignatov AI, 2022. Physicochemical parameters and in vitro antimicrobial effects of water filtrated with nano-structured carbonaceous Shungite. Journal of Chemical Technology and Metallurgy 57(5): 937-945.

Jurgelane I and Locs J, 2021. Shungite application for treatment of drinking water – Is it the right choice? Journal of Water and Health 19(1): 89-96. http://dx.doi.org/10.2166/wh.2020.139

Kaavya R, Pandiselvam R, Abdullah S, Sruthi NU, Jayanath Y, Ashokkumar C, Chandra Khanashyam A, Kothakota A and Ramesh SV, 2021. Emerging non-thermal technologies for decontamination of Salmonella in food. Trends in Food Science & Technology 112: 400-418. http://dx.doi.org/10.1016/j.tifs.2021.04.011

Karomatov ID, 2018. Antibacterial and anti-inflammatory properties of propolis. Electronic Scientific Journal "Biology and Integrative Medicine" 2(19): 313-325.

Khromushkin V, Chestnova T, Platonov V, Khadartsev A and Kireev S, 2014. Shungite as natural nanotechnology (literature review). Journal of New Medical Technologies. eJournal 1: 3-11. http://dx.doi.org/10.12737/7346

Kornilov VI, Yakovlev DS, Bagmetova VV, Bugayeva LI, Spasov AA and Getmanenko AYu, 2020. Izucheniye kumulyativnykh svoystv tabletirovannoy formy novogo protivomigrenoznogo sredstva RU-31 [Cumulative properties of the new anti-migenous compound in the tablet medical form]. Journal of Volgograd State Medical University 4(76): 46-49. https://doi.org/10.19163/1994-9480-2020-4(76)-46-49

Kuklin DN, Styazhkina SN, Ivanova MK, Tikhonova VV, Bagautdinov AL, Gilimkhanova AR and Vakhitova AI, 2022. The effect of the analyte solution on pathogenic microflora. Antibiotics and Chemotherapy 67(5-6): 10-13. https://doi.org/10.37489/0235-2990-2022-67-5-6-10-13

Kutsova AE, Sergienko IV, Alehina AV, Kosenko IS and Kutsov SV, 2020. In vivo experiments to determine the efficiency of the elementary status correction. IOP Conference Series: Earth and Environmental Science 422(1): 012076. http://dx.doi.org/10.1088/1755-1315/422/1/012076

Lenart-Boroń A, Stankiewicz K, Bulanda K, Czernecka N, Heliasz M, Hunter W, Ratajewicz A, Khachatryan K and Khachatryan G, 2024. In vitro antibacterial activity of ozonated olive oil against bacteria of various antimicrobial resistance profiles isolated from wounds of companion animals. International Journal of Molecular Sciences 25(6): 3557. https://doi.org/10.3390/ijms25063557

Li HF, Feng H, Wang Y, Pan ZC, Yin L, Qiu HL, Qiao H, Zhao JQ, Xia XY, Hou JC and Wang RX, 2024. Evaluation of hemostatic, anti-inflammatory, wound healing, skin irritation and allergy, and antimicrobial properties of active fraction from the ethanol extract of Chromolaena odorata (L.) R.M. King & H. Rob. Journal of Ethnopharmacology 331: 118330. https://doi.org/10.1016/j.jep.2024.118330

Maryam A, Anwar R, Malik AU, Ur Raheem MI, Khan AS, Ul Hasan M, Hussain Z and Siddique Z, 2021. Combined aqueous ozone and ultrasound application inhibits microbial spoilage, reduces pesticide residues and maintains storage quality of strawberry fruits. Journal of Food Measurement and Characterization 15(2): 1437- 1451. https://doi.org/10.1007/s11694-020-00735-3

McFarland J, 1907. The nephelometer: An instrument for estimating the numbers of bacteria in suspensions used for calculating the opsonic index and for vaccines. Journal of the

American Medical Association 49: 1176-1178. https://doi.org/10.1001/JAMA.1907.25320140022001F

Medved LI, Kagan YS and Spynu EI, 1968. Pestitsidy i problemy zdravookhraneniya [Pesticides and health problems]. Bulletin of the All-Russian Chemical Society named after Mendeleyev 13(3): 263-271

Miller JM, Astles R, Baszler T, Chapin K, Carey R, Garcia L, Gray L, Larone D, Pentella M, Pollock A, Shapiro DS, Weirich E, Wiedbrauk D, Biosafety Blue Ribbon Panel and Centers for Disease Control and Prevention (CDC), 2012. Guidelines for safe work practices in human and animal medical diagnostic laboratories. Recommendations of a CDC-convened, Biosafety Blue Ribbon Panel. Morbidity and Mortality Weekly Report. Supplements 61(1): 1-102.

Mussayeva A, Yegorova N, Yerishov M, Dossanova A, Suchshikh V, Namet A, Siyabekov S, Nussupova S and Yespembetov B, 2021. Molecular-biological properties of the attenuated strain of Salmonella abortus-equi E-841, used in the creation of a vaccine against abortion of mares. American Journal of Animal and Veterinary Sciences 16(2): 144–150.

Naliukhina AN, Kozlova AV, Eregin AV, Guseva YE and Kuzina NI, 2024. Responses of soil physico-chemical properties, structure of the microbial community and crop yields to different fertilization practices in Russia's conventional farming system. Brazilian Journal of Biology 84: e282493.

Nametov AM, Yerzhanov ES and Kokanov SK, 2014. The study of some physico-chemical characteristics of the biologically active sorbent Shungite, promising in the treatment of surgical wounds. Intellect, Idea, Innovation-intellect, Idea, Innovation 1: 14-18.

Nusair SD, Hammad E and Alqudah MAY, 2023. Quantification of aflatoxins and health risk assessment through intake of herbal medicine formulations. Emerging Contaminants 9(1). https://doi.org/10.1016/j.emcon.2023.100206

Piskláková L, Skuhrovcová K, Bártová T, Seidelmannová J, Vondrovic Š and Velebný V, 2024. Trends in the incorporation of antiseptics into natural polymer-based nanofibrous mats. Polymers 16(5). https://doi.org/10.3390/polym16050664

Prokopenko AA, Vanner NE and Filipenkova GV, 2019. Study of the bactericidal and disinfecting activity of a new environmentally safe medicine Anolyt Ank Super. Russian Journal "Problems of Veterinary Sanitation, Hygiene and Ecology" 3(31): 286-293. https://doi.org/10.36871/vet.san.hyg.ecol.201903008

Qosimah D, Santoso S, Maftuch M, Khotimah H, Fitri LE, Aulanni'am A and Suwanti LT, 2023. Aeromonas hydrophila induction method in adult zebrafish (Danio rerio) as animal infection models. Veterinary World 16(2): 250–257.

Ramazanova AA, Yernazarova GI, Turasheva SK and Ablaikhanova NT, 2021. Determination of the content of biologically active substances in some aquatic higher plants. Pakistan Journal of Botany 53(5): 1893-1899.

Reuter G, 1988. Requirements for disinfectants for food areas. 1. List of tested disinfectants of the German Veterinary Association. Berliner und Munchener Tierarztliche Wochenschrift 101(6): 194-199.

Rivera-Yañez N, Ruiz-Hurtado PA, Rivera-Yañez CR, Arciniega-Martínez IM, Yepez-Ortega M, Mendoza-Arroyo B, Rebollar-Ruíz XA, Méndez-Cruz AR, Reséndiz-Albor AA and Nieto-Yañez O, 2023. The role of propolis as a natural product with potential gastric cancer treatment properties: A systematic review. Foods 12(2). https://doi.org/10.3390/foods12020415

Röhm-Rodowald E, Jakimiak B and Podgórska M, 2008. The principle of registration, evaluation and testing of disinfecting preparations. Przegląd Epidemiologiczny 62(3): 661-668.

Ruiz-Hurtado PA, Garduño-Siciliano L, Domínguez-Verano P, Balderas-Cordero D, Gorgua-Jiménez G, Canales-Álvarez O, Canales-Martínez MM and Rodríguez-Monroy MA, 2021. Propolis and its gastroprotective effects on NSAID-induced gastric ulcer disease: A systematic review. Nutrients 13(9). https://doi.org/10.3390/nu13093169

Saidova LB, Karomatov ID and Rakhmatova DB, 2021. Propolis in Medicine (the Use of Propolis in Folk and Scientific Medicine). RS Global Sp. z O. O., Warsaw, Poland, pp: 109.

Selvam R, Kumar Perumal S, Subbiah L, Gowdu Viswanathan MB and Palanisamy S, 2023. Safety assessment of ethanolic extract of Canarium strictum Roxb. leaves: Acute and subchronic toxicity studies. Pakistan Journal of Pharmaceutical Sciences 36(2): 491-500.

Shevchenko TV, Ustinova YV, Ermolaeva EO, Popov AM and Uzunov GB, 2023. Extending the storage life of foods using Shungite. Advances in Life Sciences 10(2): 152-156.

Styazhkina SN, Kiryanov NA, Kuklin DN, Kutbedinov KS, Eykina ON and Strashnov DV, 2023. Evaluation of the antiseptic effect of the anolyte solution on laboratory rats. Antibiotics and Chemotherapy 68(3-4): 35-38. https://doi.org/10.37489/0235-2990-2023-68-3-4-35-38

Talib Al-Sudani B, Mahmoudi E, Adnan Shaker Al-Naymi H, Al-Musawi MH, Al-Talabanee IBN, Ramezani S, Ghorbani M and Mortazavi Moghadam F, 2024. Antibacterial and wound healing performance of a novel electrospun nanofibers based on polymethyl-methacrylate/gelatin impregnated with different content of propolis. Journal of Drug Delivery Science and Technology 95: 105641. https://doi.org/10.1016/j.jddst.2024.105641

Tremasova AM and Beletsky OS, 2012. O primenenii shungita v zhivotnovodstve [On the use of Shungite in animal husbandry]. Dostizheniya nauki i tekhniki APK 3: 72-74.

Večerková L, Mašková L, Knejzlík Z, Kašpar O and Tokárová V, 2024. Development of spray dried powder hand sanitiser with prolonged efectivity. Scientific Reports 14(1): 4827. https://doi.org/10.1038/s41598-024-55503-w

Vetkina IF, Komarinskaya LV, Ilyin IY and Solovyova MV, 2005. Sovremennyy podkhod k vyboru dezinfitsiruyushchikh sredstv v sisteme profilaktiki vnutribol'nichnykh infektsiy (VBI) [Modern approach to the choice of disinfectants in the system of prevention of nosocomial infections (VBI)]. Pharmindex-Praktik 7: 13-20.

Votavá M, Šlitrová J and Matušková Z, 2005. Microbicidal efficacy of a new foam disinfectant. Epidemiologie, Mikrobiologie, Imunologie 54(2): 84-89.

Weisbrod AV, Woodburn KB, Koelmans AA, Parkerton TF, McElroy AE and Borgå K, 2009. Evaluation of bioaccumulation using in vivo laboratory and field studies. Integrated Environmental Assessment and Management 5(4): 598-623. https://doi.org/10.1897/ieam_2009-004.1

Xue Y, Gao Y, Guo M, Zhang Y, Zhao G, Xia L, Ma J, Cheng Y, Wang H, Sun J, Wang Z and Yan Y, 2024. Phage cocktail superimposed disinfection: A ecological strategy for preventing pathogenic bacterial infections in dairy farms. Environmental Research 252(1): 118720. https://doi.org/10.1016/j.envres.2024.118720

Zashchepkina VV, 2019. Izucheniye kumulyativnykh svoystv supramolekulyarnogo kompleksa ivermektina [The study of the cumulative properties of the supramolecular complex of ivermectin]. Russian Journal of Parasitology 13(4): 72-76. https://doi.org/10.31016/1998-8435-2019-13-4-72-76