Evaluation of the antibacterial effect of *Foeniculum vulgare* Mill essential oil on opportunistic microflora: growth and enzymatic activity indicators

Abstract.

Fennel (*Foeniculum vulgare*) is an edible spice with edible value and is cultivated in both tropical and temperate regions. It is a traditional spice with important economic value and extensive medical application value. *The purpose of the research*: Antibacterial tests were conducted on four types of opportunistic bacteria in the gastrointestinal tract to determine their biochemical indicators. Fennel oil was used as raw material in this study. The object of study were opportunistic microorganisms of the gastrointestinal tract *Escherichia coli*, *Proteus mirabilis, Staphylococcus aureus, Bacillus cereus.* The study groups were fennel essential oil produced by Botanika LLC, Russian Federation (FEN-B) and IP Saules Sapnis, Republic of Belarus (FEN-SS). Collection microorganisms of the same species were used as a control. Research results have shown that fennel essential oil (*Foeniculum vulgare*) inhibits the growth of opportunistic microflora. *E. coli*, *B. cereus* and *P. mirabilis* exhibited proteolytic and amylolytic activities.

Keywords: *Foeniculum vulgáre;* essential oil; conditional pathogenic microorganisms; proteolytic activity; sacharolytic activity; zone of growth retention; gastrointestinal tract.

List of abbreviations

CFU – colony-forming unit.

EO – essential oils.

FEN-SS – Fennel essential oil (*Foeniculum vulgare*), produced by IE "Saules Sapnis" (Republic of Belarus).

FEN-B – Fennel essential oil (*Foeniculum vulgare*), produced by LLC "Botanika" (Russian Federation).

GSA agar – grain soak agar.

G+ – gram- positive bacteria.

G– – gram-negative bacteria.

MDR – multidrug resistance.

MPA agar – meat-peptone agar.

MYP agar – mannitol egg yolk polymyxin agar (agar Mossel).

SCI – isotonic sodium chloride solution.

1. Introduction

In many industries such as livestock and poultry breeding, the overuse of antibiotics has resulted in bacterial resistance, which has become a major public health problem facing mankind. In the field of modern medicine, the problem of bacterial resistance has become increasingly prominent, especially in two common bacteria, *Escherichia coli* and *Staphylococcus aureus[18,19]*. These bacteria have developed resistance to multiple antibiotic drugs, a condition widely known in the medical community as "multidrug resistance" (MDR). This resistance makes

treating the infection more difficult because patients need to take more antibiotics to fight the same bacteria, but with less effectiveness. Therefore, understanding and controlling the spread of bacterial resistance is critical to preventing and treating infections [1].

Antibiotics are the most effective means of preventing and treating livestock and poultry diseases and promoting the growth of livestock and poultry, but food additives that are safer and have no toxic side effects should also be provided for livestock and poultry production [2].

As the adverse reactions of antibiotics to humans and animals have attracted increasing attention, countries around the world have listed them as banned drugs. There is a clear need for new feed additives that can improve feed utilization and improve people's health; they not only fight pathogens but also act as immune response stimulators and antioxidants. Such as probiotics, organic acids, natural pigments and their extracts [3].

As a new means of regulation, feed additives can effectively regulate the intestinal flora structure of livestock and poultry and improve the growth and development of livestock and poultry. In the past few decades, due to the preventive application of veterinary drugs, people began to worry about the possible presence of antibiotic residues and their disease-resistant mechanisms in livestock and poultry breeding [4].

Therefore, the search for new, safe, non-toxic natural antibacterial agents is a key scientific issue that needs to be solved urgently [5].

Essential oils have a relatively complex chemical composition; an essential oil may even contain an estimated 20 to 60 biological components that are not identical to each other. However, when we measure the content of each ingredient in detail, we will find that generally the main 2 to 3 ingredients can reach between one-fifth and four-fifths of the total concentration. Compared with other ingredients accounts for a very high concentration. The chemical composition of essential oils will be affected by geographical conditions such as place of origin and environment, as well as external factors such as maturity time, harvesting season, and harvesting methods.

Plants protect themselves from biotic or abiotic factors in the environment by producing secondary metabolites – essential oils, which is their own protection mechanism. Vinyl oxide is an important chemical raw material with very complex chemical composition, mainly terpenes. In addition, many other types of aromatic small molecules and fatty compounds were discovered during this process. These three components constitute a series of ethylene oxide molecules with multiple functions and broad application prospects [6].

Foeniculum vulgare Mill, Also known as anise, it is a small genus of one-, two-year or perennial herbs. Fennel (*Foeniculum vulgare* Mill.) is a plant in the *Umbelliferae* family. It is distributed in temperate and tropical zones around the world and has a wide planting range. In Asia, fennel is not only a commonly used anise but is also used for medicinal and other economic purposes. People can extract an aromatic oil called "fennel oil" from dried fruits, which is now used in many households around the world for flavoring [7].

Fennel also has the effects of dispelling cold, removing dampness, and treating rheumatism. In addition, fennel also has the effect of promoting intestinal peristalsis and has a certain effect on improving indigestion and constipation. Its main function is to be a diuretic, regulate the water balance in the body, and have a good protective effect on the kidneys. In addition, it can also strengthen the elasticity of connecting tissues, which is very important for the health of bones and the normal function of muscles. Fennel is a natural plant with anti-aging and anti-aging properties. There are many commercial medicines based on anise oil. The antibacterial properties of essential oils are also recognized. At present, steam distillation and solvent extraction are mainly used to extract plant essential oils. The chemical composition and some antibacterial properties of *S. flexneri* have been previously studied [8].

There are two advantages to using essential oils as antibacterial agents: first, most essential oils are safer for the human body; second, the resistance of bacteria to essential oils is reduced [9].

This project plans to use 4 types of opportunistic bacteria in the gastrointestinal tract as research objects, study the antibacterial effect of fennel volatile oil on 4 types of intestinal opportunistic bacteria, and measure their biochemical indicators.

2 Material and methods

Each phase of research is carried out in accordance with relevant standards and relevant regulatory requirements.

The procedure for selecting, adapting and applying methods for isolating pure cultures of opportunistic microorganisms was carried out on the basis of the recommended procedures and described in the approved instructions for use. Deputy Minister of Health – Chief State Sanitary Doctor of the Republic of Belarus dated March 19, 2010 No. 075-0210 [10], Instructions “Microbiological methods for isolating and identifying pathogens in bacterial food poisoning” approved. Resolution of the Chief State Sanitary Doctor of the Republic of Belarus dated October 9, 2006 No. 120 [11].

Collection, storage, transportation of selected samples and work using cultures of microorganisms and biological material (copromaterial) were carried out taking into account the principles of interpretation of research results in accordance with the Methodological Guidelines, approved. Rector of VSAVM dated November 29, 2017 No. 02-1-31/33 [12], Instructions for use of the Ministry of Health of the Republic of Belarus dated March 19, 2010 No. 075-0210 [10], Order of the Ministry of Health of the Republic of Belarus “On approval of the Instructions on the procedure for organizing preanalytical stage of laboratory research" dated November 10, 2015 No. 1123 [13] and the Rules for organizing laboratory research (tests) during veterinary control (supervision), approved. Council of the Eurasian Economic Commission dated November 10, 2017 No. 80 [14].

The laboratory part of the research was organized in accordance with the requirements of the Order of the Ministry of Health of the Republic of Belarus dated April 18, 2019 No. 466 “On improving the activities of the laboratory

diagnostic service of the Republic of Belarus" [15]. The studies were carried out in triplicate.

2.1 Essential oils

Two essential oils produced at LLC "Botanika", Russian Federation ("Botanika") and IE "Saules Sapnis", Republic of Belarus ("Saules Sapnis") This is what this article uses. During this process, the harvested plants are sorted and dried to facilitate the extraction of essential oils. Table 1 lists the types of EOs investigated, the plant substances used to extract essential oils, and their sources.

Table 1. The types of essential oils studied, the botanicals used, and their origins

Code	Type of EO	The Vegetable Material from Which the Oil Is Extracted	The Origin of the Vegetable Raw Material
FEN-B	Fennel essential oil (*Foeniculum vulgare*)	Aerial parts	"Botanika"
FEN-SS	Fennel essential oil (*Foeniculum vulgare*)	Aerial parts	"Saules Sapnis"

The samples were stored at low temperature (4 °C) and without light. Ethylene oxide is stored in a refrigerated (4 °C), light-shielding, sealed glass box.

Table 2 lists the properties of ethylene oxide, including its general name, main components, botanical components, and components used for extraction.

Table 2. According to the manufacturer, the general name, plant species, main ingredients, and plant parts used to extract essential oils.

Botanical name	Common name	Oil source	Major chemical compounds	Oil %	Chemical class
Foeniculum vulgare Mill.	Fennel	Leaves, Stems	Trans-anethole Limonene	50 35	Ether Alkene
		Seeds	Trans-anethole Fenchone	75 15	Ether Ketone

The ethylene oxide selected in this paper is obtained by distillation and is stored in amber glass bottles at room temperature.

2.2 Microorganisms tested

Two Gram-negative (G–) bacteria (*E. coli* MRE-600, *Proteus mirabilis*), two Gram-positive (G+) bacteria (*Staphylococcus aureus* K2-1, *Bacillus cereus*) (see table 3): Belarusian Center for the Preservation of Non-pathogenic Microorganisms (Institute of Microbiology of the National Academy of Sciences of Belarus, Minsk, Republic of Belarus) and Department of Biology of the Belarusian State University (Minsk, Republic of Belarus).

Table 3. Standard microbial strains used for testing antimicrobial activities

No.	Microbial Strain	Source

1	*Escherichia coli* MRE-600, БИМ В-238	Gram-negative standard strain, The microorganism collection of the open access fund of the Belarusian collection of non-pathogenic microorganisms, SNU "Institute of Microbiology of the National Academy of Sciences of Belarus"
2	*Escherichia coli*	Gram-negative strain, isolated from copromaterial (intestinal contents) of cattle
3	*Proteus mirabilis,* ATCC 25933	Gram-negative standard strain, The microorganism collection of the research laboratory of molecular genetics and biotechnology, Department of Genetics, Faculty of Biology, Belarusian State University
4	*Proteus mirabilis*	Gram-negative strain, isolated from copromaterial (intestinal contents) of cattle
5	*Staphylococcus aureus* K2-1, БИМ В-1841	Gram-positive standard strains, Institute of Microbiology, National Academy of Sciences of Belarus, Belarusian Foundation for Pathogen-free Preservation of Microorganisms
6	*Staphylococcus aureus*	Gram-positive strain, isolated from copromaterial (intestinal contents) of cattle
7	*Bacillus cereus,* БИМ В-108	Gram-positive standard strain, The microorganism collection of the open access fund of the Belarusian collection of non-pathogenic microorganisms, SNU "Institute of Microbiology of the National Academy of Sciences of Belarus"
8	*Bacillus cereus*	Gram-positive strain, isolated from copromaterial (intestinal

		contents) of cattle

Pure microbial culture medium (National University) provided by the Belarusian Open Source Foundation for the Preservation of Non-pathogenic Microorganisms (Belarusian State University) and pure microbial culture provided by the Research Laboratory of Molecular Genetics and Bioengineering, Department of Genetics, Faculty of Biology (Belarusian State University).

All strains were stored in nutrient agar slopes at 4 °C (Sakharov Institute of Environmental Sciences, Belarusian State University, Minsk, Republic of Belarus). Active cultures were prepared by pipetting one-week-old cells from agar slopes into tubes containing 5 ml of bacterial nutrient medium (Sakharov Institute of Environment, Belarusian State University, Minsk, Republic of Belarus). Incubate overnight at 37 °C to enter the logarithmic growth phase. Draw a straight line on the NA plate for bacterial purity testing. Each cell suspension was measured at 600 nm and adjusted to the desired concentration (approximately 1×10^8 CFU/ml) using a McFarland standard. DuPont Tyvek (1073 D) was purchased from Changzhou Road Commercial Company, Jiangsu Province; 500 ml PP disposable plastic basin (bottom diameter 88 mm) was purchased from Chengdu Anbao Paper Co., Ltd. (Chengdu, Sichuan Province, 118 mm diameter), height 68 mm, top).

2.3 Isolation and production of pure cultures of microorganisms

Isolation of microorganism cultures was carried out from copromaterial (intestinal contents) obtained from cattle of the livestock farm of JSC "Gorodilovo" in the Molodechno district of the Minsk region.

The possibility of contamination of biological material (copromaterial) from the environment during its collection was excluded. Samples were taken from animals that did not receive phage and antibiotic therapy.

The test material (feces about 1 g) from cattle (bulls) was taken into sterile, pre-weighed penicillin vials.

In the laboratory, the test material was suspended in an isotonic sodium chloride solution (SCI) pH 7.2–7.4 in a ratio of 1:10 (at the rate of 1 g of material, 9 ml of SCI) and sown on nutrient media no later than two hours from the moment the sample was taken.

If the storage period of experimental materials needs to be extended, it must be carried out within 12-24 hours in accordance with the approved "Isolation and Identification of Animal Digestive Tract Microorganisms". Head of the Veterinary Department of the Ministry of Agriculture of the Russian Federation dated May 11, 2004 No. 13-5-02/1043 [16].

When studying the biochemical properties of test cultures, catalase activity, proteolytic activity (casein hydrolysis) were studied, hydrogen sulfide was determined, and the Voges-Proskauer test was performed.

2.4 Antibacterial effect of essential oils

Screening determination of the bactericidal effect of fennel essential oil was carried out by a series of inoculations of microorganism cultures with a Drigalsky spatula on the surface of a nutrient medium (meat-peptone agar (MPA)) with determination of the sensitivity of these microorganisms to the test substances, using standard paper discs soaked in essential oil in standard concentrations (disc-diffuse method). Antimicrobial activity was performed by disc diffusion (Whatman No. 1) with a diameter of 6 mm. It is believed that the inhibitory zone is a distinct ring-like structure without any growth within the disc. The susceptibility of microorganisms is determined by measuring the diameter (mm) of the growth retardation zone.

Daily cultures of the studied strains were suspended in sterile physiological solution. A standard inoculum corresponding to a turbidity of 10 U according to the Tarasevich standard and containing 5×10^8 CFU/ml was adjusted to a concentration of 10^2 CFU/ml. The resulting suspensions of microorganisms in a volume of 0.1 ml were applied to the surface of nutrient media of microorganism cultures (*Escherichia coli*, *Proteus mirabilis* – Endo medium, *Staphylococcus aureus* – GSA agar, *Bacillus cereus* – MYP agar). The bacterial suspension was evenly distributed with a spatula over the surface of the nutrient medium to obtain an enrichment culture for research. Microorganisms were incubated for 24 hours in an incubator at a temperature optimal for the growth of these cultures. Next, a nutrient medium for analysis (MPA) was prepared. The resulting suspensions of microorganisms in a volume of 0.1 ml were applied to the surface of the MPA. The bacterial suspension was evenly distributed over the surface of the nutrient medium

using a spatula. Standard paper discs soaked in fennel essential oil in standard concentrations were placed on the surface of the medium; the cups were placed upside down in a thermostat for a day to take into account the antibacterial effect. After 24 hours, the bactericidal effect of fennel essential oil was determined (analysis of zones of delayed growth of microorganisms in mm) [17].

2.5 Biochemical parameters of test cultures

Suspensions of microorganisms in a volume of 0.1 ml were applied to the surface of a standard Petri dish (half a dish) with a nutrient medium (MPA). The bacterial suspension was evenly distributed over the surface of the nutrient medium using a spatula. 1 ml of fennel essential oil was added to the second half of the Petri dish and distributed over the surface of the nutrient medium with a spatula. Test cultures were cultivated in the presence of essential oil for 24 hours. Next, microorganisms were selected to analyze the effect of essential oil on the biochemical parameters of test cultures.

Proteolytic activity of test cultures. The basis was a nutrient medium (MPA) with a double concentration of agar-agar, to which an equal volume of sterilized milk was added. The test bacterial culture was streaked into Petri dishes with a nutrient medium. Incubated for 14 days. Before taking into account the results, the surface of the medium was filled with a 10 % solution of hydrochloric acid. A positive result was assessed by the clearing of the environment around the colonies.

Sugar-lytic (amylolytic) activity of test cultures. The optimal agar nutrient medium for the studied microorganisms was melted, 0.2 % soluble starch was added, mixed, and brought to a boil. The medium was sterilized at 115 °C for 10 min. The test culture was inoculated in one stroke along the diameter of the Petri dish and the inoculations were incubated at the optimal temperature. Then an iodine solution was poured onto the surface of the agar. A positive reaction was determined when a colorless zone formed along the streak as a result of starch breakdown.

The amylolytic activity of the enzyme preparation of the culture liquid was studied by the Caraway method. The principle of the method is based on the colorimetric determination of amylodextrin concentration based on the intensity of the reaction with iodine and a wavelength of 650 nm on a spectrophotometer before and after enzymatic hydrolysis of starch by amylase contained in the culture medium. The ratio of substrate and enzyme component was determined experimentally. The unit of enzyme activity was taken to be the amount contained in 1 ml of the biological solution under study, which breaks down amylodextrin (μg) in 1 minute at 37 °C.

The specific amylolytic activity of the enzyme preparation of the culture liquid (μg/min×ml) in the samples was calculated by the expression (equation 1):

$$X = ((Dk - Dp) / (Dk \times t \times V)) \times 1000, \quad (1)$$

where Dk is the optical density of the control solution (nm); Dp – optical density of the prototype (nm); t – incubation time of the enzyme-substrate mixture (min); V – sample volume (ml).

2.6 Statistical analyses

In order to ensure the accuracy and reliability of the data, this study used three groups of samples for data analysis and measurement. First of all, this project plans to use Excel analysis method to statistically analyze the collected data to obtain the standard deviation (± SD) and its mean of each sample to understand the statistical characteristics of the data. Secondly, SPSS 21.0 software was used to conduct a statistical analysis on the influence of multiple independent variables on the dependent variable. On this basis, this study will conduct in-depth research on the research object from both theoretical and empirical aspects. The result was considered significant at $P < 0.05$.

3 Results and discussions

3.1 Analysis of the growth of pure cultures and biochemical parameters of microorganisms

Analysis of the bactericidal activity of fennel essential oil against opportunistic microorganisms consists of studying the zone of inhibition (suppression) of microorganism growth.

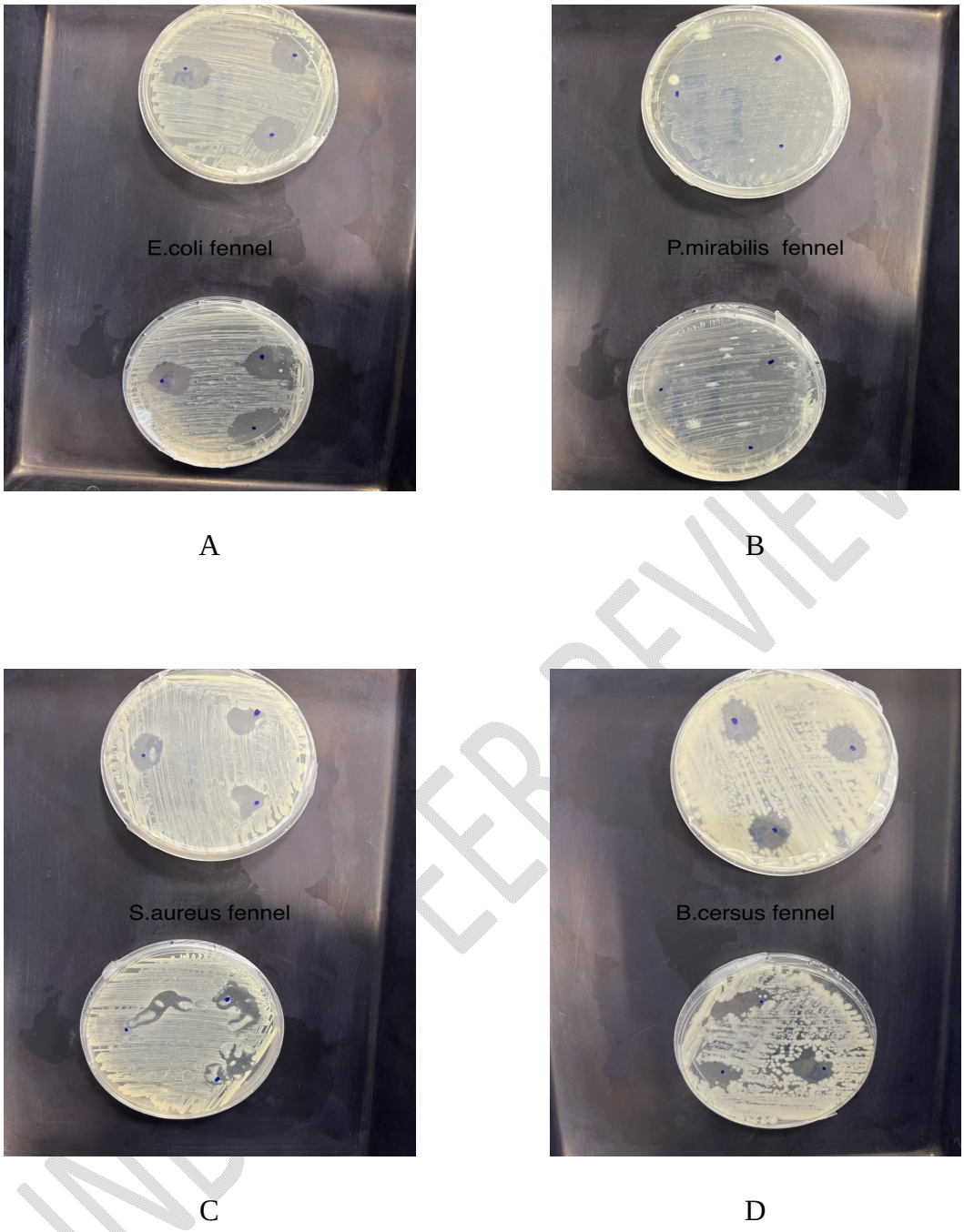
The studies showed that the essential oil had antibacterial activity against all strains taken into the experiment. The criterion for antibacterial activity is the assessment of the zone of inhibition of the growth of microorganisms during co-cultivation with the test object (Fig. 1).

The greatest antimicrobial activity (Table 4, Fig. 1 A) was observed against *Escherichia coli* and exceeded control values by 22.4-33.3 %. The sensitivity of *B. cereus*, *Staphylococcus aureus* and *P. mirabilis* (Table 4, Fig. 1 B, C, D) when treated with fennel essential oil was also at a high level, exceeding the control value by 17.6, 16.3–20.0 and 50.5 %, respectively.

Table 4. Diameter of growth retardation zone, mm

Group	*E. coli*	*P. mirabilis*	*S. aureus*	*B. cereus*
FEN-B	53.0±2.2	2.0±0.3	6.0±0.2	12.0±1.3
P value	0.079	≤1.000	0.050	≤1.000
FEN-SS	58.0±3.1	4.0±0.1	5.0±0.6	14.0±1.0
P value	0.035	≥0.001	≤1.000	0.211
Control	43.3±3.5	2.0±0.1	5.0±0.3	12.0±0.9

The growth inhibition zones of microorganisms around the discs when using fennel essential oil produced by IP Saules Sapnis (FEN-B) were larger than in the case of Botanika LLC (FEN-SS), which confirms higher antimicrobial activity.



A B

C D

Fig. 1. Zones of inhibition of the growth of microorganisms when they are co-cultivated with the studied samples of fennel essential oil (top row – FEN-SS, bottom row – FEN-B): **A** - *Escherichia coli*, **B** - *Proteus mirabilis*, **C**- *Staphylococcus aureus*, **D** - *Bacillus cereus*

During the analysis of the enzymatic activity of *Escherichia coli*, *Proteus mirabilis*, *Staphylococcus aureus* and *Bacillus cereus* cultures under the influence of fennel essential oil (Table 5), the following was shown. Thus, the amylolytic activity of *Escherichia coli* and *Bacillus cereus* after joint incubation in the presence of fennel essential oil (FEN-B) for 24 hours was lower by 25 and 32 % compared to the control, respectively.

Table 5. Amylolytic activity (zone of starch hydrolysis, mm)

Group	*E. coli*	*P. mirabilis*	*S. aureus*	*B. cereus*
FEN-B	4.5±0.1	4.4±0.3	–	6.8±0.6
P value	0.003	0.124	–	0.007
FEN-SS	4.0±0.2	4.8±0.3	–	7.0±0.3
P value	0.024	0.452	–	0.001
Control	6.0±0.2	5.1±0.2	–	10.0±0.2

Our previous study found that under the action of fennel essential oil "Saules Sapnis" (FEN-SS), after 24 hours of fermentation, the starch degradation activities of *Escherichia coli* and *Bacillus cereus* (P=0.024) decreased by 48.2 % (P=0.024) respectively and 42.9 % (P=0.001). The results show that when the essential oils from the two manufacturers work together, the starch degradation activity of *Proteus mirabilis* reaches 12.7-13.7 %. When fennel oils from two different manufacturers were co-cultured, there was no starch hydrolysis zone fermented by *Staphylococcus aureus*.

The proteolytic activity of *Escherichia coli* and *Bacillus cereus* (Table 6) after joint incubation in the presence of fennel essential oil (FEN-B) for 24 hours) was also lower by 15.0 and 20.0 % compared to the control, respectively.

Table 6. Proteolytic activity (casein hydrolysis zone, mm)

Group	*E. coli*	*P. mirabilis*	*S. aureus*	*B. cereus*
FEN-B	5.1±0.1	6.9±0.2	10.4±0.1	8.0±0.3
P value	0.047	0.678	0.055	0.005
FEN-SS	4.5±0.2	6.8±0.1	10.6±0.2	7.9±0.2
P value	0.014	0.230	0.230	0.002
Control	6.0±0.3	7.0±0.1	11.0±0.2	10.0±0.2

The proteolytic activity of *Escherichia coli* and *Bacillus cereus* after joint incubation in the presence of fennel essential oil IP "Saules Sapnis" (FEN-SS) for 24 hours was lower by 32.4 (P=0.014), 27.4 % (P=0.002) compared to the control, respectively. The casein hydrolysis zone in the *Staphylococcus aureus* culture after joint incubation in the presence of fennel essential oil from two manufacturers was lower by 4.6–6.0 %, in *Proteus mirabilis* – by 1.4–3.0 %.

The value of specific amylolytic activity (Table 7) was the highest in cultures of microorganisms *Bacillus cereus* and *Escherichia coli* when incubated in the presence of fennel essential oil produced by IP Saules Sapnis (FEN-SS) and was inferior to the control by 58.3 (P=0.013) and 46.4 % (P=0.019).

Table 7. Amylolytic activity, μg/min×ml

Group	*E. coli*	*P. mirabilis*	*S. aureus*	*B. cereus*
FEN-B	4.8±0.4	4.8±0.2	–	4.1±0.2
P value	0.074	0.609	–	0.024
FEN-SS	4.1±0.1	5.1±0.2	–	3.8±0.1
P value	0.019	0.795	–	0.013
Control	6.0±0.3	5.0±0.3	–	6.0±0.5

At a high level, specific amylolytic activity was observed during incubation in the presence of essential oil produced by Botanika LLC (FEN-B) in *Bacillus cereus* and *Escherichia coli* cultures and was lower than the control value by 31.7 (P=0.024) and 20.0 % (P=0.074). In the *Proteus mirabilis* culture, the value of specific amylolytic activity incubated in the presence of essential oils from two manufacturers was the lowest and was 2–4 % lower than the control. The specific amylolytic activity of *Staphylococcus aureus* after joint incubation in the presence of fennel essential oil from two manufacturers was absent in all groups.

4 Conclusion

In conclusion, many essential oils show significant antibacterial activity. It has broad-spectrum antibacterial activity and can effectively kill various bacteria, viruses and other microorganisms. This product has a strong antibacterial effect and is a valuable resource that can be used in medical and health care. The molecular structure of aromatic oils is closely related to their antibacterial

properties. The mechanism of action of aromatic plant essential oils involves multiple targets, especially target molecules on the cell membrane, which can often penetrate the cell membrane and cause component leakage.

Fennel oil is an important biologically active substance with antibacterial and enzymatic activities. Its chemical composition gives it medicinal properties. Phenolics are some of the most active and reactive compounds in anise. As a natural plant, fennel contains physiologically active substances with significant medical value. Not only do these substances have a long history of use in traditional medicine, but modern research has shown that they are potentially helpful in the treatment of a variety of diseases. Therefore, these active ingredients extracted from fennel have become important raw materials for the production of various drugs, playing a vital role in promoting human health and maintaining overall function.

The bactericidal effect of fennel essential oil was established during the cultivation of opportunistic microflora, which contributed to the inhibition of the growth of *Escherichia coli* by 22.4–33.3 % (P=0.035-0.079), *Bacillus cereus*, *Staphylococcus aureus* and *Proteus mirabilis* – by 17.6, 16.3–20.0 and 50.5 %, respectively, relative to the control group. Zones of retarded growth of microorganisms around the discs of fennel essential oil produced by IP Saules Sapnis (FEN-SS) showed higher antimicrobial activity.

The enzymatic activity of cells of cultivated opportunistic microorganisms under the influence of fennel essential oil was revealed, which is as follows. It was established that the casein hydrolysis zone was suppressed by 15.0–32.4 %

(*Escherichia coli*), 20.0–27.4 % (*Bacillus cereus*), 4.6–6.0 % (*Staphylococcus aureus*), 1.4–3.0% (*Proteus mirabilis*); zones of starch hydrolysis by 32.0–42.9 % (*Bacillus cereus*), 25.0–48.2 % (*Escherichia coli*), 12.7–13.7 % (*Proteus mirabilis*); amylolytic activity by 31.7–58.3 % (*Bacillus cereus*), 20.0–46.4 % (*Escherichia coli*), 2.0–4.0 % (*Proteus mirabilis*) relative to the control value. *Staphylococcus aureus* culture cells showed a complete absence of enzymes that destroy starch.

Therefore, further research on this plant as a treatment for various chronic diseases is necessary.

Data availability

No data was used for the research described in the article.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

References

1. Poolman JT, Anderson AS. *Escherichia coli* and *Staphylococcus aureus*: Leading bacterial pathogens of healthcare associated infections and bacteremia in older–age populations. Expert Rev. Vaccines. 2018;17:607–618. https://doi.org/10.1080/14760584.2018.1488590.

2. Imbabi T, Sabeq I, Osman A, Mahmoud K, Amer SA, Hassan AM, Kostomakhin N, Habashy W, Easa AA. Impact of Fennel Essential Oil as an Antibiotic Alternative in Rabbit Diet on Antioxidant Enzymes Levels, Growth Performance, and Meat Quality. Antioxidants. 2021;10:1797. https://doi.org/10.3390/antiox10111797.

3. El-Araby DA, Amer SA, Attia GA, Osman A, Fahmy EM, Altohamy DE, Alkafafy M, Elakkad HA, Tolba SA. Dietary Spirulina platensis phycocyanin improves growth, tissue histoarchitecture, and immune responses, with modulating immunoexpression of CD3 and CD20 in Nile tilapia, *Oreochromis niloticus*. Aquaculture. 2021;546:737413.

4. Ghiasvand AR, Khatibjoo A, Mohammadi Y, Akbari Gharaei M, Shirzadi H. Effect of fennel essential oil on performance, serum biochemistry, immunity, ileum morphology and microbial population, and meat quality of broiler chickens fed corn or wheat-based diet. British Poultry Science. 2021;62(4):562–572. DOI: 10.1080/00071668.2021.1883551

5. Galgano M, Capozza P, Pellegrini F, Cordisco M, Sposato A, Sblano S, Camero M, Lanave G, Fracchiolla G, Corrente M, et al. Antimicrobial Activity of Essential Oils Evaluated *In Vitro* against *Escherichia coli* and *Staphylococcus aureus.* Antibiotics. 2022;11:979. https://doi.org/10.3390/antibiotics11070979.

6. Swamy MK, Akhtar MS, Sinniah UR. Antimicrobial Properties of Plant Essential Oils against Human Pathogens and Their Mode of Action: An Updated Review. Evidence-based complementary and alternative medicine: eCAM. 2016:3012462.

7. Hassaan MS, Soltan M. Evaluation of Essential Oil of Fennel and Garlic Separately or Combined with *Bacillus licheniformis* on the Growth, Feeding Behaviour, Hemato-biochemical Indices of *Oreochromis niloticus* (L.) Fry. J. Aquac. Res. Dev. 2016;7:422–429.

86. Sajid M, Ohran I, Ahsan Z, Arslan S, Gulfraz M. Fatty acid composition of *Sorghum bicolor* varieties. Food Chem. 2008;109:855–859.

9-4. Hong Z, Xinping C, Jingnan L. *In vitro* antifungal activity and mechanism of essential oil from fennel (*Foeniculum vulgare* L.) on dermatophyte species. Journal of Medical Microbiology. 2015;64:93–103.

10. Microbiological methods for studying biological material: instructions for use No. 075-0210: approved. Deputy Minister of Health of the Republic of Belarus – Chief State Sanitary Doctor of the Republic of Belarus 03/19/2010. Minsk. 2010. 123 p. (in Russian)

11. Kolomiets ND, Tonko OV, Gray-eyed TI, Mareyko AM, Litunovskaya LG, Ermakova TS, Kolodkina VL, Sergeychik NL, Levshina NN, Slavinskaya AA, Tochko NI, Voitik SB, Novomlinova LV, Shitikova PV, Klyuiko NL, Kulichkovskaya IV. Microbiological methods for studying biological material: Instructions for use. Approved Deputy Minister of Health – Chief State Sanitary Doctor of the Republic. Belarus No. 075-0210 dated 03/19/2010. Minsk. 2010. 122 p. (in Russian)

12. Petrovsky SV, et al. Guidelines for the selection of biological material for laboratory research. Vitebsk: Educational Institution VGAVM. 2017. 48 p. (in Russian)

13. On approval of the Instructions on the procedure for organizing the preanalytical stage of laboratory tests: Order of the Ministry of Health of the Republic of Belarus dated November 10. 2015. No. 1123. (in Russian)

14. On approval of the Rules for organizing laboratory research (tests) during veterinary control (supervision): Decision of the Council of the Eurasian Economic Commission dated November 10. 2017. No. 80.

15. On improving the activities of the laboratory diagnostic service of the Republic of Belarus: Order of the Ministry of Health of the Republic of Belarus dated April 18. 2019. No. 466. (in Russian)

16. Voronin ES, Gryazneva TN, Tikhonov IV, et al. Isolation and identification of bacteria in the gastrointestinal tract of animals: methodological recommendations. Approved. Head of the Veterinary Department of the Ministry of Agriculture of Russia dated May 11, 2004 No. 13-5-02/1043. 84 p. (in Russian)

17. Roby MHH, Sarhan MA, Selim KA, Khalel KI. Antioxidant and antimicrobial activities of essential oil and extracts of fennel (*Foeniculum vulgare* L.) and chamomile (*Matricaria chamomilla* L.). Industrial Crops and Products. 2012;44:437–445.

18 Viuda-Martos M, Mohamady MA, Fernández-López J, Abd ElRazik KA, Omer EA, Pérez-Alvarez JA, Sendra E. In vitro antioxidant and antibacterial activities of essentials oils obtained from Egyptian aromatic plants. Food control. 2011 Nov 1;22(11):1715-22.

19 Das T, Nandy S, Mukherjee A, Nongdam P, Dey A. Plant essential oils for combating antimicrobial resistance via re-potentiating the fading antibiotic arsenal. Antimicrobial Resistance: Underlying Mechanisms and Therapeutic Approaches. 2022:419-85.