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The applicability of bioluminescent bacteria for preliminary screening of antibacterial activity: Comparative analysis of aqueous and ethanol extracts from plant raw material

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ABSTRACT

This study aimed to assess the effectiveness of whole-cell luminous biosensors in detecting the antibacterial activity of plant extracts. The biosensors included bioluminescent genetically modified *Escherichia coli* MG1655 pXen7 and naturally occurring *Aliivibrio fischeri* F1 bacterial strains. Initially, chemical substances known for their antibacterial properties, such as ethanol, quercetin, and zinc sulfate, were used to evaluate the functionality of the biosensors. Subsequently, 17 herbal extracts were screened for antibacterial activity in both aqueous and ethanol forms. As a result, extracts from *Coreopsis grandiflora*, *Thymus vulgaris*, and *Monarda x hybrida* demonstrated significant antibacterial potential, resulting in a 50% reduction in bioluminescence. Notably, *A. fischeri* F1 exhibited higher sensitivity compared to recombinant *E. coli* MG1655 pXen7 in detecting antimicrobial compounds at lower concentrations. The variations in the effects of extracts from different species within the same and different plant families were observed, utilizing both biosensors. These findings align with existing literature data regarding the antibacterial activity of the investigated plant species against pathogenic bacteria.

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Overall, the results underscore the efficacy of bioluminescent bacteria for the rapid screening of antibacterial properties in crude plant extracts.

Keywords: antibacterial activity, *lux*-biosensors, *Aliivibrio fischeri* F1, *Escherichia coli* MG1655, crude plant extracts

INTRODUCTION

One of the most pressing problems in the modern world is that microorganisms are adjusting to antibacterials faster than these are being developed by pharmaceutical companies, which results in an enormous rise in the death rate all over the globe from earlier treatable infections[1]. However, this problem can potentially be addressed by expanding the scope of investigation to explore novel active compounds and optimizing the time and financial requirements associated with the initial screening stages.

By broadening the investigation area, researchers can explore a wider range of sources, including natural substances, synthetic compounds, and their combinations, which may possess potent antibacterial properties. In this regard, medical plants continue to hold immense potential as a source of future medications. Plants are rich in secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, and other compounds known for their antibacterial characteristics[2]. Moreover, utilizing natural sources can help reduce the cost of developing and producing medicines. Notably, The World Health Organization highlights that 50% of prescribed medications worldwide originate from plants, and traditional medicine still plays a prominent role in illness prevention and treatment, with approximately 80% of the population relying on it[3-4].

On the other hand, the current methods used to test antibacterial activity are often time-consuming and inadequate for screening certain bioactive substances, especially those of plant origin. The widely used diffusion into agar method, commonly employed for evaluating antimicrobial activity[5-6], has its limitations, particularly in measuring the antibacterial effects of plant extracts. The water-based agar matrix used in diffusion methods hampers the diffusion of non-polar substances into its layers compared to polar compounds, resulting in incomplete diffusion of complexes with intermediate polarity, which are considered to possess the highest antibacterial activity[7-8]. Moreover, this method requires a significant investment of time and resources, posing constraints on conducting efficient screening tests[9]. Therefore, there is a need for more uniform methods to measure antibacterial activity. One potential solution lies in incorporating widely utilized in toxicological studies methodologies based on bacterial luminescence

into the screening of potential antibacterial compounds. Above all, biotests utilizing bioluminescent bacteria have long been used in environmental and toxicological studies, demonstrating their high sensitivity to various biologically active substances[10-12]. In recent years, these methods have also shown promise in the field of pharmacy, indicating their potential applicability[13-15]. Furthermore, the sensitivity of bioluminescent bacteria allows the examination of hundreds of samples within several hours, whereas traditional procedures often require days or even weeks to achieve comparable results. By reducing screening time, valuable resources such as consumables and personnel are conserved, resulting in cost savings for the study. Consequently, with decreased time and funding requirements, the development and production of treatments can be accelerated, potentially saving countless lives[11-13].

The aim of this study was to assess the applicability of whole-cell bacterial bioluminescent biosensors for the preliminary evaluation of the antimicrobial activity of aqueous and ethanol extracts derived from raw materials of medicinal plants.

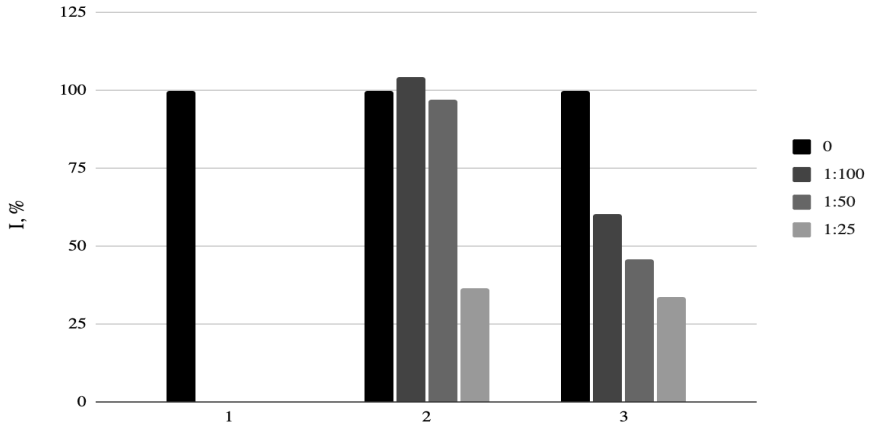
METHODOLOGY

This study assessed the antibacterial activity of extracts from seventeen plant species including *Echinacea purpurea* (L.) Moench, *Echinacea pallida* (Nutt.). Nutt., *Echinacea angustifolia* A.Heller, *Echinacea tennessesnsis* (Beadle) Small, *Calendula officinalis* L.*, Coreopsis grandiflora* Hogg ex Sweet, *Hyssopus officinalis* L., *Monarda fistulosa* L., *Monarda didyma* L., *Monarda* x *hybrida* hort., *Myrtus communis* cv. Yuzhnoberezhny, *Satureja montana* L.*, Thymus serpyllym* L., *Thymus vulgaris* cv. Fantasia, *Thymus striatus* cv.Yubileynyy, *Rosmarinus officinalis* cv. Gorizont, *Vitex agnus-castus* L. These species were harvested within the territory of the Federal State Budgetary Institution "Nikitsky Botanical Garden – National Scientific Center of the Russian Academy of Sciences" in Yalta, Republic of Crimea, Russia. Aqueous and ethanol extracts of these plant species were obtained following established pharmacopoeial methods for tinctures and decoctions[16]. Biosensors used for the testing of antimicrobial activity of plant extracts included naturally ccurring bioluminescent strain of *Aliivibrio fischeri* F1 from the collection of the Institute "S.I. Georgievsky Medical Academy" FSAOU VO "V.I. Vernadsky CFU", and genetically engineered luminescent strain of *Escherichia coli* MG1655 pXen7[17-18]. Bacterial strain cultivation and inoculum preparation were carried out in accordance with previously described techniques[18-20]. To assess the functionality of the bacterial bioluminescent strains, ethanol (OAO Flora Kavkaza, RF), quercetin (Quercetin >95%, Sigma-Aldrich), and zinc sulfate (LenReaktiv, Russia) was used as positive controls in the study.

Ethanol solutions were prepared at a 70% concentration, similar to the ethanol concentration used for the preparation of herbal tinctures. An ethanol solution of quercetin was prepared by dissolving quercetin powder in 70% ethyl alcohol to achieve a concentration of 10 mg/mL. A zinc sulfate solution was prepared by dissolving it in distilled water to reach a concentration of 0.175 mg/mL[21-22]. The assessment of antibacterial activity was conducted in accordance with established eco-toxicity testing standards[23], which, notably, demonstrated remarkable efficacy in evaluating the antimicrobial properties of substances from diverse sources, including those of plant origin[24-26]. The testing was conducted as follows: Aqueous solutions of sodium chloride at concentrations of 3% and 1% were prepared for *A. fischeri* F1 and *E. coli* pXen7, respectively, and dispensed into the bioluminometer cuvettes in volumes of 950, 940, 930, and 910 μL. To these sodium chloride solutions, samples of plant extracts were added in quantities of 10, 20, and 40 μL, resulting in dilutions of 1:25, 1:50, and 1:100, respectively. Positive control samples contained solutions of quercetin, ethanol, and zinc sulfate in the same volumes as the tested plant extracts. The negative control consisted of suspensions of bacterial cultures in sodium chloride solutions, excluding the test samples. The resulting systems were thoroughly mixed at 100 rpm for 15 minutes using an orbital shaker (OS-20, biosan, Latvia) to ensure an even distribution of substances in the sample. After that, 50 μL of bacterial suspension was added with a cell concentration corresponding to 0.5 McFarland turbidity standard. The prepared samples were incubated at 25°C and 37°C in a thermostat (TCO -1/80 SPU, Russia) for *A. fischeri* F1 and *E. coli* pXen7, respectively, for 30 minutes with constant stirring, after which the intensity of bioluminescence of the systems was measured using a biochemiluminometer (BHL-06, Nizhny Novgorod, Russia). The results of measuring the bioluminescence of the test strains were presented as the bioluminescence intensity index (I), which was calculated by the formula: $I = Io/Ic \times 100\%$ (expressed as a percentage), where Io – is the luminescence intensity of the test strain in the test sample after a certain time of incubation (30 min), and Ic – is the intensity luminescence of the test strain in the control sample after a certain time of incubation (30 min)[21,26]. The antimicrobial activity of plant extracts was assessed by the effective dilutions of the plant extracts, which reduced the luminescence of microorganisms by 50% (ED_{50}). Statistical processing of the research results was carried out in accordance with the pharmacopoeial standards “Statistical processing of experimental results” using Microsoft Excel 2003.

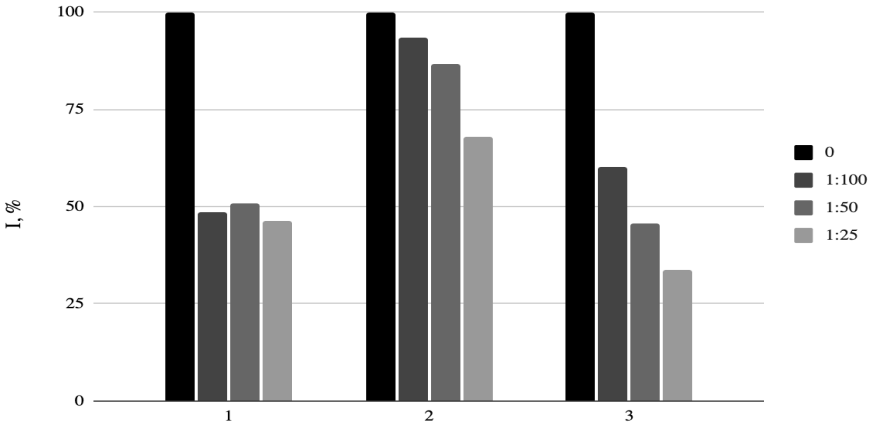
RESULTS and DISCUSSION

The sensitivity evaluation of *A. fischeri* F1 and *E. coli* pXen7 to quercetin, ethanol, and zinc sulfate demonstrated that both strains exhibited sensitivity to all tested agents, displaying a dose-dependent response. However, a difference in sensitivity was distinguished between the natural strain, *A. fischeri* F1 (Figure 1), and the genetically engineered strain, *E. coli* pXen7 (Figure 2), with the natural strain showing higher sensitivity to the tested agents. The minimum concentration of zinc sulfate solution required to inhibit bioluminescence by 50% in both *A. fischeri* F1 and *E. coli* pXen7 strains was 0.0035 mg/mL. In contrast, *A. fischeri* F1 strain showed greater sensitivity to ethanol solution, inhibiting bioluminescence at a dilution of 1:25 (corresponding to a 2.8% concentration in the sample). Ethanol solution caused a slight dose-dependent inhibition of *E. coli* bioluminescence, but none of the used in the study concentrations reduced it by 50% or more. Notably, *A. fischeri* F1 strain exhibited significantly higher sensitivity to the quercetin solution, with complete inhibition of luminescence starting from a minimum tested concentration of 0.1 mg/mL. In comparison, the same concentration of quercetin inhibited bioluminescence in *E. coli* pXen7 by 51.43%, without a significant dose-dependent increase within the applied concentrations in the experiment.



1- quercetin solution 10 mg/mL, 2- ethanol solution 70%, 3- zinc sulfate solution 0.175 mg/mL. I-bioluminescence intensity index, calculated by the formula: I = Io/Ic × 100%, where Io – is the luminescence intensity of the test strain in the test sample, and Ic – the bioluminescence intensity of the test strain in the negative control sample.

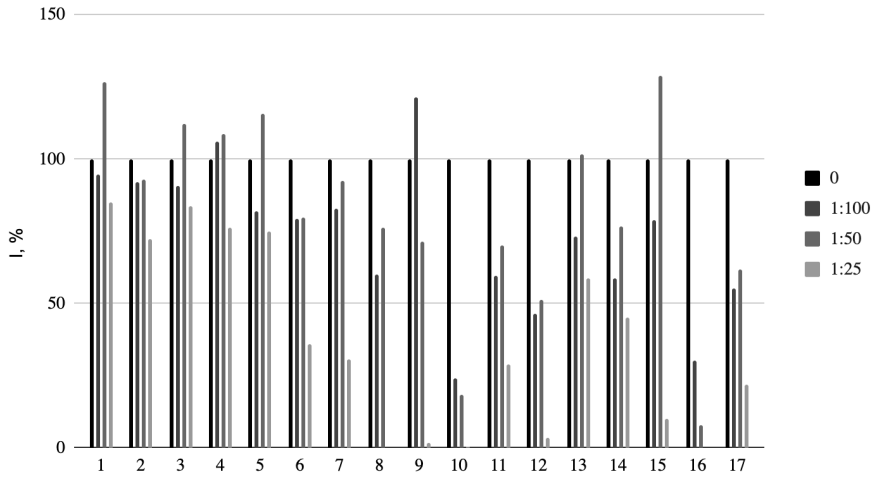
Figure 1. The effect of different concentrations of quercetin, ethanol and, zinc sulfate solutions on the bioluminescence of *A. fischeri* F1 strain



1- quercetin solution 10 mg/mL, 2- ethanol solution 70%, 3- zinc sulfate solution 0.175 mg/mL. I-bioluminescence intensity index, calculated by the formula: I = Io/Ic × 100%, where Io – is the luminescence intensity of the test strain in the test sample, and Ic – the bioluminescence intensity of the test strain in the negative control sample

Figure 2. The effect of different concentrations of quercetin, ethanol, and zinc sulfate solutions on the bioluminescence of *E. coli* pXen7 strain

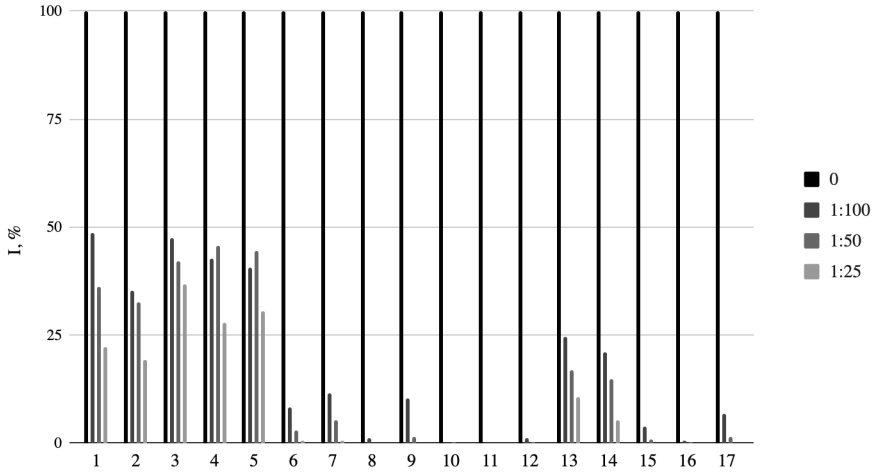
The next step involved utilizing natural and genetically engineered bioluminescent biosensors to evaluate the antibacterial properties of aqueous and ethanol plant extracts. The assessment of the effects of aqueous plant extracts on the bioluminescence of the natural marine strain *A. fischeri* F1 revealed that extracts from *E. purpurea, E. pallida, E. angustifolia, E. tennessesnsis, C. officinalis*, and *Vítex agnus-castus* did not reduce the luminescence intensity of the test strain by more than 50% at a minimum dilution of 1:25 (Figure 3).



1– *E. purpurea* (L.) Moench; 2– *E. pallida* (Nutt.). Nutt.; 3– *E. angustifolia* A.Heller; 4– *E. tennessesnsis* (Beadle) Small; 5– *Calendula officinalis* L.*;* 6– *Thymus serpyllym* L.; 7– *Satureja montana* L.*,* 8– *Monarda fistulosa* L.; 9– *Myrtus communis* cv. Yuzhnoberezhny; 10– *Coreopsis grandiflora* Hogg ex Sweet; 11– *Rosmarinus officinalis* cv. Gorizont; 12 – *Thymus vulgaris* L.; 13– *Vítex agnus-castus* L.*,* 14– *Hyssopus officinalis* L.; 15– *Monarda didyma* L.; 16– *Monarda* x *hybrida* hort.; 17– *Thymus striatus* cv. Yubileynyy. I- bioluminescence intensity index, calculated by the formula: I = Io/Ic × 100%, where Io – is the luminescence intensity of the test strain in the test sample, and Ic – the bioluminescence intensity of the test strain in the negative control sample.

Figure 3. The effect of different dilutions of aqueous plant extracts on the bioluminescence of *Aliivibrio fischeri* F1

Aqueous extracts of *T. serpyllym*, *S. montana*, *M. fistulosa*, *M. communis* cv. Yuzhnoberezhny; *R. officinalis* cv. Gorizont, *Hyssopus officinalis*, *Monarda didyma* и *Thymus striatus* cv. Yubileynyy reduced the intensity of bioluminescence by 50% or more at a dilution of 1:25. Aqueous extracts of *C. grandiflora*, *T. vulgaris* cv. Fantasia and *Monarda* x *hybrida* hort. Inhibited the bioluminescence of the *A. fischeri* F1 strain by 50% or more at the highest dilution of 1:100. As a result of the testing of the ethanol extracts, it was found that all the extracts were inhibiting the intensity of bioluminescence of *A. fischeri* F1 by 50% or more (Figure 4). At the same time, ethyl alcohol in similar dilutions did not reduce bioluminescence intensity by more than 35.64% of the control values.



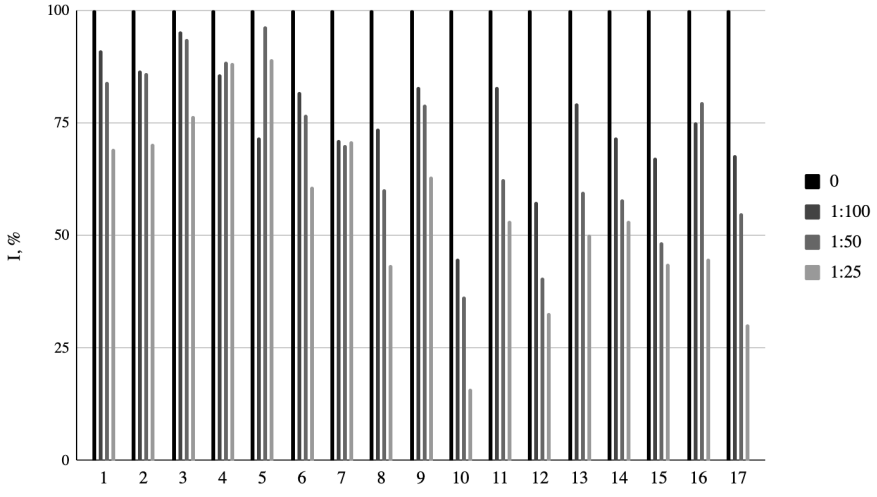
1– *Echinacea purpurea* (L.) Moench; 2– *Echinacea pallida* (Nutt.). Nutt.; 3– *Echinacea angustifolia* A.Heller; 4– *Echinacea tennessesnsis* (Beadle) Small; 5– *Calendula officinalis* L.*;* 6– *Thymus serpyllym* L.; 7– *Satureja montana* L.*,* 8– *Monarda fistulosa* L.; 9– *Myrtus communis* cv. Yuzhnoberezhny; 10– *Coreopsis grandiflora* Hogg ex Sweet; 11– *Rosmarinus officinalis* cv. Gorizont; 12– *Thymus vulgaris* cv. Fantasia; 13– *Vítex agnus-castus* L.; 14– *Hyssopus officinalis* L.; 15– *Monarda didyma* L.; 16– *Monarda* x *hybrida* hort.; 17– *Thymus striatus* cv. Yubileynyy. I- bioluminescence intensity index, calculated by the formula: I = Io/Ic × 100%, where Io – is the luminescence intensity of the test strain in the test sample, and Ic – the bioluminescence intensity of the test strain in the negative control sample.

Figure 4. The effect of different dilutions of ethanol plant extracts on the bioluminescence of *Aliivibrio fischeri* F1

Ethanol extracts of *T.serpyllym*, *S. montana*, *M. fistulosa*, *M. communis* cv. Yuzhnoberezhny, *C. grandiflora*, *R. officinalis*, *T. vulgaris*, *Vítex agnus-castus*, *H. officinalis*, *M. didyma*, *Monarda* x *hybrida* hort., *T. striatus* cv. Yubileynyy was reducing bioluminescence intensity by more than 75% at a maximum dilution of 1:100. When comparing the complete dataset of the effect of aqueous and alcohol extracts on the *A. fischeri* F1 test strain, a moderate linear positive correlation was observed with a Pearson correlation coefficient of 0.65. This finding suggests a general similarity in the effects of aqueous and ethanol extracts from plants. However, it is important to note that differences were identified in the overall action of the components extracted using different solvents. For instance, it was determined that ethanol extracts from *E. purpurea*, *E. pallida*, *E. Angustifolia, E. tennessesnsis, C. grandiflora*, *T. vulgaris*

cv. Fantasia had no considerable difference in effect with the aqueous extracts of these plants. For alcohol extracts of *E. purpurea*, *E. pallida*, *E. angustifolia*, *E. tennessesnsis*, ED_{50} was only 1:25, which indicates their low antimicrobial potential in both aqueous and alcohol forms. The activity of *C. grandiflora*, *T. vulgaris* cv. Fantasia was up to 4 times more both in alcohol and aqueous forms, their ED_{50} was 1:100, which indicates their higher antimicrobial potential. The differences were found in the action of aqueous and ethanol extracts from *C. officinalis, T.serpyllym*, *S. montana*, *M. fistulosa*, *M. communis* cv. Yuzhnoberezhny, *R. officinalis* cv. Gorizont, *Vítex agnus-castus, H. officinalis*, *M. didyma* и *M.* x *hybrida* hort., *T. striatus* cv. Yubileynyy. Ethanol extracts of these plants inhibited bioluminescence up to 4 times more than aqueous extracts. The ED_{50} of alcohol extracts was 1:100, and of aqueous extracts – 1:25.

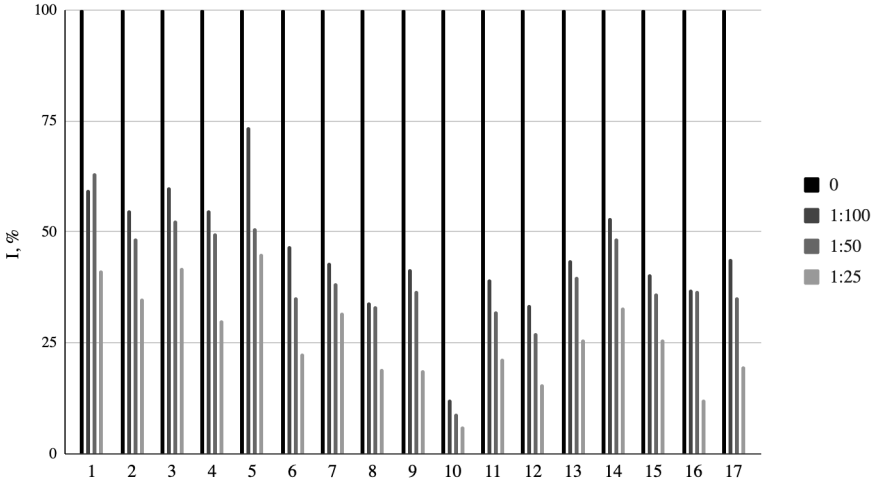
As a result of studies on the effect of aqueous plant extracts on the bioluminescence of *E. coli* pXen7, it was found that aqueous extracts of *E. purpurea*, *E. pallida*, *E. angustifolia*, *E. tennessesnsis*, *C. officinalis*, *T. serpyllym*, *S. montana,* and *M. communis* cv. Yuzhnoberezhny did not reduce bioluminescence intensity by more than 50% at a minimum dilution of 1:25 (Figure 5). Aqueous extracts from *M. fistulosa*, *R. officinalis* cv. Gorizont, *Vítex agnus-castus*, *H. officinalis*, *M.* x *hybrida* hort., and *T. striatus* cv. Yubileynyy reduced bioluminescence genetically engineered test strain by 50% or more at a dilution of 1:25. Aqueous extracts from *T. vulgaris* cv. Fantasia, *M. didyma*, reduced bioluminescence intensity at ED_{50} = 1:50 and only an aqueous extract from *C. grandiflora* inhibited the bioluminescence of the *E. coli* pXen7 at a dilution of 1:100.



1– *Echinacea purpurea* (L.) Moench; 2– *Echinacea pallida* (Nutt.). Nutt.; 3– *Echinacea angustifolia* A.Heller; 4– *Echinacea tennessesnsis* (Beadle) Small; 5– *Calendula officinalis* L.*;* 6– *Thymus serpyllym* L.; 7– *Satureja montana* L.*;* 8– *Monarda fistulosa* L.; 9– *Myrtus communis* cv. Yuzhnoberezhny; 10– *Coreopsis grandiflora* Hogg ex Sweet; 11– *Rosmarinus officinalis* cv. Gorizont; 12– *Thymus vulgaris* cv. Fantasia; 13– *Vítex agnus-castus* L.*;* 14– *Hyssopus officinalis* L.; 15– *Monarda didyma* L.; 16– *Monarda* x *hybrida* hort.; 17– *Thymus striatus* cv. Yubileynyy. I- bioluminescence intensity index, calculated by the formula: I = Io/Ic × 100%, where Io – is the luminescence intensity of the test strain in the test sample, and Ic – the bioluminescence intensity of the test strain in the negative control sample.

Figure 5. The effect of different dilutions of aqueous plant extracts on the bioluminescence of *Escherichia coli* pXen7 strain

As a result of testing of ethanol extracts on the bioluminescence of the *E. coli* pXen7 strain, it was found that 15 extracts reduced intensity of bioluminescence by 50% or more at a maximum dilution of 1:100, and only *E. angustifolia* and *C. officinalis* reduced I at ED_{50} = 1:50, and the alcohol extract from *E. purpurea* was characterized by ED_{50} = 1:25, despite the fact that alcohol in similar dilutions did not reduce bioluminescence by more than 8.69% of the control values (Figure 6).



1– *Echinacea purpurea* (L.) Moench; 2– *Echinacea pallida* (Nutt.). Nutt.; 3– *Echinacea angustifolia* A.Heller; 4– *Echinacea tennessesnsis* (Beadle) Small; 5– *Calendula officinalis* L.*;* 6– *Thymus serpyllym* L.; 7– *Satureja montana* L.*;* 8– *Monarda fistulosa*; 9– *Myrtus communis* cv. Yuzhnoberezhny; 10– *Coreopsis grandiflora* Hogg ex Sweet; 11– *Rosmarinus officinalis* cv. Gorizont,

12– *Thymus vulgaris* cv. Fantasia; 13– *Vitex agnus-castus* L.*;* 14– *Hyssopus officinalis* L.; 15– *Monarda didyma* L.; 16– *Monarda* x *hybrida* hort.; 17 – *Thymus striatus* cv. Yubileynyy. I – bioluminescence intensity index, calculated by the formula: I = Io/Ic × 100%, where Io – is the luminescence intensity of the test strain in the test sample, and Ic – the bioluminescence intensity of the test strain in the negative control sample.

Figure 6. The effect of different dilutions of ethanol plant extracts on the bioluminescence of *Escherichia coli* pXen7 strain

A moderate linear positive correlation (Pearson correlation coefficient = 0.78) was observed when comparing the effects of aqueous and ethanol extracts on the test strain *E. coli* pXen7. This suggests a general similarity in the results, although the differences were noted between the effects of the two extract types. For example, when evaluating the effect of alcohol and aqueous extracts on the luminescence of the test strain *E. coli* pXen7, it was found that when using 70% ethyl alcohol as a solvent, the ED_{50} value for *E. purpurea, E. pallida*, *E. angustifolia*, *E. tennessesnsis, C. officinalis* increased to 1:100 compared to aqueous extracts, for *T. serpyllym* increased to 1:50. For *M. fistulosa*, *R. officinalis* cv. Gorizont, *V. agnus-castus, M.* x *hybrida* hort.*, T. striatus* cv. Yubileynyy ED_{50} has changed from 1:25 to 1:100. The strength of the effect has not changed for *C. grandiflora a*nd *H. officinalis*. ED_{50} for *T. vulgaris* cv. Fantasia and *M. didyma* became 1:100. And only for *S. montana* and *M. communis* cv. Yuzhnoberezhny ED_{50} change occurred up to 1:100. The data obtained confirm that ethanol is more optimal for extracting the amount of substances with antimicrobial activity. When the overall data on the effect of aqueous extracts on the *A. fischeri* F1 test strain and the *E. coli* pXen7, strain were compared, a moderate linear direct correlation with a Pearson correlation coefficient of 0.67 was obtained. This indicates that the results obtained from both strains were similar. When comparing the data on the effect of alcohol extracts on the test strain *A. fischeri* F1 and the strain *E. coli* pXen7, a moderate linear direct correlation was established with a Pearson correlation coefficient of 0.77, which indicates that both strains were similarly more sensitive to the action of ethanol extracts of plants then aqueous.

The experimental results are supported by literature data regarding the activity of the plants tested in the experiment. For instance, Echinacea, which extracts have not shown high inhibitory activity in the experiments, is known to contain polysaccharides and hydroxycinnamic acids as the main bioactive compounds. When using water as a solvent, the proportion of polysaccharides is 72%, and hydroxycinnamic acids 53% in the samples. When water-alcohol

mixtures are used as a solvent, 77% of polysaccharides and 46% of hydroxycinnamic acids are extracted[27]. Therefore, the choice of the solvent can considerably impact the activity of the plant extract. Echinacea purpurea extracts, the main action of which is immunomodulatory[28], according to modern scientific studies, inhibit the growth of *Candida albicans, Saccharomyces cerevisiae, Streptococcus pyogenes, Haemophilus influenzae*, *Legionella pneumophila*. However, many pathogens such as *Acinetobacter baumannii, Bacillus cereus, Bacillus subtilis, Enterococcus faecalis, Aspergillus niger, Escherichia coli, Klebsiella pneumoniae*, *Pseudomonas aeruginosa,* and *Candida albicans* were found to be insensitive to Echinacea preparations[29]. The Coreopsis extracts have demonstrated a significant inhibitory effect on bacterial luminescence in both aqueous and alcohol forms. This outcome can be attributed to the presence of phenolic compounds and phlavonoids, including chalcones, aurones, anthocyanins, flavanones, flavonols, and phenylpropanoids within *C. grandiflora*, which are found to possess diverse pharmacotherapeutic properties, such as antioxidant, antibacterial, antiviral, and anti-inflammatory[30]. Flavonoids' antibacterial mechanism of action involves forming complexes with bacterial cell walls. Additionally, flavonoids that are highly lipophilic have the capacity to disrupt bacterial membranes. The most common flavonoid – quercetin, which is also present in Coreopsis grandiflora has shown high inhibitory activity against Streptococcus pyogenes, and mild activity against various Gram-positive and Gram-negative bacteria. Its mechanism of action includes the inhibition of biofilm and Beta-lactamase formation. The average minimum inhibitory concentration (MIC) of quercetin is reported to be 58.7 μg/mL[31-33]. According to the research data Coreopsis extracts exhibit high activity against pathogenic flora, with *Enterococcus faecalis* and *Bacillus cereus* being the most sensitive[33].

High inhibitory activity in both aqueous and ethanol forms was noted for *T. vulgaris* cv. Fantasia, with an ED50 of 1:50 for the aqueous extract and 1:100 for the alcohol extract. While *T. serpyllym* produced a significant inhibitory effect only in the ethanol form, ED_{50} = 1:100. Thyme is approved for use as an antimicrobial, antiseptic, antifungal, expectorant, enveloping, antispasmodic, and reducing gas formation in the intestine. In plants of the genus *Thymus* L., the main active substances are phenolic compounds, essential oils, and triterpene compounds. The difference in the manifested biological effects is confirmed by the data on the chemical composition of these species. It has been reported that most of the thyme species are superior to *T. serpyllym* in terms of the total content of essential oil[34]. The MIC of the thymol, the major component of Thyme species, varies from 2-10 μg/mL[35]. Calendula flowers

are used in official medicine as an anti-inflammatory agent for diseases of the gastrointestinal tract, kidneys, and urinary tract[36]. Carotenoids and polysaccharides are the major components of Calendula flowers; its aqueous extract's major components are flavonoids and saponins, and alcohol extracts contain alkaloids, flavonoids, and saponins[37]. 70% ethanol extracts of *Calendula officinalis* had a strong inhibitory effect on bacterial luminescence with ED_{50} = 1:100, while aqueous extracts had no inhibitory effect even at a minimum dilution of 1:25. This effect can be explained by the fact that 70% ethanol solution is considered to be more suitable for extracting the sum of flavonoids contained in calendula flowers[38].

The main biologically active compounds of the investigated species of the genus *Monarda* are thymol, carvacrol, paracymol, and their derivatives[39]. The known biological activity of these compounds against pathogens is consistent with the traditional use of *Monarda* L. species for the treatment of wounds, skin infections, colds, and fevers. The significant antibacterial activity of *Monarda* species is supported by literature data[40]. According to the study's findings, the species *Monarda x hybrida* hort., which belongs to the chemotype with a higher content of geraniol, demonstrated stronger activity among the *Monarda* samples tested. *Satureja montana* (savory) contains as main components terpenes and terpenoids, which are known to play a key role in antibacte'©al action. Studies have shown that savory extracts are active against both Gram-positive (*Listeria monocytogenes*, *Staphylococcus aureus*, *Staphylococcus haemolyticus*) and Gram-negative (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* и *Serratia marcescens*) bacterial strains[41]. The primary constituents of *V. agnus-castus*are are essential oils with a predominance of 1,8-cineol and α-pinene, flavonoids, iridoids, diterpenoids, and steroids[42].

Vtex is currently utilized as a dietary supplement for estrogen hormone imbalances. A number of studies have noted a high antibacterial activity of *V. agnus-castus*, including antibiotic-resistant strains of *Pseudomonas aeruginosa* and *Escherichia coli*[43]. The literature review on the biologically active components of Hyssopus showed the presence of polyphenolic compounds, primarily flavonoids, apigenin, quercetin, diosmin, luteolin, and their glucosides, followed by other phenolic compounds, such as chlorogenic, protocatechin, ferulic, syringic, p-hydroxybenzoic and caffeic acid. In essential oils isolated from the aerial part of *H. officinalis*, several major components have been identified, including the terpenoids pinocamphone, isopinocamphone, and β-pinene. Hyssop has moderate antioxidant and antimicrobial activity against gram-positive and negative bacteria, as well as antifungal, insecticidal, and antiviral properties *in*

vitro. This plant has been found in animal experiments to exhibit muscle relaxant, antiplatelet, and alpha-glucosidase inhibitory properties[44-45].

Summarising the experimental data, it can be concluded that both strains displayed sensitivity to individual substances and plant extracts in the form of tinctures and decoctions. The natural bacterial strain A. *fischeri* F1 exhibited higher sensitivity to both aqueous and ethanol plant extracts compared to the recombinant strain *E. coli* pXen7, although the data on the effect of plant extracts correlated between the two strains. The study specifically identified extracts from *C. grandiflora*, *T. vulgaris* cv. Fantasia, and *M. x hybrida* hort as having the strongest inhibitory effect (ED_{50} = 1:100) in the forms of decoctions and tinctures. Extracts from *T. serpyllym, S. montana, M. fistulosa, M. communis* cv. Yuzhnoberezhny, *R. officinalis* cv. Gorizont, *H. officinalis, M. didyma, T. striatus* cv. Yubileynyy, and *Vitex agnus-castus* showed moderate inhibitory activity (ED_{50} = 1:25 to 1:100). *E. purpurea, E. pallida, E. angustifolia,* and *E. tennessesnsis* exhibited weak inhibitory activity with minimal ED_{50} values ranging from 1:25 to 1:50. Notably, the inhibitory activity of most plant extracts was enhanced when using 70% ethanol as a solvent. However, their inhibitory activity exceeded that of the 70% ethanol solution at the same dilutions. These findings align with the literature data, further affirming the confirmation of the validity of the obtained results.

Overall, the investigation demonstrated the potential of whole-cell bacterial bioluminescent biosensors for evaluating the antimicrobial activity of aqueous and ethanol extracts from medicinal plant raw materials, with *A. fischeri* F1 strain exhibiting higher sensitivity and the ability to detect variations in the antibacterial action of different plant species.

STATEMENT OF ETHICS

This study did not involve experiments on animals or humans, and, therefore, ethical approval was not required. Nevertheless, the research methods employed adhered to ethical standards, ensuring data integrity, compliance with professional codes of conduct, and adherence to institutional policies regarding research practices.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Yuliia Yu Havrychenko designed and carried out the experimental work and wrote the article with support from Andrei M. Katsev. Andrei M Katsev super-

vised the findings of this work and contributed to the final manuscript. Sergei L Safronyuk contributed to the analysis of the results. Dhruv Vashisht contributed to sample preparation and designed the figures.

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