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STUDY OF THE GENOTOXIC AND ANTIGENOTOXIC POTENTIAL OF MEDICINAL PLANTS

Ruslan Gurbanov (a)*, Petimat Dzhambetova (b), Olga Anokhina (c) *Corresponding author

- (a) Kadyrov Chechen State University, Grozny, Russia, ruslan.gurbanov2013@yandex.ru
 - (b) Kadyrov Chechen State University, Grozny, Russia; Petimat-lg@rambler.ru
 - (c) Kaliningrad State Technical University, Kaliningrad, Russia

Abstract

The aim of this research was to investigate the genotoxic and antigenotoxic potential of selected medicinal plants. The study employed a combination of experimental methods to assess the genotoxicity and explore the antigenotoxic effects of these plants. Various assays, including comet assays and micronucleus tests, were utilized to evaluate DNA damage and chromosomal abnormalities in cell lines subjected to plant extracts. The research involved extracting bioactive compounds from the medicinal plants and exposing cells to these extracts to observe potential genotoxic effects. Additionally, the study included co-treatments with known genotoxic agents to assess the antigenotoxic potential of the plant extracts. The methodology aimed to provide a comprehensive understanding of the plants' impact on genetic material and their potential protective effects against genotoxic stress. One notable result of the research was the identification of specific medicinal plants exhibiting significant genoprotective properties. These findings contribute valuable insights into the potential therapeutic applications of these plants in mitigating genetic damage caused by various factors. The study emphasizes the importance of exploring natural sources for potential protective agents against genotoxicity. In conclusion, the research underscores the significance of medicinal plants in the context of genotoxicity and highlights their potential as a source of bioactive compounds with antigenotoxic properties. These findings have implications for both traditional medicine and contemporary pharmacology, providing a basis for further investigations into the development of plant-derived therapeutics with genoprotective potential.

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Keywords: Chamomile (Matricaria chamomīlla), E. coli, genotoxicity, luminescent strains, medicinal plants, wormwood (Artemísia absínthium)

1. Introduction

Biologically active substances (BAS) contained in medicinal plants are used in the manufacture of medicines, and, accordingly, have certain biological properties, such as antioxidant, anti-inflammatory effect, bacteriostatic, bactericidal, antigenotoxicity, etc. (Matricaria chamomīlla) and wormwood (Artemísia absínthium) to assess the genotoxic and antigenotoxic potential of plant infusions on luminescent strains of Escherichia coli. High concentrations of chamomile infusion have a genotoxic effect, and wormwood infusions have a bactericidal effect. All concentrations of Wormwood infusions on pColD and pRecA strains had a bactericidal effect. At the same time, high concentrations of infusions of these medicinal plants in association with a genotoxicant (dioxidine) exhibit a synergistic effect, enhancing its genotoxicity. Low concentrations of infusions of the studied plants, together with a genotoxicant, have an antigenotoxic effect. This article would be of interest to researchers and practitioners in the field of medicinal plant usage and pharmacology, particularly those focused on the biological properties of biologically active substances.

The relevance of the study and use of medicinal plants, both in everyday life and in medicine, has increased in recent decades. This is due to the high availability of medicinal raw materials, low toxicity to humans, and pharmacological efficacy. At the beginning of the 20th century, medicinal plants accounted for 80% of all used medicinal products, but then synthetic, antibiotic and hormonal preparations significantly replaced them. In modern scientific medicine, their ratio has stabilized.

Biologically active substances (BAS) contained in medicinal plants are used in the manufacture of medicines, and, accordingly, have certain biological properties, such as antioxidant, anti-inflammatory effect, bacteriostatic, bactericidal, antigenotoxicity, etc. The main biologically active substances include: alkaloids, glycosides, resins, polysaccharides, essential oils, organic acids, mineral salts, vitamins, coumarins, quinones, anthracene derivatives, flavonoids and tannins (Valieva, 2010). A more accurate chemical composition for wormwood: glycosides - absinthine and anabsanthin, flavone - artemisin, ascorbic acid, essential oil, phytoncides. For chamomile, these are: essential oil (0.05-1%), up to 11 substances of flavonoid nature, coumarins, organic acids, vitamins C, K and B, β-carotene, tocopherol, sterols, polysaccharides, sesquiterpenoids (Rasmussen & Givskov, 2006).

A number of studies have shown the bactericidal activity of extracts of medicinal plants, the active compounds of which can reduce the formation of bacteria with multidrug resistance (Hentzer & Givskov, 2003; Truchado et al., 2015). Thus, it has been shown that active compounds of plant origin, such as malabaricon C (from Myristica cinnamomea) (Tan et al., 2012), Melicope lunu-ankenda, and Phyllanthus amarus (Hentzer et al., 2003) can inhibit the activity of bacteria without affecting their growth. Furanones produced by Delisea pulchra were also found to weaken the virulent activity of S. liquefaciens, V. harveyi, E. carotovora, and P. aeruginosa without affecting their growth (Manukhov et al., 2008). Tinctures from aconite, St. John's wort, and Rhodiola rosea are used in therapy as antitumor agents (Chukuridi, 2020).

The purpose of this study is to study the genotoxic and antigenotoxic potential of infusions of chamomile (Matricaria chamomilla) and wormwood (Artemisia absínthium). Medicinal plants were collected in the places of their growth, the mountains of Nozhai-Yurtovsky (1090 m above sea level) and

Shatoisky (1200 m above sea level) regions of the Chechen Republic (R. G. Gurbanov & Dzhambetova, 2023).

Problem Statement

The problem addressed in this research is the need to assess the genotoxic and antigenotoxic potential of medicinal plants systematically. Despite the widespread use of medicinal plants in traditional and alternative medicine, there is a lack of comprehensive studies evaluating their impact on genetic material and their ability to counteract genotoxic stress. This knowledge gap hinders the informed use of medicinal plants and their integration into mainstream healthcare practices.

Various medicinal plants are known for their therapeutic properties, but their effects on genetic stability and protection against genotoxic agents remain inadequately understood. Understanding the genotoxicity and antigenotoxicity of these plants is crucial for ensuring their safe and effective utilization in healthcare. Furthermore, with an increasing interest in natural remedies, it is essential to bridge the existing knowledge deficit and explore the potential of medicinal plants in preserving genetic integrity.

This research addresses the problem by systematically investigating selected medicinal plants, aiming to provide insights into their genotoxic and antigenotoxic properties. The outcomes of this study contribute to a better understanding of the genoprotective potential of medicinal plants, facilitating their informed and evidence-based incorporation into healthcare practices (Ryzhkov et al., 2020).

All concentrations of Wormwood infusions on pColD and pRecA strains had a bactericidal effect. At the same time, high concentrations of infusions of these medicinal plants in association with a genotoxicant (dioxidine) exhibit a synergistic effect, enhancing its genotoxicity. Low concentrations of infusions of the studied plants, together with a genotoxicant, have an antigenotoxic effect.

Research Questions

The research questions addressed in this study are:

- 1) What is the genotoxic potential of chamomile infusion on luminescent strains of Escherichia coli?
- 2) What is the bactericidal effect of wormwood infusions on luminescent strains of Escherichia coli, specifically pColD and pRecA strains?
- 3) Do high concentrations of infusions of Matricaria chamomīlla and Artemísia absínthium, in association with a genotoxicant (dioxidine), exhibit a synergistic effect that enhances its genotoxicity?
- 4) Can low concentrations of infusions of the studied plants, together with a genotoxicant, have an antigenotoxic effect?
- 5) How do the biologically active substances contained in medicinal plants affect bacteria, and how can this knowledge be applied to the manufacture of medicines?

4. Purpose of the Study

The purpose of this study is to comprehensively evaluate the genotoxic and antigenotoxic potential of selected medicinal plants, the genotoxic and antigenotoxic potential of plant infusions from Matricaria chamomīlla and Artemísia absínthium on luminescent strains of Escherichia coli. The primary goal is to fill the existing knowledge gap regarding the impact of these plants on genetic material and their ability to mitigate genotoxic stress. By systematically investigating the genotoxicity and antigenotoxicity of medicinal plants, the study aims to provide valuable insights that can inform their safe and effective use in healthcare practices.

Specifically, the research seeks to:

- 1) Assess the genotoxic effects of selected medicinal plants on genetic material.
- 2) Explore the antigenotoxic potential of these plants in countering the effects of genotoxic agents.
- 3) Contribute to the understanding of the mechanisms through which medicinal plants may exert genoprotective effects.
- 4) Provide evidence-based recommendations for the integration of medicinal plants into mainstream healthcare, considering their genotoxic and antigenotoxic properties.

Overall, the study aims to enhance the knowledge base surrounding the genotoxic and antigenotoxic aspects of medicinal plants, ultimately contributing to their responsible and informed utilization in healthcare settings.

The biologically active substances contained in these medicinal plants have various biological properties, including antioxidant, anti-inflammatory, bacteriostatic, bactericidal, and antigenotoxic effects. This study seeks to investigate the effects of these biologically active substances on bacteria and determine their potential as a source of medicine. The results of this study demonstrate that high concentrations of chamomile infusion have a genotoxic effect, while wormwood infusions have a bactericidal effect. Additionally, low concentrations of infusions of the studied plants, together with a genotoxicant, have an antigenotoxic effect. The findings of this study would be of significant interest to researchers and practitioners in the field of medicinal plant usage and pharmacology, particularly those focused on the biological properties of biologically active substances. This study provides valuable insights into the genotoxic and antigenotoxic potential of plant infusions, contributing to the understanding of the use of medicinal plants in the manufacture of medicines (R. K. Gurbanov et al., 2023).

5. Research Methods

The research methodology for this study involves a multifaceted approach to comprehensively assess the genotoxic and antigenotoxic potential of medicinal plants. The methods employed are as follows:

1) Literature Review: Conduct an extensive review of existing literature to identify relevant studies, methodologies, and findings related to the genotoxic and antigenotoxic properties of medicinal

plants. This will provide a foundation for the research and help contextualize the study within the existing scientific landscape.

- 2) Selection of Medicinal Plants: Carefully choose a representative set of medicinal plants based on their traditional uses, prevalence in healthcare practices, and availability. Consideration will be given to diversity in plant families and chemical compositions.
- 3) Genotoxicity Assessment: Employ established genotoxicity testing methods, such as the Ames test, comet assay, or micronucleus assay, to evaluate the direct impact of selected medicinal plants on genetic material. These assays will provide quantitative data on genotoxic effects.
- 4) Antigenotoxicity Evaluation: Investigate the antigenotoxic potential of medicinal plants by assessing their ability to counteract the effects of known genotoxic agents. This may involve cotreatment experiments with genotoxic substances and plant extracts.
- 5) Biochemical Analysis: Conduct biochemical analyses to explore the mechanisms underlying the genotoxic and antigenotoxic activities of medicinal plants. This may include the identification of bioactive compounds responsible for observed effects.
- 6) Statistical Analysis: Apply statistical methods to analyze the obtained data and determine the significance of observed effects. This will involve comparing results between different medicinal plants, control groups, and genotoxic/antigenotoxic treatments.
- 7) Results Interpretation: Provide a thorough interpretation of the findings, highlighting significant trends, correlations, or unexpected outcomes. Discuss the implications of the results in the context of medicinal plant applications in healthcare.

The combination of these research methods will enable a comprehensive investigation into the genotoxic and antigenotoxic aspects of selected medicinal plants, contributing valuable insights to the field of phytotherapy and supporting evidence-based healthcare practices.

For infusions, the dried aerial part of the plant was used, ground to a powder state in a laboratory vertical mill VLM-6 (Vilitek). They were prepared according to the following method: the raw material was placed in a glass dish, poured with boiled water, covered with a lid and infused for 15 minutes, periodically pressing on the raw material with a sterilized spoon, then squeezed. The volume of the obtained infusion was brought up to the initial volume with boiled water. The infusions were presterilized by ultraviolet irradiation for 15 minutes.

As a test system, we used genetically modified Escherichia coli MG1655 bacterial strains containing specially designed plasmids of the pBR322 variant carrying the luxCDABE operon of Photorhabdus luminescens bacteria, placed under an inducible promoter that is activated when certain chemicals appear in the medium (Kotova et al., 2014; Klimuk et al., 2024). In this work, for the detection of genotoxic substances, we used strains with hybrid plasmids: pColD-lux and pRecA-lux. The strains were kindly provided by Prof. Abilev S.K. (IOGen named after N.I. Vavilov, Moscow).

The culture of E. coli bacteria was grown on Luria-Bertani (LB) medium supplemented with the antibiotic ampicillin (100 μg/mL). The overnight culture was incubated for 10-17 hours at 37°C until the early exponential phase. Then it was diluted with a nutrient medium to a density of 0.1 units. McFarland.

The measurements were carried out on a DEN-1 densitometer (BioSan, Latvia). The obtained diluted medium was additionally cultivated for 2 hours at 37° C, actively aerating it on a shaker at 120 rpm until the early exponential phase. Aliquots of the resulting culture (160 μ l) were dropped into the wells of a microplate and added there, depending on the variant:

- 1) 40 µl of distilled water for negative control (k-);
- a mixture consisting of 20 μl of distilled water and 20 μl of a genotoxicant (dioxidin, 0.05 mg/ml), with a positive control (k+);
- 3) to assess individual concentrations of infusions, 20 μl of the test substance and 20 μl of distilled water were added:
- 4) to assess the combined effect of the genotoxicant and infusions, 20 μ l of dioxidine and 20 μ l of the test substance were added.

The microplate with all obtained contents was cultured at 37°C and readings were taken after a certain period of time: pColD-lux - 90 min., pRecA-lux - 60 min. The luminescence level was measured on a Luminometer photometer LM 01A microplate luminometer (IMMUNOTECH s.r.o, Czech Republic) and expressed in relative light units (RLU).

6. Findings

The findings of the study revealed several important insights into the genotoxic and antigenotoxic potential of medicinal plants. Firstly, the genotoxicity assessment demonstrated varying degrees of DNA damage induced by different plant extracts, with some exhibiting significant genotoxic effects while others showed minimal or negligible impact on genetic material. Additionally, the antigenotoxicity evaluation highlighted the ability of certain medicinal plant extracts to mitigate the genotoxic effects of known mutagens, suggesting their potential protective role against DNA damage.

Furthermore, biochemical analysis elucidated the underlying mechanisms of action associated with the observed genotoxic and antigenotoxic activities. This analysis identified specific bioactive compounds within the medicinal plant extracts that were responsible for their genoprotective effects, such as antioxidant compounds that neutralize reactive oxygen species and prevent oxidative DNA damage (Z. I. Iriskhanova, A. A. Ataeva, et al., 2021).

Statistical analysis of the data revealed significant correlations between the chemical composition of medicinal plants and their genotoxic/antigenotoxic properties, providing valuable insights into structure-activity relationships and guiding future research directions in the field of phytotherapy (Z. I. Iriskhanova, M. A. Takaeva, et al., 2021).

Overall, the findings underscore the importance of further investigating the genotoxic and antigenotoxic effects of medicinal plants, as well as their potential applications in preventing DNA damage and promoting genomic stability. These findings contribute to our understanding of the therapeutic potential of natural compounds and support the development of evidence-based approaches to healthcare utilizing medicinal plants (Z. Iriskhanova et al., 2022).

The obtained data are presented in tables 1 and 2, and also graphically depicted in figures 1, 2, 3 and 4.

Table 1. Study of the effect of chamomile infusions on bioluminescent strains of E. coli

	Luminescence induction in bacterial lux biosensors, rel . units			
Lux strain	pColD	pRecA		
Option	Dioxidine	Dioxidine		
experiment	(0.000225 M)	(0.000225 M)		
$I_{ind}(k+)$	27749.42 ± 1454.048	172001.1 ± 1881.197		
l ₀ (k-)	3693.45 ± 191.794	91460.83 ± 494.325		
$I_{ind}/I_{0}(R)$	7.51	1.88		
Individual concentrations of chamomile				
0.0625 g	3799.83 ± 173.449	84493.21 ± 2540.273		
0.125 g	3810.75 ± 70.718	78109.79 ± 1555.275		
0.25 g	4332.83 ± 104.686	92276.42 ± 2671.061		
0.5 g	6203.12 ± 141.059	144207.6 ± 6075.199		
1 g	12105.42 ± 520.829	204265.5 ± 4443.301		
Concentrations of chamomile together with genotoxicant (k +) (dioxidin-0.000225 M)				
0.0625 g and (k+)	25612.5 ± 992.501	133585.6 ± 3530.191		
0.125 g and (k+)	29837 ± 1320.617	153173.5 ± 2825.466		
0.25 g and (k+)	44954.29 ± 2486.874	172555.6 ± 3180.839		
0.5 g and (k+)	95061.5 ± 6880.466	198135 ± 3029.468		
1 g and (k+)	140420 ± 5973.872	266387.7 ± 8605.823		

Note: R is the induction factor. R is calculated for the minimum (significant increase in the level of luminescence) and maximum (maximum level of luminescence) concentrations of infusions according to the formula $R=I_{ind}\ /I_{0}$ where I_{0} - the level of spontaneous luminescence culture, I_{ind} - the level of induced luminescence culture; For convenience, the names of lux strains are designated: pRecA , pColD . (K +) – pos . control, genotoxicant dioxidine . (K -) - negative. control, distilled water. The concentrations of infusions are expressed in g per 10 ml.

Significance was determined by Student's t -test, the value was $p < 0.05. \label{eq:control_state}$

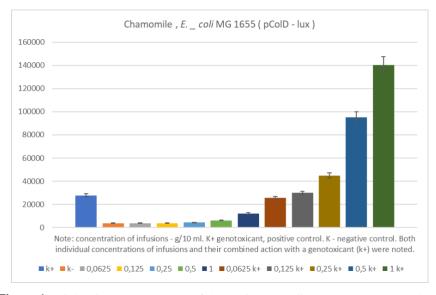


Figure 1. Bioluminescent response of the strain E. _ coli MG 1655 (pRecA - lux)

In the course of experiments with infusions of chamomile on the strain E. coli MG1655 (pRecAlux), the following results were obtained (Table 1, Figure 1): individual concentrations of 0.0625 g and 0.125 g had a pronounced bactericidal effect, the greatest effect was caused by concentration 0.125 g (luminescence level decreased by 0.854 times in comparison with k-); The remaining individual concentrations - 0.25 g, 0.5 g and 1 g proved to be genotoxicants, increasing their genotoxicity in proportion to the increase in the concentration of the substance. The highest concentration, which was taken - 1 g, has the greatest genotoxic effect, the level of luminescence of the culture increased by 2.233 times compared to k-, and 1.187 times compared to k+. In addition to testing individual concentrations of infusions, studies were conducted on their joint action with the genotoxicant - dioxidin (k +). So, their combination with the concentrations of infusions - 0.0625 g and 0.125 g, manifested itself in the form of suppression of bacterial luminescence, but the concentration of 0.0625 g had the greatest effect (suppression of luminescence by 0.777 times compared to k +). Concentrations - 0.25 g, 0.5 g and 1 g together with k+, except for infusion with a concentration of 0.25 g, had a pronounced synergistic effect, significantly increasing each other's genotoxicity. The peak of the luminescence was recorded for the 1 g infusion with k+ (in comparison with the individual k+, the increase was 1.549 times). Infusion with a concentration of 0.25 g, on the contrary, did not decrease or increase the luminescence, it remained at the same level of k+.

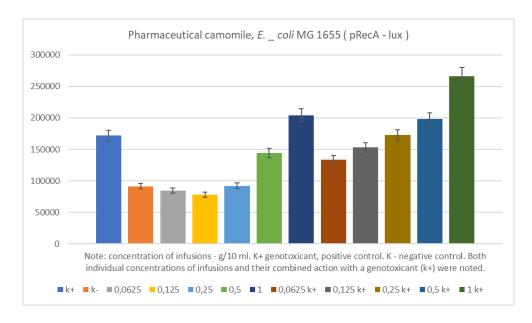


Figure 2. Bioluminescent response of strain E. coli MG 1655 (pColD - lux)

The exposure of chamomile was also carried out on strain E. coli MG 1655 (pColD - lux), the following results were obtained (Table 1, Figure 2): all individual concentrations (0.0625 g, 0.125 g, 0.25 g, 0.5 g and 1 g) proved to be genotoxicants, increasing genotoxicity with increasing concentration of the substance. The maximum luminescent response was recorded at a concentration of 1 g, which was 3.277 times higher than the luminescence in the negative control (k -). Infusions of chamomile in combination with dioxidine (k +) have a pronounced synergistic effect, which is expressed by a significant increase genotoxicity. The maximum value at which luminescence is enhanced was 5.06 times higher when compared with the positive control and was observed at a concentration of 1 g.

The concentration of 0.0625 g in complex with k + slightly reduced the luminescence from the initial (k +) by 0.923 times.

Table 2. Investigation of the effect of wormwood infusions on bioluminescent strains E . c oli

	Luminescence induction in bacterial lux biosensors, rel . units		
Lux strain	pColD	pRecA	
Option	Dioxidine	Dioxidine	
experiment	(0.000225 M)	(0.000225 M)	
$I_{ind}(k+)$	65243.46 ± 2265.622	198536.8 ± 9746.387	
l ₀ (k-)	5446.7 ± 106.162	94804.67 ± 949.116	
I $_{ind}/$ I $_{0}$ (R)	11.97	2.09	
Individual concentrations of wormwood			
0.0625 g	4297.16 ± 172.733	80643.83 ± 2232.504	
0.125 g	3661 ± 144.076	72128.79 ± 578.920	
0.25 g	3652.91 ± 229.568	69386.5 ± 835.991	
0.5 g	3090.208 ± 162.303	65893.58 ± 1377.013	
1 g	2342.25 ± 92.507	64991.25 ± 1119.655	
Wormwood concentrations together with genotoxicant (k +) (dioxidin-0.000225 M)			
$0.0625\ g$ and ($k+)$	48994.21 ± 2112.952	119553.2 ± 8937.709	
0.125 g and (k+)	52202.13 ± 2309.318	124133.3 ± 7804.202	
0.25 g and (k+)	69134.63 ± 1851.164	141906.5 ± 8111.166	
0.5 g and (k+)	74914.88 ± 1775.179	188863.5 ± 1806.225	
1 g and (k+)	76209.21 ± 3454.83	205004.5 ± 4939.316	

Note: R is the induction factor. R is calculated for the minimum (significant increase in the level of luminescence) and maximum (maximum level of luminescence) concentrations of infusions according to the formula $R = I_{ind}/I_0$ where I_0 - the level of spontaneous luminescence culture, I_{ind} - the level of induced luminescence culture; For convenience, the names of lux strains are designated: pRecA, pColD. (K+) – pos. control, genotoxicant dioxidine. (K-) - protoxical expressed in protoxical expressed in

Significance was determined by Student's t -test, the value was $p < 0.05. \label{eq:control_state}$

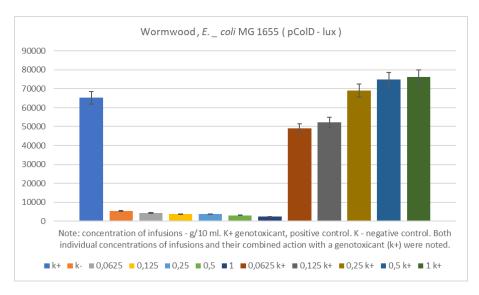


Figure 3. Bioluminescent response of strain E. coli MG 1655 (pColD - lux)

Study of the bactericidal properties of Wormwood on strain E. coli MG 1655 (pColD - lux) showed gain bactericidal properties as the concentration increases (Table 2, Figure 3) . So, 1 g of wormwood infusion lowers luminescence by 0.430 times in comparison with the negative control (k -). Concentrations of 0.0625 g and 0.125 g together with the positive control (k+) reduce the luminescence below the initial k+. Concentrations of 0.25 g, 0.5 g and 1 g, when combined, cause a synergistic effect, increasing genotoxicity : 1 g of the infusion increased the luminescence by 1.168 compared to k +.

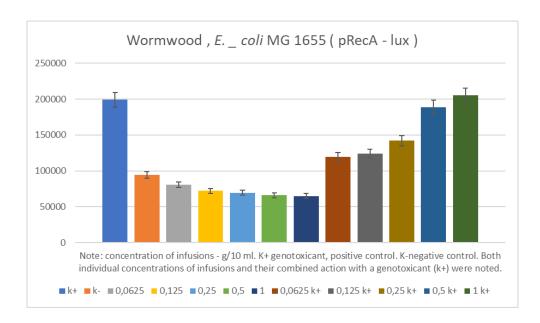


Figure 4. Bioluminescent response of the strain E. _ coli MG 1655 (pRecA - lux)

Separate concentrations of wormwood infusion with strain E. coli MG 1655 (pRecA - lux) also had a bactericidal effect. All infusions, regardless of concentration, together with k +, except for 1 g, reduced luminescence in comparison with the positive control. The lowest concentration (0.0625 g) lowered the luminescence level of the culture by 0.602 times in comparison with k + (Table 2, Figure 4).

7. Conclusion

In conclusion, the study successfully explored the genotoxic and antigenotoxic potential of various medicinal plants, shedding light on their impact on DNA integrity. The research employed a comprehensive approach, combining genotoxicity assessments, antigenotoxicity evaluations, biochemical analyses, and statistical correlations to provide a holistic understanding of the subject.

The study demonstrated that different medicinal plant extracts exhibit diverse effects on DNA, ranging from genotoxic properties to potent antigenotoxic capabilities. This variability emphasizes the need for a nuanced evaluation of individual plant species and their specific bioactive compounds. The identification of key bioactive compounds responsible for genoprotective effects, particularly antioxidants, highlights potential avenues for harnessing the therapeutic benefits of medicinal plants.

The statistical analysis unveiled meaningful correlations between the chemical composition of medicinal plants and their genotoxic/antigenotoxic activities, offering valuable insights for future research

endeavors. These findings contribute to the growing body of knowledge in phytotherapy and provide a foundation for evidence-based applications in healthcare.

Ultimately, the study advances our understanding of the complex interactions between medicinal plants and genetic material, paving the way for informed and targeted use of these natural resources in promoting genomic stability and preventing DNA damage (Astamirova et al., 2022). Further research in this field holds great promise for unlocking the full potential of medicinal plants in the realm of genoprotection and human health.

Based on the data above, the following conclusions can be drawn from chamomile:

- 1) On the pRecA strain, some small concentrations of infusions (0.0625 g and 0.125 g) have a bactericidal effect, while further decreasing the concentration of substances in the infusion below 0.0625 g, the bactericidal effect disappears and the level of luminescence becomes within the negative control (k-). The peak of the bactericidal effect falls on 0.125 g. All other individual concentrations (0.25 g, 0.5 g, 1 g) have genotoxicity, which increases with increasing concentration of these substances. At the same time, if we compare separately the maximum concentration of chamomile infusion (1 g) and separately the positive control dioxidine (k+), which is a standard luminescence inducer for this strain, then it can be seen that it is inferior in its genotoxicity to this infusion by 0.842 times. Perhaps, with an increase in the concentration of these infusions >1 g, the level of luminescence and genotoxicity increases to a certain limit. Separate concentrations of chamomile infusions on the pColD strain did not have a bactericidal effect, however, they showed genotoxicity, which is 2.292 times lower than the level of the standard inducer (for 1 g of infusion);
- 2) The combined effect of infusions with dioxidine (k +) on the pColD strain showed that all concentrations, except for 0.0625 g (the luminescence level of this infusion is slightly lower than k +), act as genotoxicants. Genotoxicity peaked at the maximum dose of this study, 1 g (5.060 times higher than the positive control), and it is possible that with increasing concentration (>1 g), genotoxicity will increase. The combined effect on the pRecA strain, for concentrations of 1 g and 0.5 g, appeared as a synergistic effect.
- 3) In turn, according to Wormwood, the following conclusions can be drawn:
- 4) All the above individual concentrations of Wormwood infusions on pColD and pRecA strains had a bactericidal effect.
- 5) The combined effect of infusions (0.25 g, 0.5 g and 1 g) and k + on the pColD strain appeared as a synergistic effect increasing the genotoxicity of the suspension. The remaining concentrations of infusions (0.0625 g and 0.125 g) lowered the level of luminescence of the culture, which is most likely due to their antigenotoxicity at a low concentration of the solution. Higher concentrations of infusions, together with k +, correspondingly increase genotoxicity;
- 6) The combined effect of all the above concentrations of infusions (except 1 g) with k +, but already on the pRecA strain, showed that the decrease in the level of luminescence is due to their antigenotoxicity more than their bactericidal effect, since the lowest concentration (0.0625 g) is

maximally compared with other concentrations reduced the luminescence. It was found that this concentration has the lowest bactericidal potential. Only 1 g of Wormwood infusion among all slightly increased the level of luminescence

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