Oxidative and antioxidative properties of medicinal flora of the Chechen Republic

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Abstract. The oxidative and antioxidative properties of medicinal infusions of Chamomile (Matricaria chamomilla) and Wormwood (Artemísia absínthium) were studied on luminescent strains of Escherichia coli. The studied plants grow in the mountains of the Nozhai-Yurt and Shatoi regions of the Chechen Republic. Various concentrations of infusions showed a pronounced bactericidal effect. Wormwood suspension reduced the level of oxidative stress caused by hydrogen peroxide. Concentrations of chamomile (0.0625, 0.125 and 0.25 g/10 ml) together with hydrogen peroxide on the pKatG-lux strain increased oxidative stress within acceptable limits. However, all concentrations of chamomile had an antioxidant effect.

1 Introduction

Oxygen is one of the most common chemical elements. It is present both in the surrounding atmosphere and is part of the macromolecules of living organisms. Oxygen is vital for energy reactions in the cell, however, its active forms and free radicals can cause significant harm by modifying the structures of biological molecules. Changes in biomolecules can subsequently lead to various pathological processes in the body [1, 2]. In this regard, an important task is to find effective and cheap substances that inhibit oxidative stress (antioxidants). These substances include: vitamins A and E, ascorbic acid, carotenoids, lipoic acid, flavonoids, melatonin, etc. Some of them can be found in medicinal plants, food products, and also synthesized inside the body. But it should be taken into account that the synthesis of antioxidant substances by the body itself is usually limited, and they are unevenly distributed in different foods [3]. Based on this, this study examined the oxidative and antioxidative effects of the medicinal plants Chamomile (Matricāria chamomīlla) and Wormwood (Artemísia absínthium) of the Chechen Republic using luminescent strains of E. coli

Chamomile and Wormwood were collected in their places of growth, the mountains of Nozhai-Yurtovsky (1090 m above sea level) and Shatoysky (1200 m above sea level) regions of the Chechen Republic.

Wormwood contains the following main biologically active substances (BAS): lactones, terpenoids (for example, trans-thujone, myrcene γ-terpinene, bornyl acetate, 1,4-terpeniol, etc.), essential oils, organic acids, resins, phenols, tannins substances, flavonoids, flavonoid

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glycosides, as well as phenolic acids (coumaric, chlorogenic, salicylic, syringic and vanillic acids), which contribute to the mechanism of scavenging free radicals [4, 5].

Chamomile contains the following main biologically active substances: sesquiterpenes, coumarins (eg, umbelliferone), flavonoids, essential oils and polyacetylenes [6]. More than 120 chemical components have been identified in chamomile flower as secondary metabolites [7, 8].

2 Research Methodology

When making infusions, the dried aerial part of the plant (stem, leaves, flowers) was used, which was ground to a powdery state in a laboratory vertical mill VLM-6 (Vilitek). It was prepared according to the method most often used in everyday life: ground medicinal raw materials were poured with hot distilled water (1000 C), tightly closed and infused (15-20 minutes), pressing with a sterilized spoon, finally squeezed out and filtered through sterile medical gauze. The volume of the resulting infusion was adjusted with boiled sterile distillate to the original volume [9].

As a living biological test system, genetically modified strains of Escherichia coli MG1655 were taken, into the cells of which the constructed plasmids pBR322 with the inducible luxCDABE promoter of the bacterium Photorhabdus luminescens were introduced. The activity of the promoter depends on the influence of a number of chemical compounds [10, 11]. In our work, we used E. coli strains with plasmids pKatG-lux and pSoxS-lux. Strains were kindly provided by Prof. Abilev S.K. (IOGen named after N.I. Vavilov, Moscow).

To grow a culture of luxury strains of E. coli, Luria-Bertani (LB) medium was used, supplemented with 100 μg/ml of the antibiotic ampicillin. Cultivation was carried out in a thermostat at 37°C for 10-17 hours. By adding a nutrient medium, the density was adjusted to 0.1 units. McFarland (densitometer DEN-1 (“BioSan” Latvia).

The resulting diluted medium was further cultivated for 2 hours at 37°C, actively aerating it on a shaker at 120 rpm until the early exponential phase.

160 μl of the resulting culture was placed into the wells of a microplate and, depending on the option, the following was added:

- 40 μl of distilled water with negative control (k-);
- 20 μl of distillate and 20 μl of oxidant (hydrogen peroxide, 10-3 M), with positive control (k+);
- to evaluate individual concentrations of infusions, 20 μl of the test substance and 20 μl of distillate were added;
- to assess the combined effect of the oxidant and infusions, 20 μl of hydrogen peroxide and 20 μl of the test substance were added.

Aliquots in the microplate were cultured at 37°C and data were collected after 45 min. for pKatG-lux strains and 60 min for pSoxS-lux strains.

For the study, a microplate luminometer Luminometer photometer LM 01A (IMMUNOTECH s.r.o, Czech Republic) was used and expressed in relative light units (RLU) [12].

The induction factor R is calculated for the minimum and maximum concentrations of infusions using the formula $R = Iind / I0$, where I0 is the level of spontaneous luminescence of the culture, Iind is the level of induced luminescence of the culture.

Significance was determined by Student's t-test ($p<0.05$).

3 Results and Discussions

The data obtained during the study on luminescent bacteria are presented in Tables 1 and 2, and are also displayed in Figures 1, 2, 3, 4.

Table 1. Effect of Chamomile infusions on E. coli strains.

Strain	Luminescence induction, rel. units	
	pSoxS	pKatG
Option	Hydrogen peroxide (10^{-3} M)	Hydrogen peroxide (10^{-3} M)
I_{ind} (k+)	100213,5±5239,052	47775,96±2102,902
I_0 (k-)	10569±414,3639	6795,833±102,9342
I_{ind}/I_0 (R)	9,4818	7,03
Individual concentrations of chamomile		
0,0625 g	9435,25±440,9294	5248,042±163,7229
0,125 g	8480,875±350,5653	4659,236±297,6198
0,25 g	8400,708±373,3398	4559,542±95,6457
0,5 g	7455,375±323,0422	4194,542±123,7829
1 g	6246,208±223,0952	4017,292±95,9001
Concentrations of chamomile together with oxidant (k+) (hydrogen peroxide, 10-3 M)		
0,0625 g and (k+)	66195,96±2621,353	47864,25±1142,368
0,125 g and (k+)	71000,75±2581,929	50047,42±1457,325
0,25 g and (k+)	75759,96±2729,822	50817,54±1553,653
0,5 g and (k+)	61854,29±1966,568	41631,08±1253,674
1 g and (k+)	49209,83±1414,629	37655,79±1139,441

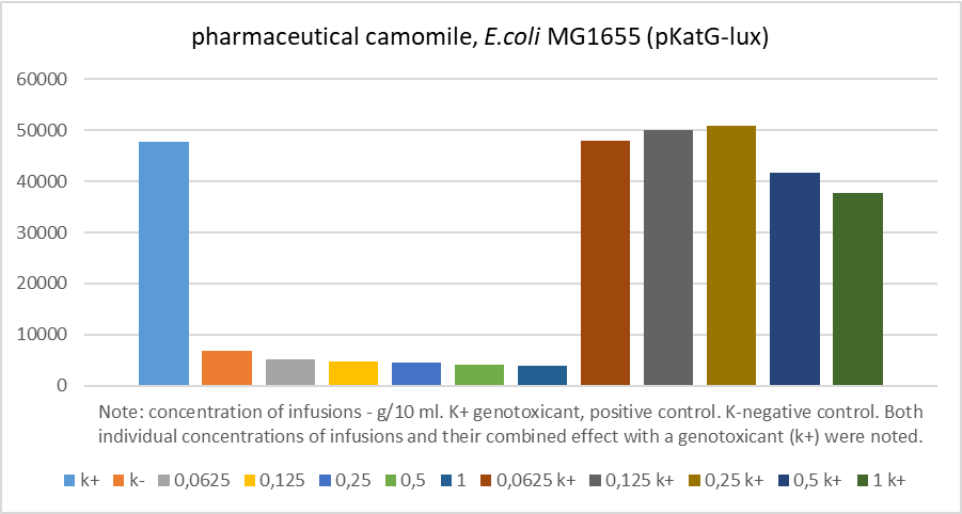

Fig. 1. Bioluminescent response of E. coli strain MG1655 (pKatG-lux)

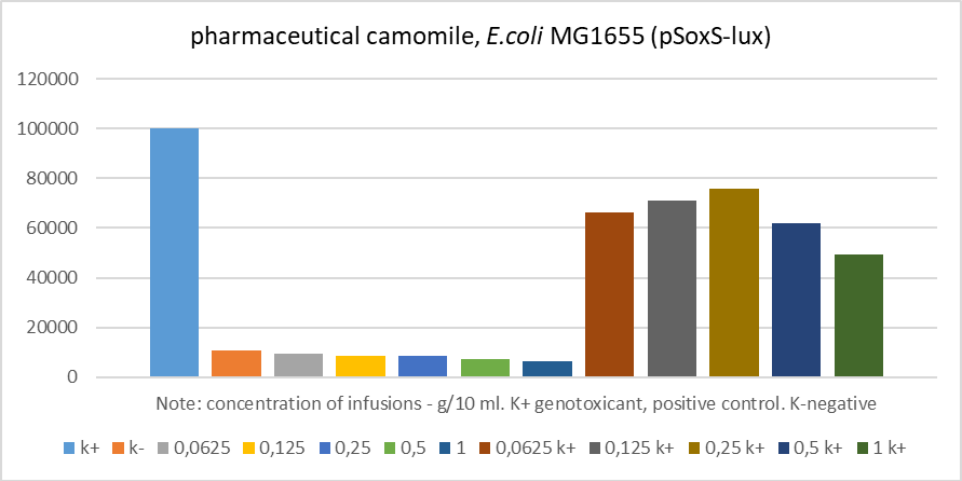

Fig. 2. Bioluminescent response of E. coli strain MG1655 (pSoxS-lux)

Based on the data obtained in a series of experiments with chamomile on the pKatG and pSoxS strains (Table 1, Fig. 1 and 2), we can say the following: all individual concentrations of infusions exhibit a bactericidal effect. With increasing concentration, the bactericidal effect also increases, respectively, the maximum occurred per 1 g of infusion. For pKatG and pSoxS for this infusion (1g/10ml), the induction factor (calculated using the formula in the table note) was 0.591; artificially induced oxidative stress caused by hydrogen peroxide (k+) on these strains was inhibited by the majority of the presented concentrations of chamomile infusions. However, on the pKatG strain with concentrations of 0.125 and 0.25 g, a slight increase in oxidative stress was recorded, within the acceptable values of 1.047 and 1.064 compared to the positive control (k+). For the same strain, as for pSoxS, the maximum inhibition of oxidative stress was recorded at a concentration of 1 g, the value was 0.788 (for pKatG) and 0.491 (pSoxS).

Experiments with wormwood infusions on strains pKatG and pSoxS (Table 2, Figs. 3 and 4) gave the following results: all individual concentrations had a bactericidal effect, the peak of bactericidal activity occurred at the maximum concentration (1g/10ml). For pKatG, the induction factor at this concentration was 0.589, for pSoxS – 0.677; all concentrations of wormwood together with hydrogen peroxide, which caused oxidative stress, proved to be antioxidants. Just like the bactericidal effect, the peak of antioxidant activity occurred at the maximum concentration of infusions of 1 g. For pKatG, the value for this concentration was 0.377, for pSoxS – 0.336, in comparison with the positive control.

Table 2. Effect of wormwood infusions on bioluminescent E. coli strains.

Strain	Luminescence induction, rel. units	
	pSoxS	pKatG
Experiment Option	Hydrogen peroxide (10^{-3} M)	Hydrogen peroxide (10^{-3} M)
I_{ind} (k+)	158888,8±4720,166	122123,5±5347,189
I_0 (k-)	13574,13±288,9711	8896,375±207,7673
I_{ind}/I_0 (R)	11,7053	13,7273
Individual concentrations of wormwood		
0,0625 g	10789,42±309,8519	7068,375±272,949
0,125 g	9298,75±198,1526	6281,333±203,0625
0,25 g	8712,5±143,4192	5868,708±197,5628
0,5 g	8477,083±184,8211	5327,625±167,0802

1 g	9185,083±145,2268	5239,792±169,4081
Concentrations of wormwood together with oxidant (k+) (hydrogen peroxide, 10-3 M)		
0,0625 g and (k+)	115960,3±4903,199	93667,38±2948,925
0,125 g and (k+)	106747,9±3694,599	85452,75±2442,496
0,25 g and (k+)	105057,7±3998,162	82650,29±2215,522
0,5 g and (k+)	86913,04±3283,594	67562,42±2095,295
1 г и (k+)	53463,92±1327,803	46066,96±741,6572

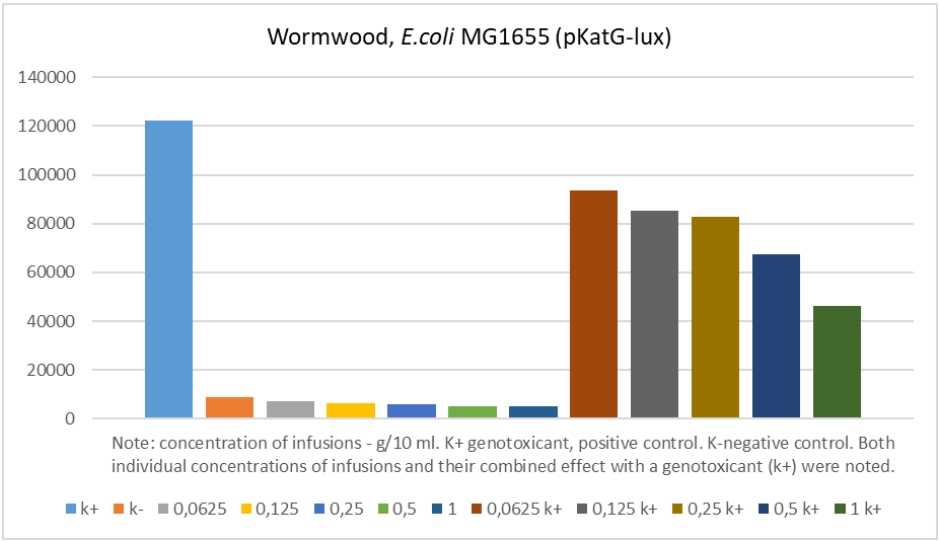

Fig. 3. Bioluminescent response of E. coli strain MG1655 (pKatG-lux).

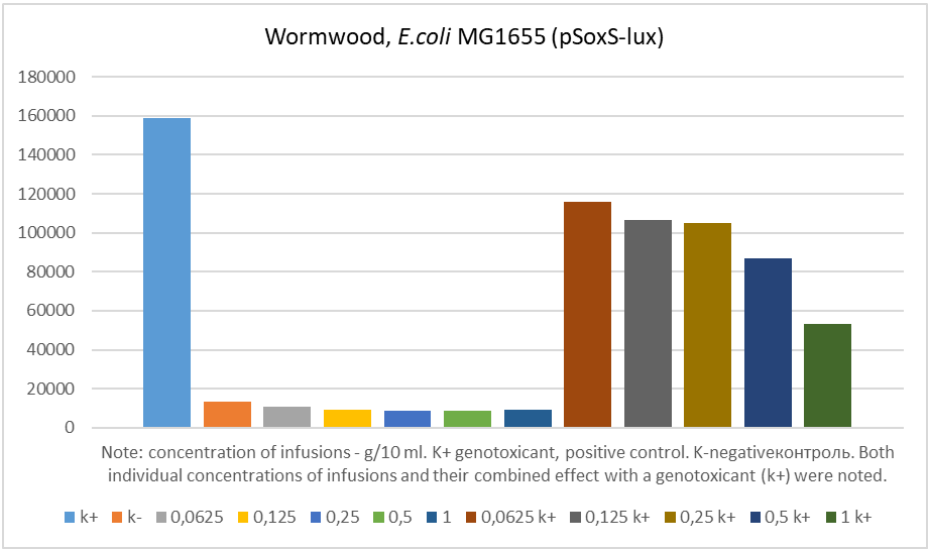

Fig. 4. Bioluminescent response of E. coli strain MG1655 (pSoxS-lux)

4 Conclusions

Thus, analysis of the joint carriage of alleles/genotypes of the studied polymorphic regions in patients and healthy individuals revealed combinations of haplotypes of the XRCC1

Arg194Trp/Gln399Arg and XPD Asp312Asn/Lys751Gln gene haplotypes that are positively associated with breast cancer. Combinations of two haplotypes XRCC1 Arg399Gln-Arg194Trp (T/C+G/G) and XPD Lys751Gln and Asp312Asn (G/G +TC)– turned out to be a breast cancer risk marker.

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