Investigating the effect of subjecting the Zinacef (750mg) antibiotic to water, tea, milk, grapefruit juice, coffee, and energy drink on its effectiveness in inhibiting growth of *Lactobacillus Rhamnosus*.

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Abstract: Antibiotics are the medications used for treatment of bacterial infections or inhibiting their spread, most frequently by interfering with bacteria metabolism. For instance, the Zinacef antibiotic used in the investigation is a cefuroxime, which is a substance that belongs to cephalosporins antibiotics. Peptidoglycan is responsible for providing structural stability to the cell wall in bacteria. In gram-positive bacteria, such as Lactobacillus Rhamnosus, peptidoglycan is a vital component with a thickness of more than 10 layers, without which the cell cannot function properly. β-lactams antibiotics have been proven to inhibit the last step of peptidoglycan synthesis, thereby depriving the cell of the main protective layer.. However, as mentioned in Fundament of interest, there is evidence that some substances can decrease the efficiency of antibiotics in fighting bacteria. Moreover, some of them have both antimicrobial properties and properties that inhibit the effectiveness of antibiotics at the same time. In this work, an attempt was made to check What is the effect of water, tea, milk, grapefruit juice, coffee, and energy drink on the effectiveness of Zinacef antibiotic in inhibiting growth of Lactobacillus Rhamnosus, investigated by measuring the zones of inhibition with use

of a vernier calliper? The results and conclusions of the experiment were as follows: water does not contain substances that could somehow affect the effectiveness of the Zinacef antibiotic, tea properties have the smallest effect on Zinacef antibiotic effectiveness in inhibiting Lactobacillus Rhamnosus growth, in the experiment milk indeed negatively influenced the activity of Zinacef antibiotic. On the other hand energy drinks have blocked the activity of the antibiotic to a greater extent than coffee.

Keywords: Zinacef; Lactobacillus Rhamnosus; effectiveness in inhibiting growth

------- Introduction ----------

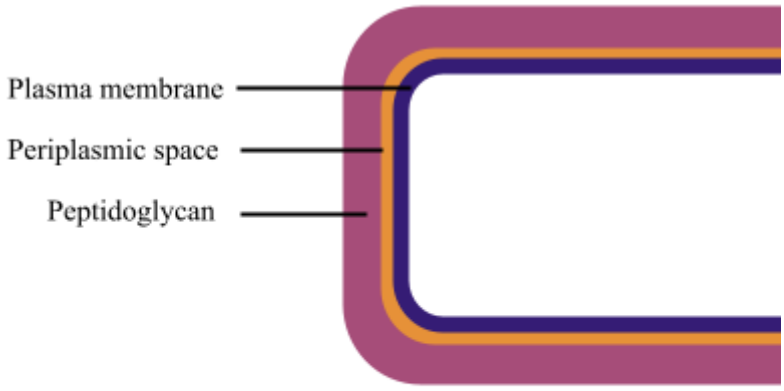
— Fundament of interest —

Along with the progression of time, comes a development of scientific research, the results of which expands our consciousness and awareness of things of everyday matter. As some of the observable outcomes of such investigations one may point at the studies concerning medicaments, healthy lifestyle, or drug abuse and its consequences on our reality. For instance, explorations concerning the topic of alcohol have shown its negative impacts on our health and behaviour (“Alcohol”), as well as on the other factors that play a major role in our lives, such as medical treatment (Mergenhagen et al.). Due to its harmful effects, contraindications have been introduced, and now it can be said that avoiding the combination of alcohol with meds, especially antibiotics, has become a widely respected standard in today’s society. However, most of the norms are being treated subconsciously and little do people think why and what is the reason behind it. Therefore, alcohol is automatically put aside when the medicine is introduced. Nevertheless, people tend to reach for other drinks, like: tea, coffee, or juices, without reflecting on their mechanisms. That makes me wonder to what extent is that a proper act, and whether the types of substances used, other than extensively inspected alcohol, can also affect the way the medicament, in the form of antibiotics, works in our organism.

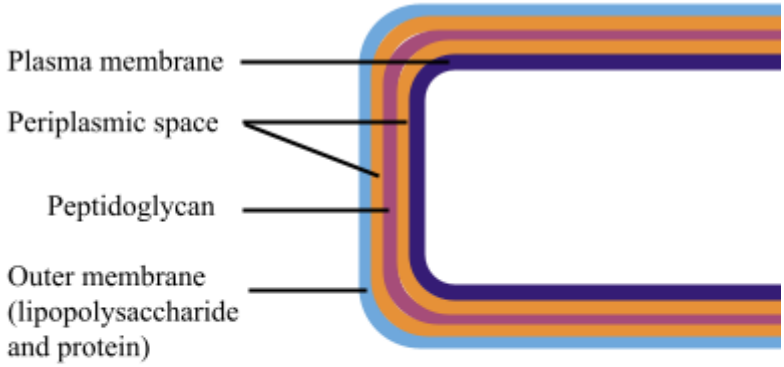
— Background knowledge —

Antibiotics are the medications used for treatment of bacterial infections or inhibiting their spread, most frequently by interfering with bacteria metabolism. They cannot fight

diseases of viral nature, since viruses do not have their own metabolism and are not considered living organisms (Felman and Begum). In accordance with the mechanism of functioning, antibiotics can be assigned into different categories. The classification consists of antibiotics that may interfere with cell wall synthesis, or protein synthesis, or nucleic acid synthesis (Kapoor et al.). Besides the mechanisms of fighting bacteria, the antibiotics can be also sorted into groups and subgroups (Kapoor et al.). For instance, the Zinacef antibiotic used in the investigation is a cefuroxime, which is a substance that belongs to cephalosporins antibiotics. Cephalosporins, in turn, is a group of β-lactams antibiotics, most of which act as a stopper of cell wall synthesis in bacteria (RxList). Depending on the type of bacteria, gram-positive or gram-negative, the cell wall has different structure (*Figure 1.*) (Silhavy et al.).



GRAM-POSITIVE



GRAM-NEGATIVE

Figure 1. Structure of gram-positive and gram-negative bacteria.

Peptidoglycan is responsible for providing structural stability to the cell wall in bacteria. In gram-positive bacteria, such as *Lactobacillus Rhamnosus*, peptidoglycan is a vital

component with a thickness of more than 10 layers, without which the cell cannot function properly. β-lactams antibiotics have been proven to inhibit the last step of peptidoglycan synthesis, thereby depriving the cell of the main protective layer. (Pandey and Cascella). However, as mentioned in *Fundament of interest*, there is evidence that some substances can decrease the efficiency of antibiotics in fighting bacteria. Moreover, some of them have both antimicrobial properties and properties that inhibit the effectiveness of antibiotics at the same time. Tea and coffee contain tannic acid, which has been proven to be able to react with different medications, making them less effective, or even dangerous (Manfredi). On the other hand, tannic acid exhibits strong antimicrobial and antiviral properties itself (Kaczmarek). Additionally, coffee and energy drinks contain caffeine as their major component, which can also affect antibiotics effectiveness in many ways. Caffeine has a huge impact on absorption of meds in our organism due to its overall ability to make complexes with drugs, which influences their absorption (Belayneh and Molla). Furthermore, bacteria metabolism strongly relies on enzymatic reactions (Todar), while caffeine has been shown to saturate some enzymes or induce them (Belayneh and Molla). Milk is a dairy product, which has a significant amount of calcium in its content. Calcium, in turn, is capable of interacting with some kinds of antibiotics creating insoluble and unable to absorb formings (Allison Gilchrist, Associate Editor). When it comes to grapefruit juice, in humans it is to blame for interacting with enzymes, mainly CYP3A4, and inhibiting them, increasing the antibiotics absorption for even 47%, which leads to extremely high and dangerous antibiotics concentrations in the bloodstream (“Grapefruit and Medication”). On the other hand, water is described not to interfere with drugs at all. To verify the above mentioned information, an experiment investigating the effect of those substances on antibiotics effectiveness has been conducted, and the results have been compared with no treatment plates - control group. To perform the examination, the spread plate technique was used. Spread plate technique is a simple procedure of imposition of a small amount of bacteria colonies in fluid form onto the agar plate and spreading it evenly on its surface (Dahal). The discs soaked in antibiotic solution and examined substances were put on the surface. After the incubation time, zones of inhibition have been measured by a vernier calliper. Zones of inhibition are circular areas, formed around the discs, in which the bacteria do not grow - are inhibited.

— Research question —

What is the effect of water, tea, milk, grapefruit juice, coffee, and energy drink on the effectiveness of Zinacef antibiotic in inhibiting growth of *Lactobacillus Rhamnosus*, investigated by measuring the zones of inhibition with use of a vernier calliper?

— Hypothesis —

Milk, coffee, and energy drinks will significantly decrease the effectiveness of the Zinacef antibiotics in inhibiting growth of *Lactobacillus Rhamnosus* when compared to no treatment conditions - control group. Grapefruit juice will enhance the activity of the antibiotic. At the same time there will be no significant difference in results between water treatment and control group. We predict that energy drinks will decrease the effectiveness of the Zinacef antibiotic the most, and that tea, excluding water, will have the smallest impact on the effectiveness of the Zinacef antibiotic.

Caffeine has a negative effect on the absorption of antibiotics making bacteria less vulnerable to the treatment. Since the energy drinks contain the most caffeine of all substances in their content, they will decrease the effectiveness of the Zinacef antibiotic the most, coffee a little bit less. Grapefruit juice was shown to enhance antibiotic absorption for even 47%, so it will positively affect the Zinacef antibiotic activity. Water is free of components affecting the antibiotics effectiveness, hence, it will not decrease the zones of inhibition. Tea contains only the tannic acid, which only slightly blocks antibiotics and itself has antimicrobial properties, hence, its antibiotics inhibiting properties will be much smaller than the properties of the other substances. Calcium present in the milk will bind to antibiotics creating non absorbable forms.

------- Methodology used in the experiment ----------

— Independent and dependent variables —

Independent variable: Substances (water, tea, milk, grapefruit juice, coffee, energy drinks) mixed with the antibiotic solutions.

Dependent variable: Radius of the zones of inhibition formed around the discs put on the agar plate, measured with a vernier calliper.

— Controlled variables —

Table 1.1. Controlled variables, method of controlling variables and their importance for the investigation.

Controlled variables	Importance of the variables	Method of controlling
Source of the *Lactobacillus Rhamnosus*.	Different sources of *Lactobacillus Rhamnosus* may provide me with bacteria of different resistance and properties that could affect both its inhibition by antibiotics and overall growth.	Buying *Lactobacillus Rhamnosus* from the same source (Dicoflor 6 probiotic from pharmacy doz.pl) and preparing the solution for investigation from only one tablet, ensuring that the same colony was placed on every MRS agar plate.
Amount of *Lactobacillus Rhamnosus* solution put on each MRS agar plate.	Different amounts of *Lactobacillus Rhamnosus* solution put on MRS agar plates would cause the differences between plates in the extent to which the bacteria grew. Hence, making the zones of inhibition to be overgrown in random places due to the excess of bacteria.	Putting exactly the same amount of *Lactobacillus Rhamnosus* solution ($0.2cm^3$) on each MRS agar plate.

Sources of substances affecting the effectiveness of Zinacef antibiotic	Different brands of separately water, tea, milk, grapefruit juice, coffee, and energy drinks may provide products with unlike compositions. Non-identical ingredients, for example: in two different brands of coffee, may affect the effectiveness of the Zinacef antibiotic in various ways, making the results unreliable	Preparing the solutions for the experiment from only one product for each substance category: water brand - Cisowianka, tea brand - Tetley; type - green tea, milk brand - Mlekovita; type - 3.2% fat milk without lactose, grapefruit juice brand - Tymbark; type - grapefruit nectar, coffee brand - Jacobs; type - Cronat Gold Instant, energy drink brand - Monster; type - ultra violet. All products were bought in supermarket Auchan.
Time of soaking discs in the examined substances.	Different amounts of time of soaking discs in prepared solutions would result in different amounts of solution remaining on the discs, hence, different inhibiting properties of the discs.	Soaking each disc for the exact same time (5s) in the examined substances solutions.
Source of the Zinacef antibiotic.	Different sources of antibiotics may provide me with antibiotics with different properties. Moreover, inhibiting properties may slightly vary among tablets, which can falsify the results.	Buying Zinacef antibiotics from the same source (pharmacy - doz.pl) and preparing the solution from only one tablet.

Table 1.2. Controlled variables, method of controlling variables and their importance for the investigation (Continued).

Controlled variables	Importance of the variables	Method of controlling
Concentration of the Zinacef antibiotic solution.	Different concentrations of the Zinacef antibiotic would result in different effectiveness of the solution in fighting bacteria.	Concentration was not calculated precisely, however, only one solution was prepared and used for all trials.
Time of soaking discs in the Zinacef antibiotic solution.	Different amounts of time of soaking discs in Zinacef antibiotic solution would result in different amounts of solution remaining on the disc, hence, the effectiveness of the antibiotic in the trial.	Soaking each disc for the exact same time (5s) in Zinacef antibiotic solution.
Size and shape of the discs.	Both size and shape of the disc affect the size and shape of the zones of inhibition. Inconsistencies make the results incomparable to each other.	Preparing disc of exact size (0.5cm) and shape (round). Prepared with a hole puncher.
The distance between discs and the edges of the MRS agar plate.	Too small distance between discs and edges of the MRS agar plate would result in overlapping of the zones of inhibition, hence, making the result immeasurable.	Verifying potentially the biggest obtainable zones of inhibition of Zinacef antibiotic in online sources. Keeping the same distance between edges (15mm) and discs (30mm) on a plate.
Method and time of measuring results.	Changing methods and time of measuring the zones of inhibition between trials, would result in different, incomparable results.	Each zone of inhibition was measured by the same method, with the same vernier calliper and after the same amount of time (72h).
Environment of the experiment.	Factors like temperature and access to light may affect the growth rate of the bacteria, hence, exposing each trial to different conditions would cause undesired inaccuracies in the results.	The entire experiment, for all trials, was conducted under the same conditions (limited access to light and room temperature).
Sterility of the equipment used in the experiment.	Using unsterile equipment would result in instant contamination of the MRS agar plates, and disturbance in the measurements due to the existence of different colonies at the same plate.	Using the sterile syringes and tweezers for each plate separately. Sterilising the cell spreader after each plate with use of ethanol and fire.

— Apparatus used in the investigation —-

Table 2. Apparatus and its uncertainty used during the investigation. Number of apparatus needed given in brackets.

Apparatus	Uncertainty	Apparatus	Uncertainty
(19) 2cm^3 sterile syringes	± 0.05cm^3	(1) Protective gloves, glasses and coat	-
(14) Sterile tweezers	-	(1) Vernier calliper	± 0.05mm
(11) 100cm^3 sterile containers	-	(1) Cell spreader	-
(8) 10cm^3 sterile syringes	± 0.10cm^3	(1) Electronic scale	± 0.01g
(1) Knife	-	(1) Marker	-
(1) Stopwatch	± 0.01s	(1) Kettle	-
(1) Hole punch	-	(1) Burner	-

— Materials used in the investigation —-

Table 3. Materials used in the investigation.

Materials	Amounts	Materials	Amounts
Zinacef antibiotic (750mg)	1 tablet	Tymbark grapefruit juice	2.00cm^3
MRS agar plates	14 plates	Monster ultra violet energy drink	2.00cm^3
(Dicoflor 6) *Lactobacillus Rhamnosus*	1 tablet	Cisowianka water	10.00cm^3
Tetley green tea	1 bag of tea	Distilled water	270.00cm^3
Jacobs Cronat Gold Instant coffee	1.00g	Sheets of filter paper	2 pieces
Mlekovita 3.2% fat without lactose milk	2.00cm^3	70% alcohol	~1 glass

— Safety, environmental and ethical issues —-

- **Safety issues:** While performing the experiment, the protective gloves, glasses and coat were worn in order to maintain protection while operating with the bacteria. All the procedures were conducted carefully and precisely. During the preparation of the tea and coffee solutions, extra caution was preserved due to dealing with the water in high temperature (>100^{o}C). MRS agar plates only for lactic bacteria were used in the experiment, preventing growth of other undesired, and potentially pathogenic bacteria. Only lactic and safe bacteria were sown during the experiment.
- **Environmental issues:** To avoid potential biohazard, after the conduction of the experiment, all the remaining MRS agar plates were sunk in 70% alcohol solution. After killing the bacteria, the plates were disposed of into a proper container for biological waste.
- **Ethical issues:** There were not any ethical issues during the experiment, since there were no animal species involved in the investigation.

------- Method used in the experiment ----------

— Preparation of concentrations used in the investigation —

- **Tea:** To prepare a tea solution, distilled water was boiled. Tea bag was placed in the sterile container and then poured with 100.00cm^3 of boiling water. After around 2 minutes the tea bag was removed from the sterile container. The tea was put aside until it reached room temperature. With the use of the sterile syringe 2.00cm^3 of tea were moved to a separate container and poured with 10.00cm^3 of distilled water, measured with 10.00cm^3 sterile syringe. The container was labelled.
- **Coffee:** To prepare a coffee solution, distilled water was boiled. 1.00g of Instant coffee was put in the sterile container and then poured with 100.00cm^3 of boiling water. The solution was put aside until reaching the room temperature. After reaching room temperature, the solution of coffee was filtered to the next sterile container with the use of 1 filter paper. This step allowed me to avoid obtaining the coffee grounds which would clog the syringe. 2.00cm^3 of filtered and cool coffee were moved into a separate container and poured with 10.00cm^3 of distilled water. The container was labelled.
- **Milk:** 2.00cm^3 of milk was poured into the sterile container. After this, 10.00cm^3 of distilled water was added to the milk in the container. The container was labelled.

- **Grapefruit juice:** 2.00cm^3 of grapefruit juice was poured into the sterile container. After this, 10.00cm^3 of distilled water was added to the grapefruit juice in the container. The container was labelled.
- **Energy drink:** 2.00cm^3 of energy drink was poured into the sterile container. After this, 10.00cm^3 of distilled water was added to the energy drink in the container. The container was labelled.
- **Water:** 10.00cm^3 of Cisowianka water was poured into the sterile container. The container was labelled.
- **Zinacef antibiotic:** The tablet of Zinacef antibiotic was cut in half. One half of the tablet was chopped into a powder and added to the sterile container. The powder was poured with 10.00cm^3 of distilled water and mixed until dissolved. The container was labelled.
- ***Lactobacillus Rhamnosus*:** Powder from one half of Dicoflor 6 probiotic capsule was added to the sterile container, poured with 10cm^3 of distilled water and mixed until dissolved. The container was labelled.

— Conduction of the experiment —

Following steps had been applied only once before the process of spreading the bacteria.

- With the use of the hole punch, 70 rounded discs with a diameter of 5.00mm were pinned from the second filter paper piece.
- Pinned discs were put into the oven and set for 200°C for 10 minutes in order to make them sterile.

Following method was applied for the plate 1 of the tea sample and was repeated with the remaining plates and substances. Control group omits the steps with soaking discs in the substance's solution.

- MRS agar plate was taken and labelled: “tea solution”.
- With the use of the calliper, exact distances were measured (*Figure 2*) and labelled with use of the marker at the reverse sides of the plates.

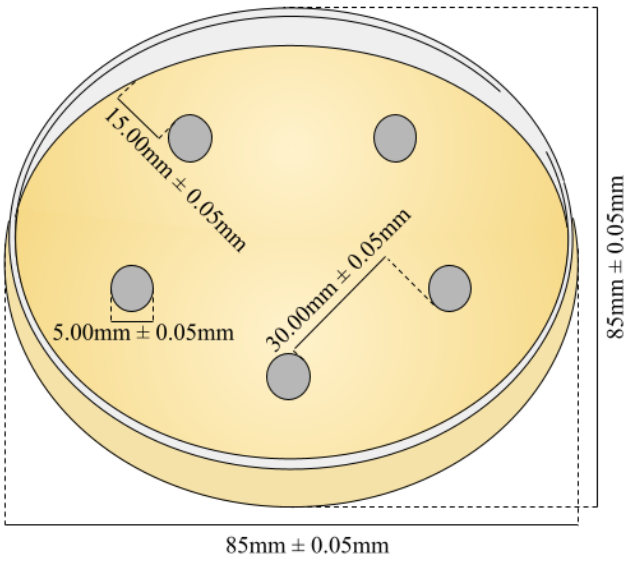


Figure 2. Distribution of discs on the plate.

Figure 3 shows control group.

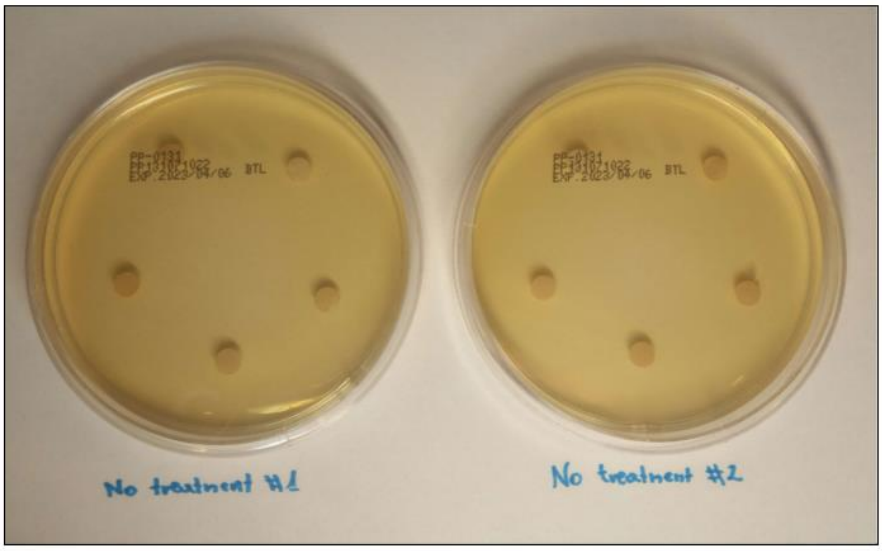


Figure 3. Control group prepared for incubation.

The spreading part of the cell spreader was soaked in 70% alcohol and fired in the burner in order to make it sterile.

- 0.2cm^3 of bacteria solution was measured with the 2.00cm^3 sterile syringe and poured into the middle of the MRS plate.
- With use of the spreader, the bacteria was spread with circular movements evenly over the surface of the agar plate.
- With the use of sterile tweezers, 5 discs, one by one, were taken and soaked in the antibiotic solution for 5.00 seconds and then soaked in the tea solution for 5.00 seconds.
- Each soaked disc was put in the labelled place on the plate.
- The spreader was soaked in 70% alcohol and fired in the burner in order to make it sterile.

- The plate was closed and put aside at the final spot.
- All the steps were repeated with the next MRS plate.
- After 72 hours, the zones of inhibition from the plate were measured with a calliper and noted.

------- Results ----------

— Observations —

- In most cases there were noticeable differences in zones of inhibition between examined substances.
- None of the zones of inhibition overlapped at the other zone of inhibition or edge of the plate.
- All discs were at the same spots where they have been labelled and put.
- Control group had very similar zones of inhibition to the water samples.
- Grapefruit juice samples had very similar zones of inhibition to the coffee samples.
- There were easily observable irregularities in zones of inhibition in milk samples.

— Raw data —

Table 4. Zones of inhibition, mm, (± 0.05mm) of *Lactobacillus Rhamnosus* for the Zinacef antibiotic.

Condition	Zones of inhibition, (mm), (± 0.05mm)									
	Number of disc on MRS agar plate 1					Number of disc on MRS agar plate 2				
	Disc 1	Disc 2	Disc 3	Disc 4	Disc 5	Disc 1	Disc 2	Disc 3	Disc 4	Disc 5
No treatment	27.74	25.74	28.38	28.92	29.38	27.06	28.40	28.86	25.84	27.88
Water	27.78	24.54	28.14	27.64	27.12	28.38	27.96	27.92	27.62	26.26
Tea	24.26	23.28	23.04	22.78	22.62	22.74	24.60	22.94	23.16	23.84
Milk	13.52	18.58	12.96	20.70	24.02	18.12	17.36	18.48	22.46	12.48
Grapefruit juice	15.02	15.94	14.58	14.86	16.08	14.58	14.94	16.68	15.38	14.50
Coffee	14.38	12.72	13.84	14.12	13.74	15.78	14.34	12.96	14.56	16.32
Energy drink	10.22	9.78	8.04	9.72	10.92	10.42	12.02	10.46	9.74	12.44

------- Processed data ----------

The data was taken from *Table 4*. Exemplar calculations are based on no treatment condition.

After conducting the calculations, all of the results were gathered and presented in *Table 5*.

Table 5. Mean zones of inhibition, mm, (± standard deviation) of *Lactobacillus Rhamnosus* for the Zinacef antibiotic.

	Mean zones of inhibition [mm]	± Standard deviation [mm]
No treatment	27.82	± 1.26
Water	27.34	± 1.15
Tea	23.33	± 0.68
Milk	17.87	± 3.95
Grapefruit juice	15.26	± 0.74
Coffee	14.28	± 1.12
Energy drink	10.38	± 1.24

— Visual representations of the obtained results —

To better understand the data, its visualisation was prepared in Microsoft Excel (*Figure 4*).

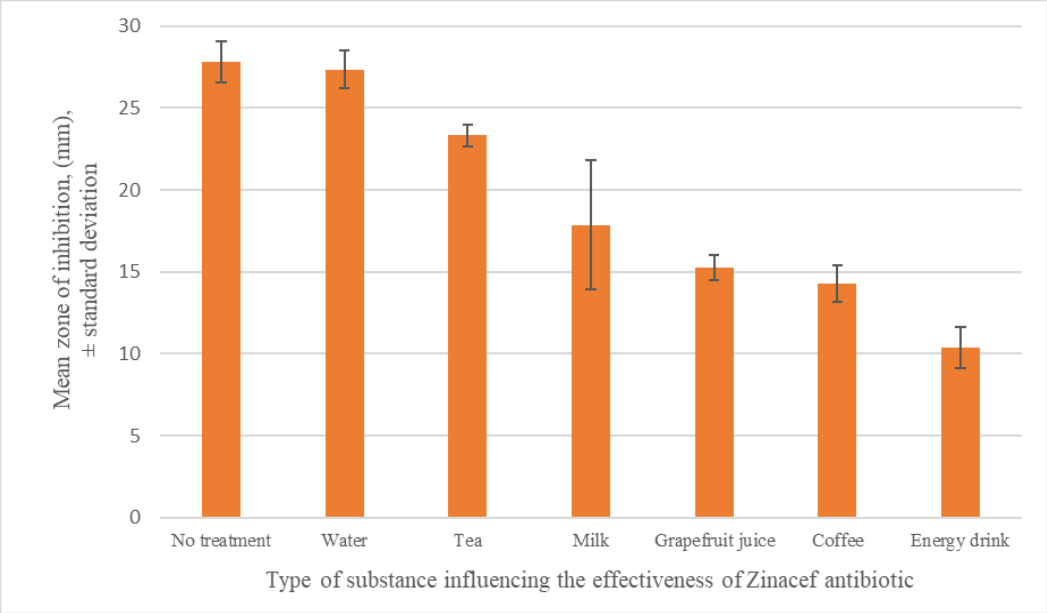


Figure 4. Mean values of zones of inhibition, (mm), (± standard deviation) of Zinacef antibiotics subjugated to different substances (water, tea, milk, grapefruit juice, coffee, energy drink). The graph presents results derived from all trials.

------- Statistical analysis of the results ----------

Since there are several samples in my results that have nearly identical means and where the error bars overlap (*Figure 4*), a statistical test was conducted to check the validity of the gathered values. There are two tests that could be carried out: T-test and ANOVA test. T-test is used to compare the results of two groups, while ANOVA is used for three and more groups. Seemingly, ANOVA test suits my needs more, as We have 7 groups to compare. However, ANOVA test shows significant difference between means of all groups when at least two of these groups exhibit the statistical difference, hence, it ignores the rest of the samples in the statistics, even if there are no differences in them (Mishra et al.). In my results there are clear differences between some groups, example: water and coffee, so ANOVA would show significant differences among results without considering less obvious cases, like: coffee and grapefruit juice, which make this test unreliable in my experiment. Therefore, for greater certainty, instead of ANOVA test, the T-test was conducted between groups

where results were not clear. These groups include: no treatment and water, tea and milk, milk and grapefruit juice, milk and coffee, grapefruit juice and coffee.

- In order to conduct T-test and analyse correlations between samples, two hypotheses were introduced and applied to each test:

Null hypothesis, H_0: There is no significant difference between mean values of samples.

Alternative hypothesis, H_1: There is a significant difference between mean values of samples.

- T-test concentrates on calculation of T-value, which later permits for P-value estimation. If the P-value is smaller than 0.05, the null hypothesis is rejected and the alternative hypothesis is proven.

- Exemplar calculations were conducted for the 'no treatment - water' pair. Data was taken from *Table 5*:

Degrees of freedom *(df)*	0.2	0.15	0.1	0.05	0.025	0.01	0.005	0.001	0.0005
1	3.078	4.165	6.314	12.706	25.452	63.657	127.321	636.619	1273.239
2	1.886	2.282	2.920	4.303	6.205	9.925	14.089	31.599	44.705
3	1.638	1.924	2.353	3.182	4.177	5.841	7.453	12.924	16.326
4	1.533	1.778	2.132	2.776	3.495	4.604	5.598	8.610	10.306
5	1.476	1.699	2.015	2.571	3.163	4.032	4.773	6.869	7.976
6	1.440	1.650	1.943	2.447	2.969	3.707	4.317	5.959	6.788
7	1.415	1.617	1.895	2.365	2.841	3.499	4.029	5.408	6.082
8	1.397	1.592	1.860	2.306	2.752	3.355	3.833	5.041	5.617
9	1.383	1.574	1.833	2.262	2.685	3.250	3.690	4.781	5.291
10	1.372	1.559	1.812	2.228	2.634	3.169	3.581	4.587	5.049
11	1.363	1.548	1.796	2.201	2.593	3.106	3.497	4.437	4.863
12	1.356	1.538	1.782	2.179	2.560	3.055	3.428	4.318	4.716
13	1.350	1.530	1.771	2.160	2.533	3.012	3.372	4.221	4.597
14	1.345	1.523	1.761	2.145	2.510	2.977	3.326	4.140	4.499
15	1.341	1.517	1.753	2.131	2.490	2.947	3.286	4.073	4.417
16	1.337	1.512	1.746	2.120	2.473	2.921	3.252	4.015	4.346
17	1.333	1.508	1.740	2.110	2.458	2.898	3.222	3.965	4.286
18	1.330	1.504	1.734	2.101	2.445	2.878	3.197	3.922	4.233
19	1.328	1.500	1.729	2.093	2.433	2.861	3.174	3.883	4.187
20	1.325	1.497	1.725	2.086	2.423	2.845	3.153	3.850	4.146

Figure 5. Critical values of T-value and their corresponding significance level (Turney)

In the case of 'no treatment - water' relationship, the significance level cannot be read directly from this particular table, since there are no critical T-values low enough to express the significance level of this example. However, the P-value, significance level, can be estimated to be around 0.38, which was checked in Microsoft Excel with use of data analysing add-in: Data Toolpak. P-value bigger than 0.05 (0.38 > 0.05). suggests that the result is not statistically significant, hence, the H_0 can be rejected and H_1 considered as proven. Ultimately, there is no significant difference between no

treatment and water samples.

- The remaining samples were taken into T-test verification, the results are shown in *Table 6.*

Table 6. Results of T-tests conducted in the experiment.

Group 1	Group 2	T-value	Estimated P-value
No treatment	Water	0.889	0.380
Tea	Milk	4.309	0.002
Milk	Grapefruit juice	2.057	0.067
Milk	Coffee	2.770	0.020
Grapefruit juice	Coffee	2.311	0.034

------- Conclusions ----------

— Interpretation of statistical significance analysis —

- Results obtained from T-test (*Table 6*) indicate P-values of 0.380, 0.002, 0.067, 0.020, and 0.034. According to the rule that P-value has to be smaller than 0.05 in order to reject null hypothesis and prove the alternative hypothesis, following assumptions were introduced:

- In case of ‘no treatment - water’, and ‘milk - grapefruit juice’ pairs, the null hypothesis cannot be rejected, hence, there is no statistically significant difference between means of paired samples.

- In case of ‘tea - milk’, ‘milk - coffee’, and ‘grapefruit juice - coffee’ pairs, the null hypothesis can be rejected and alternative hypothesis accepted, hence, there is a statistically significant difference between means of paired samples.

- Moreover, the results that have not been tested were assumed to be statistically significant in advance, since on *Figure 4* between those groups there were easily observable differences and no overlapping of error bars, so there was no need to additionally test their reliability with the T-test.
- All in all, the obtained results show that there exists a relationship between the substance that affects the Zinacef antibiotic and the Zinacef antibiotic effectiveness in inhibiting the growth of *Lactobacillus Rhamnosus*. Moreover, with very few exceptions, the results of the experiment are statistically significant.

— Experiment results —

Water: Evidence presented in *Observations*, *Table 5*, *Figure 4*, *Table 6* supports the statement that control group, no treatment plates, have nearly identical results as water samples, and that these results are statistically significant (P-value < 0.05). As it was written in *Hypothesis*, water does not contain substances that could somehow affect the effectiveness of the Zinacef antibiotic. In fact, there are calcium cations in the content of mineral water, however, in case of Cisowianka water it is only 130.3mg of Ca^{2+} ions per 1 litre of product ("Cisowianka Niegazowana"), whereas in case of Mlekovita milk it is 1050.0mg of Ca^{2+} ions per litre of product ("Mleko UHT Bez Laktozy 3,2%"). With 8 times less calcium cations than in milk, water is unable to inhibit the effectiveness of antibiotics to a significant extent.

Tea: Despite quite huge standard deviation in data concerning milk, T-test has shown the significant difference between tea and milk samples (P-value = 0.002), hence, just after the water, tea properties have the smallest effect on Zinacef antibiotic effectiveness in inhibiting *Lactobacillus Rhamnosus* growth. It was mentioned that despite negatively affecting the antibiotics activity, tannic acid has antimicrobial properties itself. Tannic acid has an ability to pass through the cell walls of both gram-positive and gram-negative bacteria and interfere with their metabolism, which results in death of the bacteria (Kaczmarek). Tea was not shown to demonstrate a great impact on the Zinacef antibiotic effectiveness, which suggests that antimicrobial properties of tannic acid are stronger than the ability to block antibiotics activity. Nevertheless, it is hard to explain if the tea blocked the antibiotic and inhibited the growth of bacteria itself, or whether it affected the Zinacef only to a small extent and also supported it in fighting the bacterial growth. Also, the calcium present in the tea might have interfered with the antibiotics or other substances.

Milk: This group was described as a substance that is able to block antibiotics effectiveness due to high calcium content in their composition. Calcium is a mineral that is able to form a bond with the antibiotics and change their way of binding, making them unable to fight the pathogens ("Why Some Antibiotics Should Be Avoided With Milk?"). In the experiment milk indeed negatively influenced the activity of Zinacef antibiotic - mean zone of inhibition of 17.87mm, however, it also generated the highest standard deviation - ± 3.95mm. Although there was no sample in which the antibiotic disc would be overgrown in bacteria, We still predict that the MRS plates could stimulate the growth of lactic bacteria originally present in the milk, at the same affecting the results obtained on certain discs.

Grapefruit juice: The group was predicted to enhance the activity of Zinacef antibiotic due to grapefruit's juice ability to dangerously increase the antibiotic absorption of drugs by inhibiting CYP3A4 enzymes. Nevertheless, the growth of bacteria was strongly inhibited - mean zone of inhibition is 15.26mm. The data about inhibiting CYP3A4 activity was obtained from human's metabolic pathways analysis, which may not

reflect the reaction of bacteria in laboratory environment. Yet, after deeper research, a different source stated that grapefruit juice may interact with some drugs by decreasing their effectiveness (Anderson), leading me to the conclusion that in case of Zinacef antibiotic and *Lactobacillus Rhamnosus* grapefruit juice does not interact with CYP3A4 enzymes, but it blocks antibiotic Zinacef activity and acts as inhibitor of bacterial growth itself. Furthermore, the average pH of grapefruit juice is 3.0, which is relatively strongly acidic, also supports the thesis of grapefruit juice inhibiting bacterial growth itself (Sinclair and Eny).

Coffee and energy drinks: Coffee and energy drinks were both tested in terms of caffeine impact on Zinacef activity in this investigation. In line with expectations, energy drinks have blocked the activity of the antibiotic to a greater extent than coffee. The difference between energy drink and coffee's abilities to block the Zinacef activity is much higher than assumed - 14.28mm to 10.38mm - nearly four millimetres. Zones of inhibition measured in energy drinks samples were on average 2.7 times smaller than in no treatment plates. One plausible reason for that is that the tannic acid contained in coffee may bind to caffeine and affect its binding making its absorption and inhibition of antibiotics less effective (C. Cyril). Moreover, such a big difference results from the fact that energy drinks have on average 4 times more caffeine than coffee itself ("Energy Drinks Worse for Your Heart Than Caffeine Alone"). Caffeine has an ability to interfere with antibiotics in many different ways, which makes her the greatest inhibitor or antibiotic activity. In the investigation, the caffeine could make a complex with antibiotics and reduce its bioavailability by up to 33% (Belayneh and Molla), as well as it could saturate CYP450 enzymes of bacteria (Belayneh and Molla). Also known as CYP1A2 enzymes play a crucial role in metabolising both caffeine and drugs, making caffeine able to compete with antibiotics for the enzymes (Belayneh and Molla), so act like a normal metabolic inhibitor.

— Final conclusions —

The aim of this experiment was to investigate the effect of water, tea, milk, grapefruit juice, coffee, and energy drink on the effectiveness of Zinacef antibiotic in inhibiting growth of *Lactobacillus Rhamnosus*. The hypothesis with different assumptions was put forward (*Table 7*).

Table 7. Assumptions made in the hypothesis, evidence for them, and overall impact on validity of the main hypothesis.

Assumption	Evidence	Hypothesis
Milk, coffee, and energy drinks significantly decrease the effectiveness of Zinacef antibiotic.	*Table 5*: 17.87mm, 14.28mm, 10.38mm zones of inhibitions of examined substances are significantly smaller than the control (27.82mm).	Confirmed
Grapefruit juice enhances the activity of the Zinacef antibiotic.	*Table 5*: 15.26mm zone of inhibition is much smaller than control (27.82mm).	Rejected
There is no significant difference between control group and water sample.	*Table 6*: P-value = 0.380, hence, there is no significant difference between those samples.	Confirmed
Energy drinks will decrease the activity of Zinacef antibiotic the most.	*Table 5:* Smallest mean for zone of inhibition (10.38mm). *Figure 4*: The shortest column.	Confirmed
Tea will be the weakest from the examined substances, after the water, in blocking the activity of Zinacef antibiotic.	*Table 5:* Mean for zone of inhibition smaller only than control and water sample (23.33mm). *Figure 4*: visual representation.	Confirmed

Since one assumption, the one concerning grapefruit juice, was rejected, the hypothesis cannot be fully confirmed. Ultimately, the main hypothesis was only partially confirmed.

------- Evaluation ----------

Excluding the milk sample, the standard deviations of the rest of the groups are relatively small (*Figure 4*), which suggests well designed procedure and enhances probability that similarities in some results (example: grapefruit juice - coffee) are not a work of chance. Furthermore, the results on *Figure 4* nearly completely overlap with the predictions introduced at the beginning of the work, which implies their small randomness as they are easy to foresee. Moreover, to obtain possibly the most valid data, very precise equipment has been used to gather the results. Precision that had been put into the experiment had a direct impact on the final outcomes, which are firmly supported by raw data, statistical tests (*Statistical analysis of the results*), and online sources (*Experiment results*). The reliable final results are also the effect of a large number of strictly controlled variables, which ensured an unchanged environment for the experiment and decreased the probability of the randomness of the results. However, the investigation also included some weaknesses, which as the result of my oversight, negatively influenced the outcomes of the experiment (*Table 8*).

Table 8. Evaluation of weaknesses, their importance to the investigation, and possible improvements.

Weakness	Importance	Improvement
Systematic error: Substances with antimicrobial properties used in the investigation.	Experiment aims to investigate antibiotics activity under different conditions. Using antimicrobial substances may affect not only the properties of the antibiotics but also the bacterial growth itself, making the results less reliable.	Using substances proven not to have any antimicrobial properties are advised to be used in the experiment, as they will not affect the bacterial growth to such extent.
Systematic error: Milk used on the MRS agar plate.	Milk is a substance full of lactic bacteria. MRS agar plates are designed for lactic bacteria growth, hence, there is a space for random bacterial growth among milk samples, which may falsify the results.	Using substances rich in calcium other than dairy products is advised. This will minimise chances of plate contamination with undesired bacteria.
Random error: Inaccurate reading of values and results.	Equipment used in the experiment was extremely precise and permitted for obtaining accurate results. However, improperly read values might have affected final results that had higher standard deviations.	Conducting more trials is advised in order to minimise the error caused by improperly read values.
Systematic error: Bacteria were obtained from probiotic.	The same bacteria solution was used in every trial and so the error should influence each result equally. However, probiotic bacteria normally act in our organisms as a shield against antibiotics, hence, are more resistant to external factors than normal bacteria. This might have influenced the mechanism of action of Zinacef on bacteria when mixed with the examined substances.	When we get an infection, it is rarely caused by scientifically strengthened bacteria. Therefore, using bacteria that are not obtained from any probiotic is advised in order to get results that are more suited to everyday problems.

The weaknesses presented in *Table 8*, combined with the overall results of the investigation create some interesting ways of extending the experiment. Firstly, in the experiment, only gram-positive bacteria were used. In *Background knowledge* it was described that gram-positive and gram-negative bacteria differ significantly, which may result in different results of the experiment if gram-negative bacteria was tested. In addition, the activity of only Zinacef antibiotics was tested. Different types of antibiotics represent different mechanisms of actions (*Background knowledge*), so testing different antibiotics would nicely complete the work done so far. Furthermore, action of different antibiotics may be tested on different probiotics bacteria to check which probiotic is the best in protection of our organism against negative antibiotics impacts.

------- Bibliography ----------

"Alcohol." *World Health Organization*, 9 May 2022, www.who.int/news-room/fact-sheets/detail/alcohol. Accessed 21 Dec. 2022.

Allison Gilchrist, Associate Editor. "5 Dangerous Food-Drug Interactions." *Pharmacy Times*, 5 Mar. 2021, www.pharmacytimes.com/view/5-dangerous-food-drug-interactions. Accessed 21 Dec. 2022.

Anderson, Leigh Ann. "Drug Interactions With Grapefruit Juice." *Drugs.com*, 18 May 2022, www.drugs.com/article/grapefruit-drug-interactions.html. Accessed 21 Feb. 2023.

Arsene, Mbarga Manga Joseph, et al. "Antibacterial Activity of Grapefruit Peel Extracts and Green-synthesized Silver Nanoparticles." *Veterinary World*, Veterinary World, May 2021, pp. 1330–41. https://doi.org/10.14202/vetworld.2021.1330-1341. Accessed 17 Feb. 2023.

Belayneh, Anteneh, and Fantahun Molla. "The Effect of Coffee on Pharmacokinetic Properties of Drugs : A Review." *BioMed Research International*, vol. 2020, Hindawi Limited, July 2020, pp. 1–11. https://doi.org/10.1155/2020/7909703. Accessed 21 Dec. 2022.

"Cisowianka Niegazowana." *Cisowianka*, 18 Jan. 2023, cisowianka.pl/cisowianka-ngaz-05-pet. Accessed 17 Feb. 2023.

C., Cyril. "Caffeine and Theine of Tea." *Terre Des Thés*, 16 Feb. 2009, www.terre-des-thes.fr/theine-caffeine/#:~:text=Tannins%20bind%20to%20caffeine%20and,theanine%20has%20a%20relaxing%20effect%20. Accessed 17 Feb. 2023.

Dahal, Prashant. "Spread Plate Method- Definition, Principle, Procedure, Uses." *Microbe Notes*, 19 Sept. 2022, microbenotes.com/spread-plate-technique. Accessed 17 Dec. 2022.

"Energy Drinks Worse for Your Heart Than Caffeine Alone." *NBC News*, 26 Apr. 2017, www.nbcnews.com/health/health-news/energy-drinks-worse-your-heart-caffeine-alone-study-n751686.

Accessed 17 Feb. 2023.

Felman, Adam, and Fazana Begum. "What to Know About Antibiotics." *MedicalNewsToday*, 28 Sept. 2022, www.medicalnewstoday.com/articles/10278. Accessed 21 Dec. 2022.

"Grapefruit and Medication." *Harvard Health*, 30 Mar. 2021, ww

Kaczmarek, Beata. "Tannic Acid With Antiviral and Antibacterial Activity as a Promising Component of Biomaterials—A Minireview." *Materials*, vol. 13, no. 14, MDPI AG, July 2020, p. 3224. https://doi.org/10.3390/ma13143224. Accessed 19 Feb. 2023.

Kapoor, Garima, et al. “Action and Resistance Mechanisms of Antibiotics: A Guide for Clinicians.” *Journal of Anaesthesiology Clinical Pharmacology*, vol. 33, no. 3, Medknow, 2017, p. 300. https://doi.org/10.4103/joacp.joacp_349_15. Accessed 21 Dec. 2022.

Manfredi, Theodoros. “Taking Medications With Coffee?” *HealthGuidance.org* |, 4 Feb. 2020, www.healthguidance.org/entry/15965/1/taking-medications-with-coffee.html. Accessed 22 Dec. 2022.

Mergenhagen, Kari A., et al. “Fact Versus Fiction: A Review of the Evidence Behind Alcohol and Antibiotic Interactions.” *Antimicrobial Agents and Chemotherapy*, vol. 64, no. 3, American Society for Microbiology, Feb. 2020, https://doi.org/10.1128/aac.02167-19. Accessed 19 Feb. 2023.

Mishra, Prabhaker, et al. “Application of Student’s T-test, Analysis of Variance, and Covariance.” *Annals of Cardiac Anaesthesia*, vol. 22, no. 4, Medknow, Oct. 2019, p. 407. https://doi.org/10.4103/aca.aca_94_19. Accessed 19 Feb. 2023.

“Mleko UHT Bez Laktozy 3,2%.” *Mlekovita*, mlekovita.com.pl/pl/produkt/szczegoly/produkty-bez-laktozy/ Accessed 19 Feb. 2023.

Pandey, Neelanjana, and Marco Cascella. *Beta Lactam Antibiotics*. 2022. Treasure Island (FL) StatPearls Publishing, 2022, www.ncbi.nlm.nih.gov/books/NBK545311. Accessed 21 Dec. 2022.

RxList. “Zinacef.” *RxList*, 27 May 2021, www.rxlist.com/zinacef-drug.htm. Accessed 21 Dec. 2022.

Silhavy, T. J., et al. “The Bacterial Cell Envelope.” *Cold Spring Harbor Perspectives in Biology*, vol. 2, no. 5, Cold Spring Harbor Laboratory, Apr. 2010, pp. a000414–a000414. https://doi.org/10.1101/cshperspect.a000414. Accessed 19 Feb. 2023.

Sinclair, Walton, and Eny. *The Organic Acids of Grapefruit Juice on JSTOR*. Apr. 1946, www.jstor.org/stable/4257934. Accessed 17 Feb. 2023.

Todar, Kenneth. “Regulation of Bacterial Metabolism.” *Online Textbook of Bacteriology*,

2020, textbookofbacteriology.net/regulation.html. Accessed 22 Dec. 2022.

Turney, Shaun. "Student's T-table." *Scribbr*, 17 Oct. 2022, www.scribbr.com/statistics/students-t-table. Accessed 19 Feb. 2023.

"Why Some Antibiotics Should Be Avoided With Milk?" *The Pharmaceutical Journal*, 18 Mar. 2021, pharmaceutical-journal.com/article/news/why-some-antibiotics-should-be-avoided-with-milk. Accessed 22 Dec. 2022.

"Why Some Antibiotics Should Be Avoided With Milk - the Pharmaceutical Journal." *The Pharmaceutical Journal*, 18 Mar. 2021, pharmaceutical-journal.com/article/news/why-some-antibiotics-should-be-avoided-with-milk. Accessed 22 Dec. 2022.