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Studies and conditions for the production of extractive solutions against pathogenic microorganisms with different plant species and extraction technologies

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Abstract. *Increasing antibiotic resistance threatens the effectiveness of antibiotics. They are becoming ineffective and infections are difficult or impossible to treat. As a result, one of the current trends that aims to address the emerging problem is the search for herbal medicines that have some antimicrobial activity. The object of the study were extracts and oils obtained from different fractions (roots, leaves, stems, whole plant) of six plant species (mint, lavender, thyme, oregano, basil, coriander) using different solvents (water, ethanol, vinegar, wine, olive oil) grown under greenhouse conditions and statistical processing of the obtained 360 databases on their antimicrobial activity against Escherichia coli. The results of the conducted complex studies are a good basis for the introduction of practically applicable technologies for the production of extractive solutions with high antimicrobial activity against Escherichia coli.*

Keywords: plant extracts, antimicrobial activity, hypothesis testing

Introduction

Need, purpose and objectives

Antibiotics are widely used in human and veterinary medicine. Since the beginning of the last century (1928), they have saved the lives of millions of people from infectious diseases (Sidorenko and Tishkov, 2004). In the post-2000 period, there has been a worldwide trend to seek opportunities to combat bacterial infections in humans and animals through safer and more environmentally friendly plant-derived medicines (Allen, 2014; Kharkevich, 2008). The method of treatment using synthetic antibiotics continues to be the mainstay, but over time microorganisms become resistant and antibiotics lose their effectiveness, causing therapeutic failures and complications (Howard et al., 2000; Jacoby and Archer 1991). Very often, artificial antibiotics cause additional side effects - the occurrence of allergic reactions, the presence of a serious toxic effect on the body and lowered immunity (Väänänen, et al. 2006). This gives rise to the need to search for alternative antibacterial agents combining anti-inflammatory, antioxidant and antibacterial activities with the aim of developing and sustaining the use of effective and safe drugs for the treatment and prevention of infectious and inflammatory diseases. The increased interest has led to the pharmaceutical market being flooded since the beginning of the 21st century with promotional materials and commercial offerings that, in the form of nutritional supplements, often go beyond the strict limits of medical and pharmacological standards. There is also a tendency for commercial interest to prevail over scientific and sound

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applied technology. In scientific circles, methods are being applied to the study of various plant extracts where inaccuracies and incompleteness are noted concerning taxonomic nomenclature, botanical authentication and the origin of botanical material. Of equal importance is the lack of standard methods for assessing the antibacterial activity of plant material and the technologies for extracting the extract solutions against specific pathogens. To solve these problems with a view to future production technologies, it is necessary to overcome the diversity of approaches and to introduce a common approach, order and way of communication.

From this point of view, in the present work we trace our attempt to introduce a systematic approach and order in the scientific and applied work on technology development and production of plant medicinal extracts as antimicrobial agents.

Method and methodology of the study

The method of investigation is limited to establishing the antibacterial properties of various plant species, plant fractions and technologies for the preparation of extractive solutions, creating conditions for the production of drugs designed to combat specific pathogens and includes:

- Conducting field experiments limited to the cultivation of the studied plant species in different conditions as a raw material base for future production;

- Conduct laboratory studies to determine soil and plant biogenicity and antimicrobial activity of the entire set of experimental data obtained under different conditions with respect to five species of pathogenic microorganisms;

- Statistical analysis and hypothesis testing of the influence of raw materials and technological factors on the antimicrobial activity of extraction solutions obtained under different conditions.

Material and methods

Cultivation of six plant species in different conditions as a raw material base

Phytochemical composition of the studied plant species

*Ginseng (*Mentha spicata*) contains a large number of compounds that contribute to the body's antioxidant defenses. Peppermint essential oil has been found to be effective against several types of harmful bacteria including E. coli and Listeria;

*Lavender (*Lavandula angustifolia*) - Lavender oil also has a strong antiseptic effect against methicillin-resistant strains of Staphylococcus aureus and vancomycin-resistant strains of Enterococcus sp. and Haemophilus influenzae, resistant to erythromycin, amoxicillin and cephalothin;

*Thymus. The essential oil of thyme contains the cresol derivatives thymol (1-2%) and carvacrol (30-40%), which act antiseptically and as disinfectant;

*Oregano *(Origanum vulgare)*. Oregano oil is effective against bacteria: Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Helicobacter pylori and Mycobacterium terrae. The oil alone or in combination can be useful in the prevention and treatment of severe bacterial infections, especially those that are difficult to treat or resistant to antibiotics;

*Basil *(Ocimum basilicum).* The main components in the essential oil are methylhavicol (up to 60%), L-linalool (up to 40%), eugenol (up to 4%), which is considered the main biologically active metabolite;

*Coriander *(Coriandrum sativum).* The oil obtained from the different parts of the plant differs in composition: in the leaves, stems and flowers, fatty aldehydes predominate; in the green fruit, aldehydes and linalool (up to 50%); in the ripe fruit: linalool (60-80%).

We aimed to evaluate the antibacterial activity of extractive solutions based on this raw material alone or in combination.

Growing environment as a factor of bioactive substances accumulation

The composition of the aromatic or volatile oils is influenced by the genotype (species, variety), the environment (geographical origin, climatic conditions, soil composition) and the cultivation, harvesting, storage and processing techniques of the raw material. Soil microorganisms are sensitive

to changes in their environment, and microbial biomass and enzyme activities are known indicators of soil stress or changes occurring as a result of different agricultural practices, soil contamination and other processes disturbing the ecological balance. From this point of view, an experiment was planned and conducted to analyze the antimicrobial activity against pathogenic microorganisms of different variants of plant extracts obtained from the adopted six plant species grown in different cultivation facilities.

Primary experiments aiming to supply the raw material and to prepare and specify future production technologies were conducted in two cultivation environments: in a greenhouse with uncontrolled conditions at the educational experimental field of the Technical University - Varna and in a greenhouse with controlled temperature (vegetation cell) at Dobrudja Agricultural Institute.

Developing methodology and practical methods for the preparation of non-extractive solutions in different variants of plant species, plant fractions and technological bases of extraction

The basis of the methodology for obtaining extraction solutions is solvent extraction, which is the most common technique for extracting bioactive compounds from herbs. Whereas the choice of solvent is dependent on the substances to be extracted, the use of technologies based on five different process media is envisaged:

Decoction is the liquid obtained by boiling the chopped plant product with the required solvent, usually water. This procedure is recommended for flowers, leaves, twigs or fruits, and especially when we intend that the valuable volatile oils remain in the aqueous extract.

Tincture. Therapeutic substances are extracted from the chopped herbs by soaking with ethyl alcohol at a concentration of 30%, usually for a time ranging between 8-10 days.

Medical wine. The extraction is carried out in a weak hydroalcoholic medium, at slightly acid pH. Pre-crushed herbs are soaked for 7-10 days in a good quality and well-stabilized wine, after which the preparation is filtered.

Medical vinegar. Extraction is carried out by extracting the useful substances from plant drugs with wine vinegar. Pre-crushed herbs are soaked for 7-10 days in good-quality vinegar, after which the preparation is filtered.

Medicinal oils are a form of soaking herbs in olive oil for 4-6 weeks. The resulting extractive solution is stored in well-closed glass bottles, in a dark and cool place.

The active part of the experiment at this stage is limited to the preparation of partial extracts by each of the mentioned technological media (extraction methodology) of the plant species grown under different conditions (greenhouse or vegetation cage) in the following variants - roots, stems leaves and whole plants. This has resulted in a solid raw data base of more than 5000 that will be subjected to further analysis aimed at obtaining effective therapeutic models.

Soil microbiological analyses, soil biogenicity and soil enzyme activity

As part of the intended analysis of environmental influences on the subsequent assessments of antimicrobial activities of plant species (fractions) and extraction methods, soil contents of ammonium and nitrate nitrogen, mobile phosphate, and assimilable potassium were investigated for the two types of cultivation conditions (greenhouse and growing cell). As a result of hypothesis testing (Konsulova -Bakalova et al., 2024), the differences of the useful signal (agrochemical indicator values in soils with vegetation of the studied species) from the so-called control indicator values in soils without vegetation were determined. This gives us a reason to exclude in our future research the disturbing control observations (soil without vegetation) from the groups of useful signals (soil with vegetation of the studied species). This guarantees the use in our future studies of large datasets with guaranteed quality (Naskova and Georgieva, 2024).

It was also found that the differences between the values of agrochemical indicators forming the useful signals for the two types of cultivation conditions were significant ($P= 1- \alpha=0.99$). This proved the possibility of dividing the large volume of experimental data into two groups according to the cultivation conditions of the plant species. As a result of the microbiological soil analyses, it was found that the total amount of microorganisms in

the cultivation of oregano coriander and basil was the highest, which directs our attention to these plant species in the following studies.

Ligation of different variants of extractive solutions of the studied plant species and extraction methods and evaluation of the biogenicity of plant extracts by major groups of pathogenic microorganisms

The biogenicity of the plant extracts was determined by examining four systematic groups of microorganisms(non-spore-forming and spore-forming bacteria (bacilli), actinomycetes, micromycetes (mould fungi)), on the 2nd, 10th and 20th day of preparation of the extracts when the plants were grown under greenhouse conditions and in a vegetation cell according to the scheme (Figure 1).

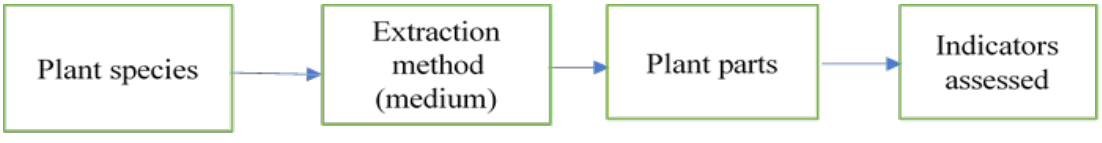


Figure 1. Scheme for the determination of the biogenicity of plant extracts

The limiting dilution method and threefold inoculations of solid nutrient media were used in the study of soil biogenicity and plant species (Nustorova and Malcheva, 2020). Surface seeding on specific nutrient media, cultivation and counting of CFU in 1 ml extract were performed. Total microflora was also determined using the formula: non-spore forming bacteria+bacilli+actinomycetes +mould fungi.

According to this scheme, the nutrient contents of the extracts of plant species grown under different cultivation conditions were determined as follows:

- plant species in the greenhouse: 360 individual and 288 combined analyses with a total of 3420 results (individual indicators and total microflora);

- plant species in a vegetation cell: 360 individual and 288 combined analyses, giving a total of 3420 results (individual indicators and total microflora).

As a result of the study of the biogenicity of plant extracts, substantial data sets have been obtained that are sufficiently representative for subsequent evaluations of the antimicrobial activity of extractant solutions based on different raw materials and process sources. Furthermore, analysis of these preliminary results shows the following trends of decreasing order of magnitude change in biogenicity:

- for plant species: coriander, basil, oregano, etc;

- for plant fractions: root, whole plant, stems, leaves;

- for the technological bases; vinegar, oil, decoction;

- for the time of application after preparation of extracts: 20th, 10th, 2nd day.

Determination of the antimicrobial activity of extraction solutions based on different raw material and technological resources against specific pathogenic microorganisms studied

At this stage, which is the last of the series of laboratory studies, the task was set to monitor and analyze the antimicrobial activity of all experimentally created extractive solutions (together and separately) against the following pathogenic microorganisms *Salmonella enterica, Listeria monocytogenes, Escherichia coli, Staphylococcus aureus* and *Clostridium perfringens*. The agar diffusion method (well method) was used by spilling 25 ml of liquefied and cooled solid culture medium specific for each microorganism species into 90 mm sterile petri dishes (Nustorova and Malcheva, 2020; Malcheva and Naskova, 2018): Deoxycholate citrate agar for *Salmonella enterica*, Listeria agar for *Listeria monocytogenes*, Endo agar for *Escherichia coli* Baird-Parker agar for *Staphylococcus aureus,* TSC agar for *Clostridium perfringens*. With a sterile instrument, 7 mm diameter wells are prepared, equidistant from each other and 2.5 cm from

the centre of the petri dish. Inoculate the wells with plant extracts, 60 ul. in volume, followed by incubation in a thermostat at 37°C for 24h. After culturing, a line was used to measure, in mm, the so-called sterile zone (retention, suppression), respectively the sensitivity of the pathogen species to a particular plant extract. The diameter of the zone is proportional to the susceptibility of the microorganism tested. Before testing the extracts and oils for antibacterial activity, a sanitary-microbiological control was conducted to verify the microbiological purity of the variants.

A total of 5600 laboratory analyses were conducted to determine the antibacterial activity of the investigated raw material and process resources (plant species, fractions and extraction technologies) for the two cultivation conditions. As a result of the preliminary data analysis, a significant antibacterial activity of the experimentally obtained extraction solutions against *Escherichia coli* was found, while no such influence was confirmed for the other pathogenic microorganisms. The overall analysis of the results confirms the trends of biogenicity changes indicated in the previous studies (section 2.4). At this stage, it was found that due to the impossibility for designing and conducting active factorial experiments (e.g. (Bojanov and Vuchkov, 1979; Nedev et al., 2012; Nedev, 2012) in primary field studies, the large data sets were in "raw" form and it was difficult to derive definite solutions and unambiguous relationships from them.

Further systematization and statistical processing of the data from the laboratory studies is necessary to solve the tasks of estimating the informative antimicrobial utility of all plant species, fractions, process media, preparation time and introduction of the solutions.

Results and discussion

Statistical processing of the results of experimental studies serves as the basis of a general technology for the production of extractive solutions for the control of *Escherichia coli.*

As a result of the conducted field and laboratory experiments, databases on the biogenicity and antimicrobial activity of the investigated extraction solutions with respect to *Escherichia coli* have been obtained in a volume of more than 10 000 laboratory analyses, which form a common raw material and technological basis for the following production technologies. It was found that by considering the cultivation conditions of the plant groups studied, it was possible to obtain local databases for each plant species of the type shown in Figure 2.

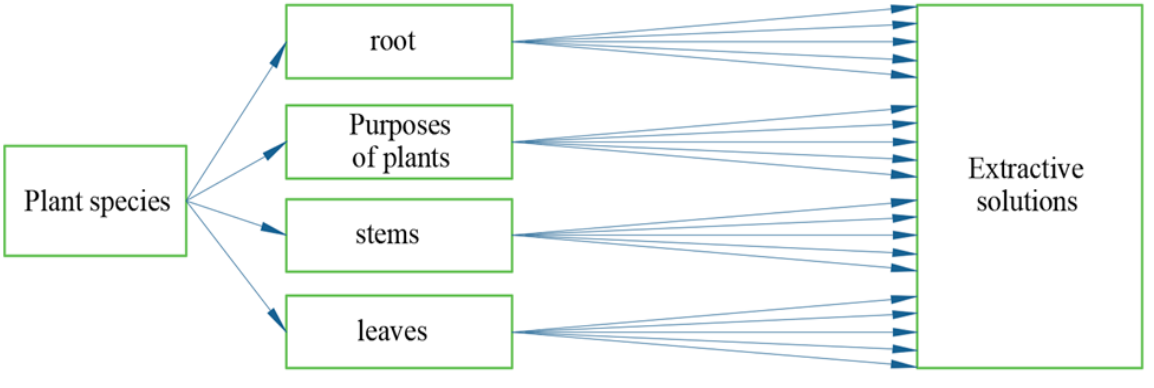


Figure 2. Local databases (models)

After conducting a systematic regrouping of the entire database (Bakalova, 2021; Konsolova et al., 2024; Nustorova and Malcheva, 2020; Naskova and Georgieva, 2024) according to the principle presented in Figure 2, 360 databases of antimicrobial activity were obtained, which can be adopted as the basis of future local models for the evaluation and production of treatment media. Such a large number of local models is unthinkable and practically unnecessary in terms of scientific and production conditions. Obviously, for the successful solution of the set task, it is necessary

to find a procedure for reducing the number and dimensionality of local models. For this purpose, we use the results of the systematic statistical analysis obtained in our cited works and presented in summary form in Figures 3, 4, 5.

Plant species Antibacterial activity X

Plant/Class	Standard x′	Good x″	Bad x‴
Coriander	X1′=0.955	X1′(′)=0.91	X1′(′)(′)(=0.)75
Basil	X2′=0,88	X2′(′)=0,85	X2′(′)(′)(=0,)75
Oregano	X3′=0,79	X3′(′)=0,73	X3′(′)(′)(=0,)68
Lavender	X4′=0,76	X4′(′)=0,74	X4′(′)(′)(=0,)67
Thyme	X5′=0,70	X5′(′)=0,71	X5′(′)(′)(=0,)61
Mint	X6′=0,69	X6′(′)=0,67	X6′(′)(′)(=0,)59
secondary	$\bar{x}'$=0,796	$\bar{x}''$=0,768	$\bar{x}'''$=0,675

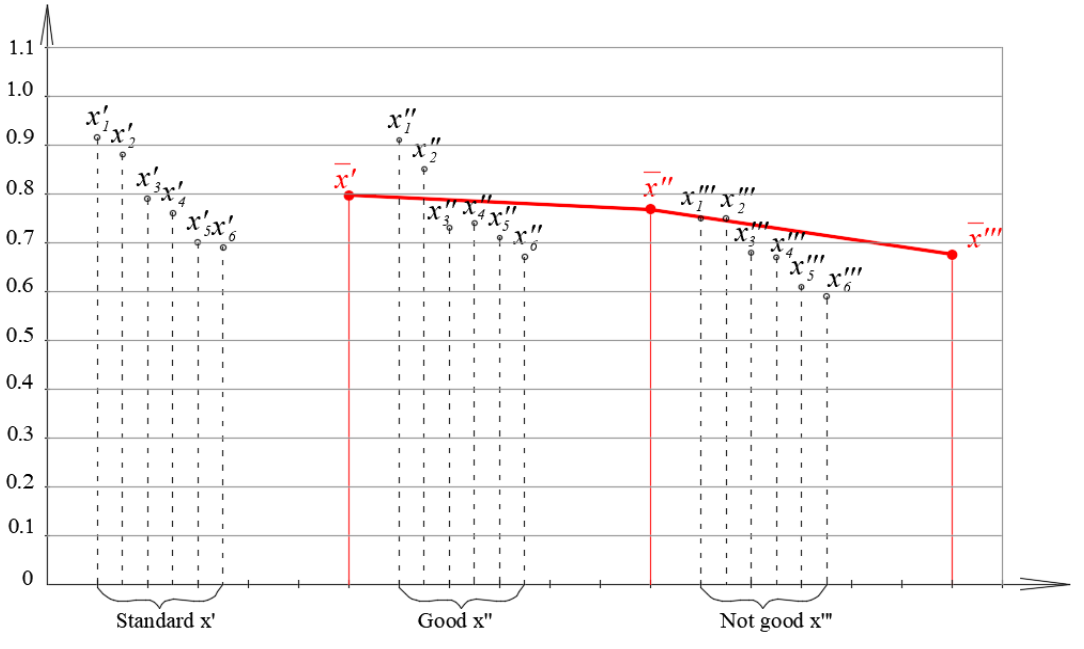


Figure 3. Plant species - statistical assessments

Technological fundamentals Y Antibacterial activity

Technical basis/Class	Standard y′	Good y″	Bad y‴
vinegar	У1′=1,11	У1″=1,09	У1‴=0.99
oil	У2′=0,93	У2″=0,91	У2‴=0,77
deco	У3′=0,70	У3″=0,67	У3‴=0,58
tincture	У4′=0,65	У4″=0,56	У4‴=0,45
wine	У5′=0,62	У5″=0,56	У5‴=0,49
secondary	$\bar{y}'$=0,80	$\bar{y}''$=0,76	$\bar{y}'''$=0,66

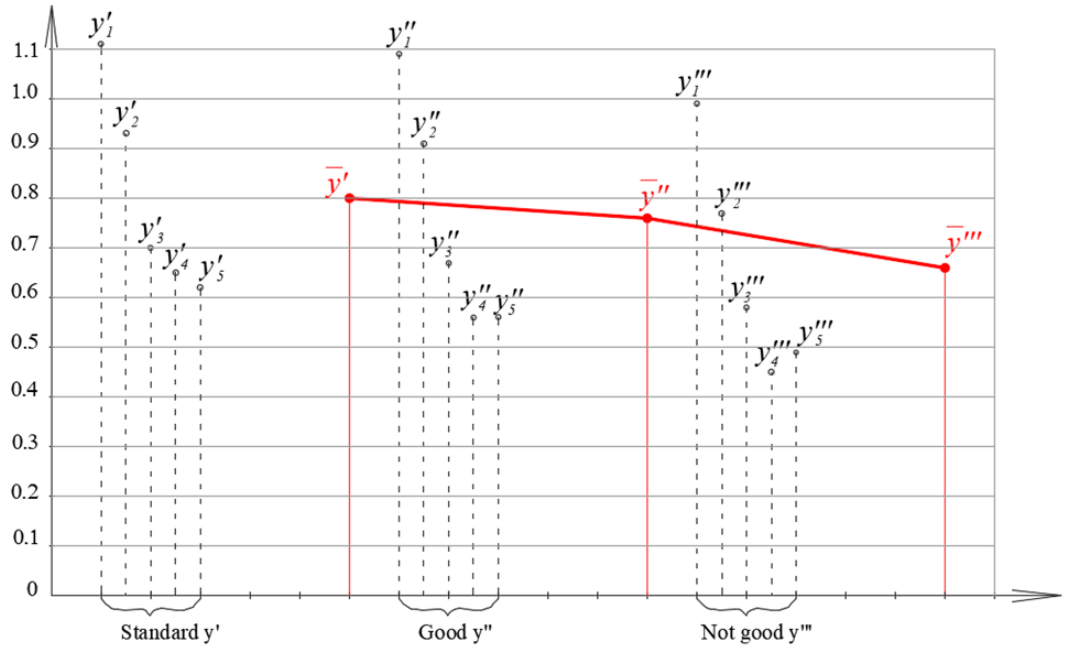


Figure 4. Technological foundations - statistical assessments

Plant fractions Z Antibacterial activity

Fraction/Class	Standard z′	Good z″	Bad z‴
roots	Z1′=0,88	Z1″=0,87	Z1‴=0.71
whole plant	Z2′=0,87	Z2″=0,86	Z2‴=0,75
stems	Z3′=0,74	Z3″=0,70	Z3‴=0,63
leaves	Z4′=0,71	Z4″=0,65	Z4‴=0,60
average	$\overline{z'}$=0,80	$\overline{z''}$=0,77	$\overline{z'''}$=0,67

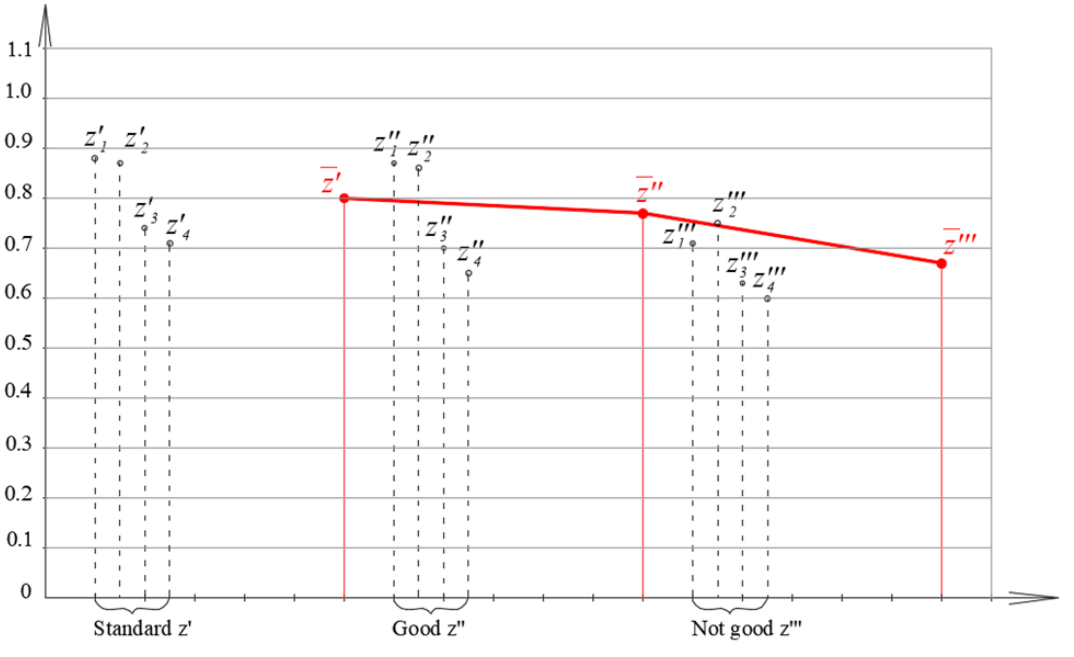


Figure 5. Plant fractions - statistical assessments

The numerical values and graphical representations of antibacterial activities in these figures correspond to the original ideas for planning and conducting the field and laboratory experiments by grouping them into three categories of data (extracts):

- plants grown in a growing cage under special conditions (reference);

- plants grown in a greenhouse ready for application after the 20th day of solution preparation (good class);

- greenhouse-grown plants ready for application after the 2nd and 10th day of solution preparation.

In an effort to reduce the dimensionality and complexity of local models, we address the following two possibilities:

- reducing the dimensionality of the local models by discarding the weakly influencing raw materials and technological factors within each class according to the plant species - fraction-solution extraction technology scheme;

- reducing the number of condition classes by proving statistical identity and adopting decisions to merge or remove one of them (e.g. the classes "reference and good" or "good and not good").

These two problems are solved by the hypothesis testing procedures.

1. Testing hypotheses for the determination of models of the type "plant species - fraction-extraction method" with minimum dimensions

1.1. Antimicrobial activities of plant species

The procedure is based on successive hypothesis tests for equality of mathematical expectations of antimicrobial activity of plant species in the following order (Figure 3) x1, x2, x3, x4, x5, x6, using the data from the "reference" class, which are the most representative.

1.1.1. Coriander x1 - basil x2 H0 : x1=x2.

RESULT: The null hypothesis is rejected by one-sided and two-sided criteria with significance level α=0.1. The alternative hypothesis H1 : x1$\geq$ x2 with P=0.9 is accepted.

1.1.2. Basil oregano. H0 : x2=x3. The null hypothesis is rejected with α=0.1. The alternative hypothesis H1 : x2$\geq$x3 is accepted with P=0.9.

Conclusion: The best antibacterial activity has the plant species coriander. Basil can be taken as a backup, and oregano and lavender as possible ones.

1.2. Antimicrobial activity of solutions obtained by extraction technologies

1.2.1. Extraction in a medium of vinegar (y1) and oil (y2) H0 : y1=y2.

The null hypothesis of equality of mathematical expectations of antimicrobial activity of vinegar and oil based extracts is accepted with probability P=0.9.

1.2.2. Extraction in oil and water medium (decoction)

The null hypothesis H0:y2=y3 is rejected. The alternative hypothesis H1:y2$\geq$ y(3) with P=0.1 is accepted.

Conclusion: The antimicrobial activities of solutions obtained in vinegar and oil process media were statistically equivalent with a slight advantage in the former case. The solutions obtained by extraction in the other media did not show high activity towards Escherichia coli.

1.3. Antimicrobial activities of solutions obtained from different plant fractions

1.3.1. Plant fractions "roots (z1) and whole plants (z2)" other things being equal H0:z1=z2. The null hypothesis is rejected and the alternative H1:z1$\neq$ z(2) with P=0.9 is accepted.

1.3.2. Whole plants (z2) and stems(z3) H0:z2=z3 other things being equal, the null hypothesis is rejected and the alternative H1:z2> z(3) with P=0.9 is accepted.

Conclusion: Extracts obtained from the roots of plant species have the best antimicrobial activity. The use of whole plants is a backup option, and the other two franks are of little use.

Testing hypotheses for statistical identity of data from different classes - possibilities to reduce the number of classes

We discuss the possibility of reducing the number of classes corresponding to different cultivation

conditions for the plant species adopted at the beginning of the study as reference (′), good ″) and poor ‴). The location of the points corresponding to the mean and other values of the antibacterial activities (Figures 3, 4, 5) gives us a reason to test the hypotheses about the statistical identity of the data from the three classes. The hypothesis testing procedures apply to both the maximum and mean antibacterial activity values for the three classes.

Data for testing the hypotheses of equality of maximum values - coriander, root, standard

χ'

Coriander, root, standard

xi′	1,3	1,4	1,4	1,5	1,4	1,4	1	1,1	1,1	1,2	1	1,1	1,4	1,5	1,5	1,4	1,5	0,7	0,8	0,8	0,9	0,8	0,9	1,4	1,5	1,5	1,6	1,5	1,6

Coriander, root, class „good“

xi″	1,3	1,4	0,9	1	1,4	1,5	1	0,8	1,5	1,5

Coriander, root, class „poor“

xi‴	1,2	1,2	0,5	0,6	0,7	0,8	0	0,4	1,2	1,3	1	1,2	0,7	0,8	0,3	0,4	1,3	1,4	0,6	0,7

2.1.Testing the hypotheses of equality of mathematical expectations of the data from the merged classes

2.1.1. Grades compared: coriander, root, standard x_1' and coriander, root, good x_1'' : null hypothesis $H_0: \overline{x_1'} = \overline{x_1''}$.

standard	source data	good
$\overline{x_1'}$=1,26; n′=30,	S′²=0,08	x_1''=1,20, n″=10, S″²=0,10

Confidence interval = $T = \frac{\overline{x_1'} - \overline{x_1''}}{\sqrt{(n'-1)s'^2 + (n''-1)\,S''^2}} \sqrt{\frac{n'n''(n'+n''-2)}{n'+n''}} = 0{,}565$

quantile of the Student's t-distribution at

v=n′+n″-2=38; α=0,1

$t = \left(\nu, \frac{\alpha}{2}\right) = t(38;\ 0{,}05) = 1{,}68$

Check

$|T| = 0{,}565 < t(38; 0{,}05) = 1{,}68$

The null hypothesis is accepted $H_0: \overline{x_1'} = \overline{x_1''}$.

The implementation of the null hypothesis shows the statistical identity of the data from the two compared classes. This gives us grounds in the next study to check whether to abandon the laborious and lengthy process of growing plant species in a vegetation cage or to use the equivalent data from the greenhouse after the 20th day.

2.1.2. Coriander, root, good x_1'' compared classes and coriander, root, bad x_1''' ; null hypothesis $H_0: \overline{x_1''} = \overline{x_1'''}$.

standard	source data	bad
$\overline{x_1'}$=1,26; n′=30,	S′²=0,08	x_1'''=$\overline{0{,}84}$, n‴=20, S‴²=0,14

Confidence interval T=4,609

quantile of the Student›s t-distribution at ν=48; α=0,1

$$t = \left(\nu, \frac{\alpha}{2}\right) = t(48;\ 0{,}05) = 1{,}678$$

Check

$|T| = 4{,}609 < t(48; 0{,}05) = 1{,}678$

null hypothesis $H_0: \overline{x''_1} = \overline{x'''_1}$ is rejected. The alternative hypotheses are accepted $H_1: \overline{x''_1} \neq \overline{x'''_1}$ и $H_1: \overline{x''_1} > \overline{x'''_1}$.

Rejection of the null hypothesis indicates statistical dissimilarity of the data from the two classes being compared. This gives us a reason to work with extracts from plant species grown in the greenhouse and applied after the 20th day from the date of preparation, under the following production conditions.

2.2. In order to determine the possibility of reducing the number of classes indicated on the basis of the plant species with maximum antimicrobial activity, we will extend the problem and test the hypotheses of statistical identity of the data from different classes using the mean values of all the factors (plant species, fractions and extraction technologies) presented in Figures 3, 4 and 5. These data are based on the whole set of 5600 laboratory results and have been obtained after systematic and statistical analysis, which proves their representativeness. In the following, hypothesis testing procedures are applied to test the equality of the mathematical expectations for the three classes of conditions.

2.2.1. Compared classes: plant species grown in a vegetative cell (x′-etalon) and in a greenhouse (x″-good) after the 20th day. Null hypothesis

$H_0: \overline{x'_1} = \overline{x''_1}$

source data on Figure 3

$\overline{x'}$=0,796; n=6; s'^2=0,024

good $\overline{x''}$=0,768, n=6, S"^{2}=0,026

checking the equality of variances:

$$F = \frac{s''^2}{s'^2} = 1{,}08 < F(0{,}1; 5; 5) = 10{,}97$$

We are working on an algorithm with equal variances (Malcheva and Naskova, 2018). Confidence interval T=0,307

quantile of the Student's t-distribution at:

$$t = \left(\nu, \frac{\alpha}{2}\right) = t(10;\ 0{,}05) = 1{,}812$$

$|T| = 0{,}307 < t(10; 0{,}05) = 1{,}812$

Null hypothesis $H_0: \overline{x'_1} = \overline{x''_1}$ is accepted with α=0,1 (P=0,9)

2.2.2. Comparable classes: plant species grown in a greenhouse after the 20th day ($\overline{x''}$--good) and after the 2nd and 10th days ($\overline{x'''}$- bad)

source data on Figure 3

good $\overline{x''}$=0,768; n=6; s''^2=0,026

bad $\overline{x'''}$=0,675, n=6, S"^{2}=0,0046

the equality of variances:

$$F = \frac{0{,}026}{0{,}0046} = 5{,}65 < F(0{,}1; 5; 5) = 10{,}97$$

We are working on an algorithm with equal variances (Malcheva and Naskova, 2018). Confidence interval T=1,302

quantile of the Student›s t-distribution at:

$$t = \left(\nu, \frac{\alpha}{2}\right) = t(10;\ 0{,}15) = 1{,}093$$

$|T| = 1{,}302 < t(10; 0{,}15) = 1{,}093$

The null hypothesis is not accepted. The alternative hypotheses are accepted. $H_1: \overline{x''} \neq \overline{x'''}$ and $H_1: \overline{x''} > \overline{x'''}$ with α=0,15

Hypothesis tests for plant fractions (Figype 3) and technological bases of extraction (Figure 4) confirmed the adopted decisions for statistical identity of the first two classes and statistical distinctness of the classes accepted as good and bad.

Conclusion

As a result of the complex laboratory, systematic and statistical studies carried out on plant species grown under different conditions and processed

by different extraction technologies, extractive solutions with proven antimicrobial activity against *Escherichia coli* were obtained.

The possibility of obtaining the best terms of future production is proven, boiling down to: Extraction of raw material products from one main plant fraction (roots or whole plant), from two to four plant species (coriander, basil, oregano and lavender) with extraction technology from one solvent (vinegar or oil). The feasibility of obtaining good quality extraction solutions by using raw material from plant species grown in the most accessible greenhouse conditions applied after the 20th day of their preparation has been demonstrated.

The results of the conducted complex studies are a good basis for the introduction of practically applicable technologies for the production of extractive solutions with high antimicrobial activity against *Escherichia coli.*

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