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DOCTORAL THESIS

STUDIES ON THE PHYSICO-CHEMICAL AND PHARMACOBOTANICAL PROPERTIES OF *LYSIMACHIA NUMMULARIA L.* SPECIES WITH THERAPEUTIC POTENTIAL IN ORO-DENTAL PATHOLOGY

SUMMARY

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INTRODUCTION

As a result of observing the general tendency to study natural biocompounds, in the doctoral thesis I chose to study the plant *Lysimachia nummularia L.* and the extracts obtained from this plant, in the perspective of generating a product with therapeutic applications in oro-dental pathology.

In the context of WHO reports, it is appreciated that oro-dental health is an integral part of the general health of the human body and that one can only talk about health in a general way only in the context of oro-dental health; thus, it is emphasized that the contribution of oral health to the well-being and quality of life is extremely important.

CURRENT STATE OF KNOWLEDGE

CHAPTER 1. GENERAL DATA OF THE SPECIES *LYSIMACHIA NUMMULARIA L.*

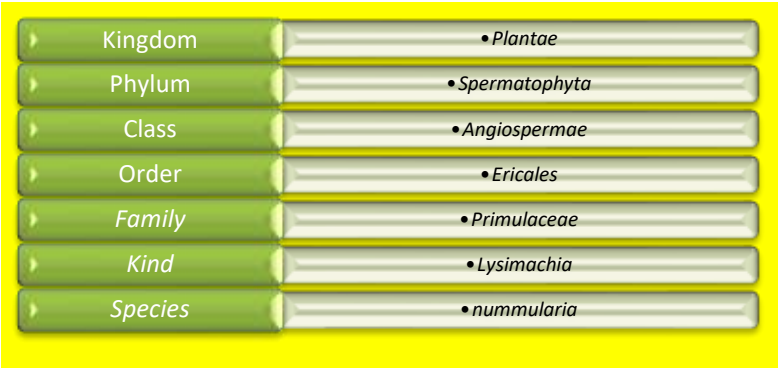
1.1. Genus *LYSIMACHIA NUMMMULARIA L.* – botanical description

Lysimachia nummularia L. (Gălbioara) is part of the *Primulacee* Family and is a perennial herbaceous plant, chamephyte, mesohydrophyte, mesothermal at amphitolerant pH, with erect stem, 10-15 cm long, glabrous, simple or weakly branched, tetrahedral, with alternate leaves, sometimes opposite, without stipules.

Lysimachia numularia L.

1.2. Classification

The *Lysimachia* genus belongs to the *Primulaceae* Family, the *Ericales* Order, contains approximately 180 species, cultivated and spontaneous, as seen in the attached figure:



Systematic classification of the genus *Lysimachia*

1.3. Geographical distribution

The species of the genus *Lysimachia* have been identified as being widely distributed in the world, there are data in the specialized literature accessed in which the presence of the plant is recorded on all continents.

CHAPTER 2. PHYTOCHEMICAL CHARACTERISTICS OF THE SPECIES *LYSIMACHIA NUMMULARIA L.*

2.1. Primary and secondary metabolites-generalities

The accumulation of secondary metabolites refers to the accumulation of biogenic substances in different plant organs, but also the accumulation of a certain secondary metabolite in certain plant organs.

Also known is the role of phytoalexins of established phenolic compounds, such as resveratrol, which is mentioned in species of the genus *Lysimachia*, determined and used as a biomarker in terms of the quality of extracts obtained from the organs of the species, *Lysimachia nummularia L.*

Flavonoids represent the most important and diverse group of natural polyphenols, with more than 9,000 structures identified to date.

2.2. The phytochemical composition of species of the genus *Lysimachia sp.* following classes of active principles:

From the specialized literature it was found that the species of the genus *Lysimachia sp*. contain the

- ✓ Phenolic acids;
- ✓ Stilbenoids: resveratrol (*L. nummularia*)
- ✓ Flavonoids;
- ✓ Sterols and saponosides;

Phenolic compounds are known for their antioxidant and antibacterial properties.

2.3. Biological properties of reported phytocompounds

2.3.1. Biological activities of *p*-coumaric acid

The antibacterial, antifungal, antiviral, anti-inflammatory and antitumor properties of p-coumaric acid have been demonstrated over time and have been the basis for the use of species containing this metabolite in allopathic medicine.

2.3.2. Biological activities of quercetol

Cell damage induced by reactive oxygen species is blocked by quercitol due to this antioxidant property.

Quercetol also exhibits pronounced anti-inflammatory capabilities.

2.3.3. Biological activities of quercitroside

The anti-inflammatory, antioxidant and regenerative properties of quercitroside have also been studied in the context of periodontal disease.

These aspects suggest that quercitroside can be regarded as a new bioactive molecule with special perspectives in dental medicine, given the fact that the possibility of regeneration of soft

tissues as well as hard tissues of the periodontium would revolutionize the treatment of periodontal disease.

2.3.4. Biological activities of isoquercitinoside

Isoquercitioside has antitumor, anti-inflammatory properties.

2.3.5. Biological activities of luteol

The undeniable antitumor properties of luteol have been demonstrated in numerous specialized studies.

2.3.6. Biological activities of rutoside

Studies aimed at evaluating the properties of rutoside have shown that it has antiplatelet, antihypertensive and anti-inflammatory activity.

2.3.7. Biological activities of myricetol

Yasaman T. and his collaborators demonstrated in the studies carried out on the properties of myricetol pro-oxidant effects translated by the reduction of trivalent iron into bivalent iron and the reduction of ROS production.

2.3.8. Biological activities of kaempferol

Recent studies by Waqas A. and his collaborators demonstrate anti-inflammatory properties.

CHAPTER 3. PHARMACOLOGICAL SPECIES OF *LYSIMACHIA NUMMULARIA L.* SPECIES.

3.1. Antibacterial properties

Among the antibacterial mechanisms, we mention the antibacterial effect by stimulating the generation of oxygenated water, changing the permeability of the cytoplasmic membrane, blocking the signals of bacterial multiplication within biofilms.

3.2. Antioxidant and anti-inflammatory properties

Similar studies on the antioxidant properties of the extracts obtained from *Lysimachia numularia L.* were also carried out by other authors; thus, Anita Toth and her collaborators who analyzed the antioxidant properties of three *Lysimachia* species, respectively, *nummularia, punctata* and *vulgaris,* and showed that the *nummularia* species has the strongest antioxidant activity.

3.3. Antitumor properties

In the specialized literature accessed, there is little data related to the antitumor properties of extracts obtained from species of the genus Lysimachia.

PERSONAL CONTRIBUTION

CHAPTER 4. OBTAINING AND QUALITY CONTROL OF VEGETABLE RAW MATERIALS

4.1. OBTAINING VEGETABLE PRODUCTS

Harvesting. The working material was represented by the *Lysimachia nummularia L.* plant, harvested in July 2020, from the edge of the Tău -Brazi lake in the Roșia Montană area.

Plant association with sedges of *Lysimachia nummularia L.*

Lysimachia nummularia L.

4.2. Determining the identity of the producing species

The identity of the species was verified both by macro and microscopic examination, as well as by chemical screening.

Materials and methods

Macroscopic examination

The macroscopic examination was carried out by examining the whole plant with the naked eye or with a magnifying glass observing: their appearance, dimensions, color, taste and smell. In order to carry out the microscopic examination, transversal sections were made through the stem and leaf.

Microscopic examination in powder

It was performed with chloral hydrate 50% according to the pharmacognostic analysis.

The groups of active principles belonging to plant products can be identified through the general qualitative chemical examination, which involves the successive and selective extraction of the active principles, with solvents of different polarities (ethyl ether, alcohol and water) followed by the performance of specific reactions with the help of which they can different groups of active principles or certain chemical constituents be identified.

Results and discussion

All characters are in accordance with those described in the specialized literature, for the species *Lysimachia nummularia L.*

Cross section through the root

The following characters are distinguished:

- ✓ the contour of the section is circular, slightly wavy;
- ✓ the structure is primary with the beginning of a secondary structure;

Transverse section through stem

The cross section through the stem shows the following characters:

- ✓ the contour of the cross section is elliptical-oval;
- ✓ the central cylinder, is formed by conductive tissues of secondary origin, arranged annularly and represented by an external ring of free secondary and an internal ring of secondary wood;
- ✓ the marrow is thick, parenchymatic-cellulosic, meaty type.

Transverse section through leaf

The transverse section through the leaf shows the following characters:

- ✓ at the level of the rib, there is a single hypodermic layer of collenchyma, fundamental parenchyma, and centrally, a large, free-woody bundle with primary structure, next to which also appears a very small bundle;
- ✓ the limbus has a dorsoventral bifacial structure.

Powder microscopic examination

The powder microscopic examination for the plant product *Lysimachiae herba* shows the following elements: pollen grains, chlorophyll parenchyma, fibers, stomata, rare unicellular peri tectors.

The powder from the root is characterized by the presence of large-caliber wood vessels (reticulated), accompanied by fragments of parenchyma with large cells and accompanying pointed elongated fibers.

The following elements were highlighted in the flower powder: endothecium, pollen grains, parenchyma fragment.

Following the general chemical analysis carried out, the following types of active principles were identified as follows:

In the roots of the species *Lysimachia nummularia L.* the following were identified: volatile oil, sterols (triterpenes), flavonic aglycones, carotenoids, coumarins, tannins (gallic tannins,

catechic tannins), flavonosides, heteroside coumarins, triterpene heterosides, ODP, reducing compounds, oses , polyoses, polyuronides, saponosides.

The following classes of active principles were identified in the plant product *Lysimachiae herba*: volatile oil, sterols (triterpenes), flavonic aglycones, carotenoids, fatty acids, coumarins, tannins (gallic tannins, catechin tannins), flavonosides, heteroside coumarins, triterpene heterosides, ODP, reducing compounds, oses, polyoses, polyuronides, saponosides.

Following the qualitative chemical analysis, the flowers of the species *Lysimachia nummularia L.* contain: volatile oil, sterols (triterpenes), flavonic aglycones, carotenoids, fatty acids, coumarins, tannins (gallic tannins, catechic tannins), flavonosides, heteroside coumarins, triterpene heterosides, ODP , reducing compounds, oses, polyoses, polyuronides.

Thus, the identification of the constituents from the groups of active principles highlighted in the researched plant product will be possible through the correlation between metabolism and phylogeny.

Preliminary conclusions

1. The analysis of the macroscopic characters of the species under study confirms that the plant is *Lysimachia nummularia L.* from the *Primulaceae* family.

2. Analysis of transversal sections through the vegetative organs of the species *Lysimachia nummularia L.*

3. Microscopic examination in powder revealed common elements and characteristic elements.

4. The presence of several groups of active principles in all the organs of the species *Lysimachia nummularia L.*, leads us to the conclusion that the species is of interest and can be researched for therapeutic use.

4.3. Purity control of vegetable products

4.3.1. Determination of impurities from the same plant

These impurities can be constituted by plant products degraded during drying or attacked by harmful insects.

Material and method

Thus, in order to determine the impurities from the same plant, proceed according to the pharmacognostic analysis.

4.3.2. Determination of foreign bodies from the producing plant

The pharmacognostic qualitative norms regarding the preliminary evaluation of plant products, provide for or exclude the presence in plant products of foreign bodies from the producing plant (parts from other plants, mineral substances, earth, sand, etc.).

Material and method

Proceed according to pharmacognostic determinations.

Preliminary conclusions

The vegetable raw material represented by the three vegetable products: *Lysimachiae herba; Lysimachiae radix* and *Lysimachiae flores*, does not contain impurities or foreign bodies.

4.4. Quality control of vegetable products

In order to control the quality of plant products, the three plant products obtained from the species *Lysimachia nummularia L.* were used, namely*: Lysimachiae herba; Lysimachiae radix* and *Lysimachiae flores.*

4.4.1. Preliminary determinations

4.4.1.1. Loss on drying

It was carried out according to the 10th edition of the Romanian Pharmacopoeia.

4.4.1.2. Determination of soluble substances

Taking into account the solubility of the active principles known from the specialized literature, as well as the extraction possibilities, we used three solvents in this determination, namely: ethanol 40% (v/v), ethanol 96% (v/v) and water.

4.4.1.3. Limit control of iron and heavy metals

Iron limit control

The iron ion (III) forms with potassium hexacyanoferrate (II) a blue complex or a blue precipitate, depending on the concentration.

Limit control of heavy metals

The lead ion forms with sodium sulphide, depending on the concentration, a brown color or a black precipitate.

Preliminary conclusions

1. The values of the loss through drying show that the plant products taken into account correspond in terms of their preservation.

2. Since the largest amount of substances are soluble in 40% ethanol, it leads us in the following research to use extracts obtained in 40% ethanol.

4. 5. Research of polyphenolic compounds

4.5.1. Determination of the content in total polyphenols for the plant products *Lysimachiae herba, Lysimachiae radix, Lysimachiae flores.*

The principle of the method

It is based on the determination of the intensity of the blue coloration of the molybdenum oxides formed by the reduction of the Folin-Ciocâlteu reagent (phosphomolybdotungstic acid) by polyphenols.

Materials and method:

The determination was carried out on all plant products according to the tannin determination protocol presented in the European Pharmacopoeia.

Results

In alcoholic solutions in 40% alcohol we determined a greater amount of total polyphenols of the plant products *Lysimachiae herba* and *Lysimachiae flores.*

Conclusions

1. The content of total polyphenols in the plant products *Lysimachiae herba, Lysimachiae radix, Lysimachiae flores* is high, compared to other plant products.

2. For all three plant products, *Lysimachiae herba, Lysimachiae radix, Lysimachiae flores,* the amount of polyphenols extracted in concentrated alcohol is lower compared to the other two solvents.

4.5.2. HPLC analysis of phenolic compounds

For the separation, identification and quantification of phenolic compounds, a standardized HPLC method for the determination of total polyphenols, described by USP monograph 30-NF25, was adapted.

For statistical analysis of differences between variables of the obtained results, Student's "t" test and one-way ANOVA test with Tukey's "post hoc" test, which allows multiple comparisons between pairs of data, were used, using GraphPad Software 9, Inc. , San Diego, CA, USA. The reproducibility of the method was assessed by the square of the correlation coefficients.

Results

Among the substances identified following the HPLC analysis, a number of substances were quantified.

1. The HPLC-DAD method was applied for the investigation of phenolic compounds, with the quantitative analysis of seven components from *Lysimachia nummularia L* plant extracts.

2. The novelty of this study is represented by the identification and quantification of phenolic compounds by the HPLC method;

3. The active principles, determined by HPLC analysis, demonstrate the possibility of using the three plant products (*Lysimachiae radix, Lysimachiae herba, Lysimachiae flores)* in phytotherapy and the presence of resveratrol is an asset, in terms of the antioxidant properties of the plant products.

4. The maximum amount detected, around 18 mg% g of dry plant product is a relatively small amount, but considering that a liter of Saugvinion wine has only 2 mg of resveratrol / liter of wine, we could say that the roots of our species are really rich in resveratrol.

CHAPTER 5. DETERMINATION OF ANTIOXIDANT CAPACITY

Materials and methods

The method is based on the discoloration of the stable DPPH (2,2,diphenyl-picryl-hydrazyl) radical, and having absorption at 517 nm by antioxidant substances.

The ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) method is a technique used in analytical chemistry to determine the antioxidant activity of compounds.

The FRAP method consists in the reduction, in an acidic medium, of the ferric 2,4,6-tripyridyl-triazine complex [Fe(III)-(TPTZ)2]3+ to the ferrous complex [Fe(II)-(TPTZ)2]2+ of intense blue color in the presence of antioxidant species.

The CUPRAC method evaluates the color of a copper complex with neocupreine (2,9-dimethyl-1,10-phenanthroline). Reduction of copper(II) ion to copper(I) iron causes the color to change from light green to reddish orange.

Results

In this research, the antioxidant activity of the extracts obtained by the four in vitro methods was evaluated: the DPPH, ABTS, FRAP and CUPRAC methods.

Preliminary conclusions

The plant products *Lysimachiae herba, Lysimachiae radix* and *Lysimachiae flor*es show marked antioxidant properties correlated with the content in total polyphenols and phenolic

compounds contained, but especially with the presence of polyphenolic compounds and resveratrol.

CHAPTER 6. TECHNOLOGICAL LABORATORY PROCEDURE FOR OBTAINING A PHARMACOLOGY ACTIVE DRY EXTRACT

6.1. Establishing the extraction parameters

The classic extraction process used to obtain dry extracts is usually maceration. Ethanol 40% meets all the conditions that a solvent must meet in order to be chosen for the production of pharmaceutical preparations with a rich content of active principles.

Material and methods

To obtain the extracts, we used *Lysimachiae herba, Lysimachiae radix* and *Lysimachiae flores* as plant products.

Results

The lowest amount of extract was obtained from the plant product *Lysimachiae herba*. A similar amount of extract was obtained from *Lysimachiae radix*.

Preliminary conclusions

Since polyphenols are soluble in ethanol, it was deduced that the optimal methodology for obtaining the dry extract consists in maceration for 14 days with 40% ethanol, a time that does not affect the stability of polyphenols.

6.2. Development of the technological process for obtaining the dry extract - laboratory phase

Therefore, the technological process (laboratory phase) to obtain the selective extract consists of maceration, for 14 days with 40% ethanol, interphase control (through specific reactions of polyphenols and their quantitative determination, reuniting the two extracts and concentration in

a rotary evaporator until to the syrup stage, then transferring to a desiccator and continuing to distill the solvent under reduced pressure to the dry extract stage.

CHAPTER 7. QUALITY CONTROL METHODOLOGY OF DRY EXTRACTS AND THEIR STANDARDIZATION.

Material and method

According to FR X, organoleptic characteristics (appearance, smell, color, consistency), solubility in the solvent used for preparation, limits of iron and heavy metals, loss through drying and content in active principles are monitored.

7.1. Determination of organoleptic characteristics

It is appreciated: the appearance and color, by visual analysis of the extract, the taste and the smell.

Loss on drying is determined on 1 g of dry extract by keeping it in an oven at 1050C for approximately 3 hours, to bring it to constant mass and refer to 100 g of extract.

7.2. Identification of active principles

Since polyphenolic derivatives (flavonosides, proanthocyanosides, stilbenoids, tannins, phenolic acids) can be extracted with 50% alcohol from the studied vegetable product, we considered it necessary to identify them, through the following reactions: the reduction of the Folin-Ciocâlteu reagent (for total polyphenols), the Schibatta reaction (for flavones), and staining with FeCl3 (tannins).

Preliminary research on the standardization of extracts

In order to standardize some dry extracts, we considered that the determination of the content in total polyphenols and caffeic and chlorogenic acid as well as the determination of resveratrol from *Lysimachiae herba* and *Lysimachiae radix* would be representative of the pharmacodynamic action.

7.3. Determination of the content in active principles

The therapeutic action of the extract being imprinted by polyphenols, we considered the determination of total polyphenols (photocolorimetric and by HPLC) as representative.

7.3.1. Determination of total polyphenols from dry extracts obtained from *Lysimachiae herba, Lysimachiae radix* and *Lysimachiae flores* by photometric method

Materials and methods

It was performed according to the previously described method.

7.3.2. HPLC analysis of polyphenolic compounds from the obtained extract

The analysis was performed according to the method described previously.

Results

Through the different extraction methods from 100 g of plant product, dry extracts were obtained in the quantities and with the organoleptic characteristics mentioned in the table below.

Table: The organoleptic characteristics of the extracts obtained by various extraction procedures

Extract code	*Solvent*	*Organoleptic characteristics of the obtained extract*			
		Appearance	*Color*	*Smell*	*Taste*
1	*Lysimachiae herba*	*powder*	*reddish brown*	*pleasant*	*pleasant*
2	*Lysimachiae radix*	*powder*	*reddish brown*	*pleasant*	*pleasant*
3	*Lysimachiae flores*	*powder*	*brown with golden reflections*	*pleasant*	*pleasant*

Preliminary conclusions

1. The HPLC method for determining polyphenols can be used to standardize extracts.

2. The amount of total polyphenols could be taken into account for the standardization of the respective extract.

CHAPTER 8. RESEARCH ON THE STUDY OF ANTIBACTERIAL AND ANTIFUNGAL PROPERTIES OF EXTRACTS OBTAINED FROM *LYSIMACHIA NUMMULARIA L.*

Working hypothesis

The results regarding the physico-chemical properties of the extracts obtained from *Lysimachia nummularia L.* presented in the previous chapter show that they contain total polyphenols in high concentrations.

The working hypothesis is that the extracts obtained from *Lysimachia nummularia L.* have antibacterial and antifungal properties.

The aim of the study is to implement the results of the antibacterial and antifungal effects in a therapeutic product usable in infectious and fungal pathology of the oral cavity.

The objectives of the study were:

1. Evaluation of antibacterial properties;

2. Evaluation of antifungal properties.

The motivation for selecting the extracts evaluated in the present study

We chose for the evaluation of the antibacterial and antifungal properties, the 40% alcoholic extract, obtained from the aerial part of the plant (leaves, stem, flowers), flowers and roots, because these had the highest content in total polyphenols, components that support the possible effects antibacterial and antifungal.

Materials and methods

8.1. Pathological products studied and study design

The pathological products used in this research pillar were collected from patients with dental caries and periodontitis, endodontic infections, acute pharyngo-tonsillitis, after the previous oro-dental clinical examination.

The samples taken according to standard methods were: dentin taken from softened dentin of dental caries, periodontal pus, product from post-extraction alveolitis, product from root canals, pharyngeal exudate.

The study was carried out in two distinct stages:

- testing the 40% alcoholic extract, obtained from the dry product of *Lysimachia nummularia L.* on lyophilized, stabilized and viable reference bacterial and fungal strains of the American Type of Culture Collection (ATCC) type.
- testing the antibacterial activity of the 40% alcoholic extract, obtained from the dry product of *Lysimachia nummularia L.* (herba, flores and radix) on bacterial and fungal strains identified from the pathological products examined, compared to the usual antibiotics and antifungals used in the conditions in which the pathological products studied were generated.

8.2. Sowing on culture media

The reference bacterial strains and the culture media on which the initial inoculation was carried out are shown in the Table below as follows:

Table - Bacterial species used in stage I

BACTERIAL SPECIES	***REFERENCE SOURCE***	***CULTIVATION ENVIRONMENT***
Streptococcus pyogenes	***ATCC 19615***	***agar-blood***
Streptococcus pneumoniae	***ATCC 46619***	***agar-blood***
Enterococcus sp.	***ATCC 23212***	***agar-blood***
Staphylococcus aureus	***ATCC 29213***	***agar-blood***
Escherichia coli	***ATCC 25922***	***agar-blood***
Klebsiella pneumoniae	***ATCC 13883***	***agar-blood***
Pseudomonas aeruginosa	***ATCC 27853***	***agar-blood***
Candida albicans	***ATCC 10231***	***Sabouraud***

8.3. Identification in the Api system of the bacterial species isolated from the pathological products under study

The pathological products were seeded on blood agar medium, and the bacterial species were identified using the Api-ident system.

Reactions are interpreted according to the analytical profile as provided by each identification kit.

8.4. Testing of bacterial sensitivity to the extracts taken in the study compared to antibiotics

The principle of the method aims at seeding a bacterial suspension to be tested on the surface of a gelatinous culture medium, followed by the subsequent deposition of microcompresses with different antibiotics or chemotherapeutics. After a 24-hour incubation at 37oC, the interpretation is made, which consists in measuring the zone of inhibition, in which the bacteria did not develop, the dimensions of this zone being directly proportional to the sensitivity of the bacteria to the respective antibiotic.

Given the fact that in the present study I wanted to assess the level of sensitivity of some bacterial strains to the obtained extracts, I adapted the diffusimetric method.

Table. Bacterial species and antibiotics/chemotherapy used in study II

WILD BACTERIAL SPECIES	*TEST ENVIRONMENT*	*Positive control - ANTIBIOTICS / ANTIMYCOTICS*	*Negative witness*
Streptococcus mitis	*agar-blood*	*Penicilină G (P)*	*Distilled water*
Staphylococcus aureus	*agar-blood*	*Penicilină G (P)*	*Distilled water*
Enterococ	*Mueller-Hinton*	*Ampicilină (AMP)*	*Distilled water*
Escherichia coli	*Mueller-Hinton*	*Gentamicină (CN)*	*Distilled water*
Pseudomonas aeruginosa	*Mueller-Hinton*	*Gentamicină (CN)*	*Distilled water*
Candida albicans	*Sabouraud*	*Fluconazol (FCA), Voriconazol (VOR)*	*Distilled water*

Results

Stage 1.

The results of the bacteriological study carried out on ATCC reference strains for the three extracts taken in the study and antibiotics are presented in the thesis.

Stage 2.

The results of the bacteriological study in stage II was carried out on a group of five bacterial strains selected from both Gram-positive and Gram-negative bacterial species, strains isolated from pathological products taken from patients with various dental conditions.

The results were expressed using the indicators S – sensitive, I – intermediate and R – resistant to the antibiotics specific to each bacterial strain used in the test.

Preliminary conclusions

1. *Lysimachia nummularia L.* extracts have antibacterial effects both on reference strains and on strains of bacteria isolated from pathological products.

2. The three extracts demonstrated the absence of antifungal effects, both on reference strains and on strains isolated from pathological products.

3. Among the referin strains, the most sensitive proved to be those from the group of Gram-positive cocci.

4. The most effective extract on reference strains was found to be that extracted from the aerial part of the plant, both for reference strains and for strains isolated from pathological products.

5. The special value regarding the antibacterial action of these extracts tested on the reference strains is proven by the antibacterial effect on species with established resistance, such as *Piocyanic bacillus, Enterococcus* and *Staphylococcus aureus*.

6. All strains isolated from pathological products were sensitive to different extracts, except staphylococcus which was equally sensitive to all three types of extracts.

7. The antibacterial effects approximately equal to those of the antibiotics used in parallel proved to be present in the case of the cariogenic flora represented in the present study by S. mitis, to which a sensitivity approximately equal to that of Penicillin was identified.

8. The predominantly bacteriostatic effects of the three extracts on bacterial species involved in the pathology of the oral cavity support the realization of clinical studies in the perspective of using this extract as a basis for obtaining pharmaceutical products widely used in the practice of dental medicine.

Chapter 9. EVALUATION OF THE CLINICAL IMPACT OF A MOUTHWASH SOLUTION WITH EXTRACT FROM *LYSIMACHIA NUMMULARIA L.* IN ORO-DENTAL DISEASES

9.1. Working hypothesis

Periodontitis and peri-implantitis are two diseases of the oral cavity with common valences in terms of bacterial etiology, pathogenic mechanisms in which oxidative stress plays an important role; for these reasons, the working hypothesis is that a mouthwash solution containing *Lysimachia nummularia L.* extract would have beneficial effects, alleviating the two conditions under study.

9.2. Objectives and activities

The primary objective of this study is to demonstrate the possible beneficial effects of the secondary metabolites contained in the mouthwash solution in the context of periodontitis and peri-implantitis.

The activities carried out within this request module were:

- ✓ Formulation of a mouthwash solution with hydro-alcoholic vegetable extract obtained from the species *Lysimachia nummularia L.*;
- ✓ Clinical examination of patients and delimitation of study groups;
- ✓ Establishing the study protocol;
- ✓ Evaluation of the NO level by performing the rapid semi-quantitative Barkeley test in patients who rinsed their mouths with a solution containing Lysimachia nummularia L.

extract for 10 days, compared to a control group that rinsed with a mouthwash solution without plant extract.

- ✓ Demonstration of the increase in antioxidant capacity in the oral cavity following the use of the mouthwash solution with a solution containing extract obtained from the species Lysimachia nummularia L. by determining the inhibitory activity of saliva against the DPPH free radical.

9.3. Material and methods

9.4.1. Obtaining the mouthwash solution

The mouthwash solution with vegetable extract in the composition was prepared by mixing 1000 g of dry extract in 2000 mL of bidistilled water; mouthwash solution with 10% dry extract was prepared and distributed in 80 brown bottles.

9.4.2. Delimitation of the study groups

For patients who presented themselves for a dental check-up with a view to periodontal treatment or for the insertion of dental implants, an initial oro-dental consultation was performed; then the heredo-collateral and personal pathological antecedents were detailed, focusing on the conditions classified as relative and absolute contraindications. The patients were examined by assessing the following periodontal indices:

1. Evaluation of the gingival tissue (by evaluating the color, shape and appearance of the gum, consistency and tone, contour, texture and the presence of bleeding), elements that are estimated in order to assess the signs of gingival inflammation;

2. The presence of bleeding on probing;

3. The degree of gingival recession;

4. Determining the existence of a lack of gingival attachment;

5. Probing the furcation area in multiradicular teeth (to determine if the periodontal pocket has extended interradicularly), the position, number and size of the furcations;

6. Evaluation of the degree of mobility of the teeth;

7. Evaluation of the existing restorations, to determine if and to what extent they contribute to the promotion of gingival inflammation;

8. Evaluation of bacterial plaque

9. Radiological examination;

For the realization of the study groups in peri-implantitis, we followed the classification below:

1. Patients with *favorable evolution of the dental implant*: the depth of the peri-implant trench < 3 mm; bleeding index absent; suppuration absent; without radiological changes.

2. Patients with *mucositis*: peri-implant trench depth ≤ 4 mm; absent/present bleeding index; suppuration absent/present; without radiological changes.

3. Patients with *peri-implantitis*, early form: peri-implant groove depth ≥ 4 mm; bleeding index present; suppuration absent/present; radiological – bone loss ≤ 25% of the implant length.

9.4.4. Evaluation of the NO level by performing the rapid Barkeley semi-quantitative test

The Berkeley Nitric Oxide Saliva Test Strips (Berkeley, CA, USA) use nitric oxide strips to indicate the level of nitric oxide in saliva; the strips are placed on the surface of the tongue for 10 seconds and then folded, the two sides of the strip are pressed and left in contact for another 5 seconds. The results are expressed semi-quantitatively, depending on the intensity of the color shift, with a mathematical equivalent expressed in μmol/L.

9.4.5. Determination of the antioxidant capacity of saliva samples against the DPPH radical

9.4.5.1. Saliva collection

Saliva samples were collected by a non-invasive method on day 1 and after 10 days of treatment. Next, each saliva sample was centrifuged at 14000 rpm for 10 minutes, and the supernatant was transferred to sterile sealed tubes and stored without freeze-thaw cycles at -20ºC until analysis.

9.5. Result

9.5.1. The variation of salivary NO values evaluated by the Berekley test in periodontitis and peri-implantitis

9.5.1.1. Periodontitis

The initial group of patients from which the study group was selected was represented by a total of 215 oro-dentally clinically evaluated patients. The study group consisted of 55 patients and was divided into two groups as follows:

- ✓ 41 patients represented the study/control group - who performed mouthwash with plant extract obtained from *Lysimachia nummularia L.*
- ✓ 14 control group patients - who performed mouthwash without vegetable extract.

Following the oro-dental clinical examination, according to the presented clinical parameters, 4 subgroups were defined as follows:

- ✓ localized acute periodontitis;
- ✓ acute generalized periodontitis;
- ✓ localized chronic periodontitis;
- ✓ chronic generalized periodontitis.

Preliminary conclusions

1. In 68% of the patients with localized acute periodontitis, an improvement in the clinical oro-dental status was recorded; in 32% of patients no change in the amount of NO was recorded between the two moments of the study.

2. Decreases in NO concentration were observed in all patients with acute generalized periodontitis.

3. In the group of patients with localized chronic periodontitis: in 50% of the patients a decrease in NO concentration was observed and in 50% of the patients there was no change in the amount of NO between the two moments of the study.

4. In patients with chronic generalized periodontitis, the NO concentration value remained constant.

- **Oral hygiene**

Objective

Given the fact that oral hygiene is an extremely important parameter monitored in the monitoring of patients with periodontal disease, we conducted a study in which we tried to identify the possible link between this parameter and the level of NO, as an indicator for evaluating the oxidative stress generated by bacteria periodontal pathogens.

The oral hygiene indices of patients with different forms of periodontal disease were studied.

Preliminary conclusions

1. At the end of the experiment, on the 11th day of the study, it was observed that in 85.36% of the patients of the control group, the value of NO decreased, in this percentage, patients with poor oral hygiene and patients with good oral hygiene had the highest share.

2. In 14.63% of patients, the concentration of NO remained unchanged between the two moments of the experiment, these belonging to the groups with very good and absent hygiene.

- **Smoking**

Objective

Since it is unanimously accepted by specialists in the field that smoking is an important risk factor in the initiation and maintenance of periodontal disease, we conducted a study in which we tried to identify the possible link between NO values and smoker/non-smoker status.

9.5.1.2. Peri-implant

The study group was represented by 102 patients, with an approximately equal weight between the sexes (54 women; 48 men), aged between 25 and 58 years, in which 2-4 implants were inserted on the same mandibular hemiarcade.

The study group consisted of 6 patients randomly chosen from the group of those with favorable evolution and the 10 patients with unfavorable evolution, respectively with mucositis and peri-implantitis. On the occasion of this first evaluation of the patients, the first determination of the NO (nitric oxide) value was also made, after which those in the mucositis and peri-implantitis group performed lavage twice a day for 10 days with the solution containing extract *Lysimachia nummularia L.*, according to the work protocol.

The NO values obtained in patients with the two conditions: mucositis and peri-implantitis, for the two moments of the evaluation are presented in the thesis.

Preliminary conclusions

1. Given the fact that there are differences with little statistical significance between the NO values quantified in patients with a favorable evolution and in those with mucositis 10 days after washing with the test solution, we can say that this had beneficial effects on the evolution of the patients in the diagnosed group with mucositis.

2. The persistence of statistically significant differences regarding NO values in patients with peri-implantitis compared to those with a favorable evolution, shows that in the case of peri-implantitis the tested solution has no effect.

3. There is a high correlation between the depth of the peri-implant groove in patients with peri-implantitis and the value of NO, so we can say that NO can be useful in assessing the clinical status in this group of patients with a higher degree of unfavorable evolution.

4. Due to the content rich in secondary metabolites with proven antibacterial, antioxidant and anti-inflammatory effects, the solution containing *Lysimachia nummularia L* extract can be used as a therapeutic adjunct in mild complications that may occur after the insertion of dental implants.

9.5.2. The results obtained in the antioxidant capacity test

In patients who were part of the control group who did not use the mouthwash solution with plant extract, the percentage of inhibitory activity did not vary.

The statistical processing of the data shows that there is a statistically significant difference between the averages of the researched categories at the 2 times day 1 of the study and day 11 of the study in patients who performed mouthwash with a solution with extract obtained from *Lysimachia nummularia L.*

Preliminary conclusions

1. The results of the assessment of the antioxidant capacity of saliva against the DPPH radical show that there are statistically significant differences between the 2 determinations for each of the types of periodontitis, with the mention that patients with localized acute periodontitis showed the best evolution after performing the lavage with *Lysimchia nummularia L.*

2. Patients with very good oral hygiene responded best after mouthwash with *Lysimchia nummularia L.*

3. Patients with periodontal disease, non-smokers showed the best DPPH inhibitory activity

4. The results obtained show that all groups of patients taken into the study, depending on the level of periodontal damage, hygiene level and smoking/non-smoking status, responded positively, in varying degrees, to the action of the extract with *Lysimchia nummularia L.,* improving the antioxidant properties of saliva.

ORIGINALITY AND INNOVATIVE CONTRIBUTIONS OF THE THESIS

I appreciate that the doctoral thesis has the following elements of originality:

1. Identification and quantification of phenolic compounds by the HPLC method, respectively gallic acid in *Lysimachia radix*, the presence of 3-O-methylgallic acid and ferulic acid in all the studied parts of the *Lysimachia nummularia L.* plant, caffeic acid in *Lysimachia flores* and trans-resveratrol in the plant products *Lysimachia radix* and *Lysimachia herba* - data that have not been mentioned in the specialized literature until now.

2. The thesis is original through the novelty elements related to the **description of the botanical** particularities of the species *Lysimachia nummularia L.* present in the national area, adapted to the geoclimatic conditions of our country.

3. Another element of originality is related to the description of **the physico-chemical properties** of *Lysimachia nummularia L.* extracts and the qualitative and quantitative identification of secondary metabolites as of particular importance in maintaining the homeostasis of the human body.

4. The thesis is also original by demonstrating and evaluating in vitro the **antibacterial and antioxidant properties** of the extracts obtained from *Lysimachia nummularia L.*

5. In vivo evaluation of the antibacterial properties of the species *Lysimachia nummularia L.* in the context of serious oro-dental diseases with a high incidence both nationally and worldwide, namely **periodontal disease, peri-implantitis.**

IMPACT OF THE RESULTS OBTAINED

I appreciate that the results obtained can have a special impact in several areas, as follows:

1. The secondary metabolites identified in the extracts obtained from *Lysimachia nummularia L.,* can optimize the treatment of some **oro-dental diseases**, respectively in the mild forms of periodontitis and peri-implantitis.

2. The obtained results support the possibility of their use in order to obtain pharmaceutical formulations, namely a **pharmaceutical product type - mouthwash**, useful in diseases of the oro-dental cavity generated by oxidative stress in the presence of bacterial species.

3. The results obtained regarding the macroscopic and microscopic study of the species *Lysimachia nummularia L.* will contribute to the development of knowledge in the field of botany.

4. Secondary metabolites, polyphenolic phytocompounds known for their therapeutic potential, qualitatively and quantitatively identified in *Lysimachia nummularia L.* extracts will enrich **knowledge in the field of pharmacognosy**.

5. Identifying the antibacterial properties of the extracts obtained from *Lysimachia nummularia L.,* can contribute to the global efforts to solve problems related to antibiotic resistance;

expanding and deepening the research from this doctoral thesis could be a starting point in **obtaining some pharmaceutical formulations complementary to antibiotics**.

GENERAL CONCLUSIONS

1. The plant products *Lysimachiae herba, Lysimachiae radix* and *Lysimachiae flores* show marked antioxidant properties correlated with the content in total polyphenols and phenolic compounds contained.

2. Since the polyphenols are soluble in ethanol, it was deduced that the optimal methodology for obtaining the dry extract consists in maceration for 14 days with 40% ethanol, a time that does not affect the stability of the polyphenols.

3. *Lysimachia nummularia L.* extracts have antibacterial effects both on reference strains and on strains of bacteria isolated from pathological products.

4. The most effective extract proved to be the one extracted from the aerial part of the plant, both for reference strains and for strains isolated from pathological products.

5. The three extracts demonstrated the absence of antifungal effects, both on reference strains and on strains isolated from pathological products.

6. Following the use of the mouthwash solution with plant extract, from *Lysimachia nummularia L.*, the increase in the antioxidant capacity of saliva was demonstrated in patients with various forms of periodontitis in smokers and non-smokers, both in those with poor oral hygiene and those with good hygiene.

7. Increased values of NO in saliva can be associated with periodontal disease and peri-implantitis, conditions in the pathogenesis of which oxidative stress plays an important role.

8. The rapid semi-quantitative Berkeley test for testing the level of oxidative stress by quantifying NO is a simple and non-invasive method that can be easily used in the dental office as a marker for the evaluation of patients with inflammatory processes in the oro-dental cavity.

9. The mouthwash solution made with Lysimachia nummularia L. extract can be used to maintain the antioxidant capacity of saliva so that the destructive processes of soft and hard processes in the context of periodontitis and mucositis can be prevented.

10. The results of this study support the continuation of research in order to obtain a standardized pharmaceutical product with wide use in the practice of dental medicine.

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