“OVIDIUS” UNIVERSITY OF CONSTANȚA

DOCTORAL SCHOOL OF MEDICINE

DOCTORAL FIELD OF RESEARCH: DENTAL MEDICINE

2020-2021

,,PHARMACOLOGICAL, PHYTOCHEMICAL STUDIES AND APPLICATIONS OF *LYTHRUM SALICARIA* L. SPECIES IN OPTIMIZING METHODS FOR OBTAINING MUCOADHESIVE MEMBRANES’’

Abstract of PhD Thesis

PhD Coordinator,

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Motto and dedication,

"Nature resembles us, education distinguishes us" (Confucius)

For my daughter **Sofia-Elena**,
Any DREAM becomes a reality after a lot of work, perseverance, confidence in your own strength and with the support of angels ... so don't forget to DREAM, to BELIEVE in yourself and your angels ... because YOU can transform THE DREAM IN REALITY.
I love you my ladybug!

And for Primary Pharmacist **Mihaela-Carmen Bâte**,
My fairy-godmother, who always watches over me from heaven and who passed on to me, in addition to her holy name, the love for this noble profession.
You will always remain in our souls!

My gratitudes,

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L plant., purple loosestrife, confirmed the identity of the species taken into account and helped me to the microscopic cross-section interpretations.

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CONTENTS

KEYWORDS: *LYTHRUM SALICARIA* L., LYOPHILIZED AQUEOUS EXTRACT, CHITOSAN, MUCOADESIVE MEMBRANES, *ARTEMIA SALINA* L., ANTIBACTERIAL ACTIVITY, GENOTOXICITY, HEMOLYTIC ACTIVITY.

INTRODUCTION

It is known that plants cannot defend themselves by fleeing or using active weapons when attacked by herbivores, be they molluscs, worms, insects or vertebrates. Under the attack of bacteria, the human species benefits from defense based on the innate and acquired immune system. Such an immune system does not exist in plants. However, the plants are over 400 million years old on this planet and have survived, although they have been attacked by herbivores and bacteria. Thousands of structurally different secondary metabolites have apparently evolved during plant development, as a means for plants to defend themselves against herbivores and bacteria, fungi and viruses [1-5]. Some secondary metabolites also serve to attract pollinating animals and insects or to protect against ultraviolet rays. From the point of view of evolutionary pharmacology, the secondary metabolites of plants represent an interesting library of bioactive compounds filtered by natural selection, which have been used by humans to treat infections and other health problems, or as spices, perfumes, poisons for arrows, toxins and pesticides [6]. Thus, the capitalization of plants led to the emergence of the first form of medicine, namely traditional medicine or phytotherapy.

Plants have always played an essential role in human life, being able to improve or treat various simple or severe diseases. Nowadays we notice a strong tendency to integrate traditional medicine in modern medicine and to introduce on the market along with synthetic pharmaceuticals a large number of products of herbal origin. This increased interest in returning to natural resources and making the most of them was the basis for choosing this theme. Poor oral hygiene, incomplete or incorrect treatment and the abuse of antibiotics have led to the development of bacterial resistance with the emergence of multidrug-resistant bacterial strains in the current pharmaceutical market and the more difficult eradication of pathogenic bacterial species.

Lythrum salicaria L. is a species found under the name of purple loosestife or fairy flower in the Romanian flora, known worldwide since antiquity for its beneficial astringent and hemostatic properties in cases of diarrhea, hemorrhoids and hemorrhages [7-8]. *Lythri herba* plant material was registered in the European Pharmacopoeia in 2001, and according to its monography, the quantitative standardization of *Lythri herba* is based on the determination of the total tannin content, which should be at least 5% on

the dry mass [9]. Previous studies performed on the *Lythri herba* plant material showed that it has antioxidant, anti-inflammatory, anti-nociceptive and hemostatic activity [10-11], in vitro antibacterial and fungistatic activities [7, 12-13], on animal models has hypoglycemic effects [8] and has modulatory effects on the composition of the intestinal microbiota through its postbiotic metabolites (urolithines) [14].

Knowing the therapeutic value of the *Lythri herba* plant extract, the current study aims to achieve a pharmaceutical form with applicability in the pathology of the oral cavity. There is a growing scientific interest in the development of biodegradable films through basic and easy methods. Biopolymer films have become a very popular choice due to many different advantages. Biopolymers are biodegradable natural polymers, used in regenerative medicine, in implantable materials, in drug delivery systems ("carriers") or as artificial tissues for tissue engineering. Natural polymers such as cellulose, chitin, chitosan, gelatin and alginate are widely used in all medical fields [15 - 17].

In order to make a pharmaceutical form, chitosan was taken into account, known as a cationic biopolymer indispensable in its use as a carrier of therapeutic substances, due to its non-toxic, biodegradable, biocompatible, antibacterial and antioxidant nature [18]. At the same time, the aspects of abundant availability and low cost of chitosan were taken into account, attractive aspects for various fields in the food, medical and pharmaceutical industry [19]. Some of the most recent studies evaluate chitosan-based membranes in which plant extracts [20] or their secondary metabolites have been incorporated [21-22], precisely due to the very good ability of chitosan to form membranes [23].

Another important property of chitosan is mucoadhesivity, explained by its ability to interact with negatively charged mucins by electrostatic attraction [24], because chitosan is the only polysaccharide in the world positively charged by the presence of amino groups in its chemical structure [19]. Another great advantage of chitosan is its wide applicability in various fields of dentistry, starting from prevention and reaching the top branch, namely oral and maxillofacial surgery. [25].

Given the above presented, the purpose of the study was to make membranes from weakly acidic solutions of standard chitosan in which the *Lythri herba* lyophilized aqueous extract was incorporated in different concentrations. Subsequent studies tested the biocompatibility, cytotoxicity and genotoxicity of the *Lythri herba* aqueous extract, as well as the hemolytic capacity of these standard chitosan membranes impregnated with the aqueous extract of the plant species used.

CURRENT STATE OF KNOWLEDGE

This part of the doctoral thesis includes a chapter of overview of the plant species *Lythrum salicaria* L., purple loosestrife. We collected data from the literature starting from the history of therapeutic uses of the *Lythrum salicaria* L plant species, its botanical particularities, chemical composition and pharmacological properties.

PERSONAL CONTRIBUTION

This part of the thesis includes eight chapters of multidisciplinary research, in which information and working methods from pharmacognosy, microbiology, toxicology and genetics fields were completed. The personal part presented studies such as:

1. pharmacognostic analysis, qualitative and quantitative chemical analysis of the *Lythri herba* plant material,
2. cytotoxicity analysis of *Lythri herba* plant extracts,
3. applications of lyophilized aqueous extract - obtaining mucoadhesive membranes by combinations of *Lythri herba* extract with chitosan biopolymer,
4. study on antibacterial and antifungal activities for *Lythri herba* lyophilized aqueous extract and membranes,
5. genotoxicity evaluation of the *Lythri herba* aqueous extract by the SOS-CHROMO TEST method,
6. hemolytic activity evaluation of standard chitosan membranes with *Lythri herba* aqueous extract.

STUDY 1. PHARMACOGNOSTIC ANALYSIS, QUALITATIVE AND QUANTITATIVE CHEMICAL ANALYSIS OF THE *LYTHRI HERBA* PLANT MATERIAL

1.1.Obtaining the plant material

The plant material (floral tips of the *Lythrum salicaria* L. species) was harvested in August 2019 from the Pirates' Inn area, Năvodari, Constanța County, where the plant grows spontaneously. After harvesting, the plant material was cleaned of impurities, then a few specimens were kept in 70% alcohol to make cross sections, and the rest of the material was dried at room temperature, protected from sunlight, to be used in future phytochemical analyzes.

1.2.Macroscopic and microscopic examination of the *Lythri herba* plant material

1.2.1. Material and method

The working material is represented by the floral tops (Lythri herba) of the species Lythrum salicaria L., which was examined with the naked eye or with a magnifying glass (macroscopic analysis) and with the help of the Novex-Holland microscope (microscopic analysis in sections and powder) of the stem, leaf and flower.

1.2.2. Results and discussions

Both macroscopic examination and microscopic examination identified elements specific to the aerial part of the *Lythri herba* plant material, thus establishing the identity of the plant species used, namely *Lythrum salicaria* L., an aspect also confirmed by Associate professor Dr. Arcuș Mariana, head of the Department of Pharmaceutical Botany of the Faculty of Pharmacy from Constanța.

1.3.Qualitative chemical analysis of the *Lythri herba* plant material

1.3.1. Material and method

The extractive solutions of the *Lythri herba* plant material were subjected to the identification reactions common and / or specific to each group of active principles followed and to the separation method such as Thin Layer Chromatography (TLC).

1.3.2. Results and discussions

The result of the **identifications** shows that the *gallic tannins* are present, due to the appearance of blue in the reaction with iron (III) chloride and anthocyanosides by the appearance of red in the specific reaction of color change depending on pH. The results of the **Thin Layer Chromatography** (TLC) **method** revealed compounds from the *phenolic acid* class, the *flavonoid* class and the *tannin* class.

1.4.Quantitative chemical analysis of the *Lythri herba* plant material

1.4.1. Loss on drying determination of *Lythrum salicaria* L.

- Material and method

Dried and crushed flower tips represent the plant material and the method used for this preliminary determination is provided both in the European Pharmacopoeia 10.0th edition [106] and in the Romanian Pharmacopoeia X edition [115].

- Results and discussions

The value of the loss by drying determination is very important, because all subsequent determinations will relate to the mass of dried plant material. The value obtained in this study was within the limits imposed in the European Pharmacopoeia 10.0th edition [106].

1.4.2. **Total ash determination**

- Material and method

Dried and crushed flower tips of *Lythrum salicaria* L. represent the plant material and the method used for this preliminary determination is provided in the European Pharmacopoeia 10.0th edition [106].

- Results and discussions

The value obtained falls within the standards of the *Lythri herba* monography from the European Pharmacopoeia 10.0th edition of the percentage of total ash [106].

1.4.3. **Determining water-soluble substances and 50% methanol study methods**

- Material and method

The dried and pulverized floral tops of the *Lythri herba* plant material was used in this determination and the method performed is according to the Romanian Pharmacopoeia X edition [115].

- Results and discussions

As the focus point of the research is the obtaining of an oral product and given the small difference between water-soluble substances and 50% methanol-soluble substances, it is imperative the use of water as the main extraction solvent.

1.4.4. **Determination of active compounds contents of the *Lythri herba* fluid aqueous extract**

1.4.4.1. **Determining total polyphenols content**

- Material and method

The dried and pulverized floral tops of the *Lythri herba* plant material was used in this determination and we performed the Folin-Ciocâlteu adapted method according to the European Pharmacopoeia 10.0th edition [106].

Principle of the method: it represents a colorimetric method based on determining the intensity of the blue coloration of molybdenum oxides formed by the reduction by polyphenols of the reagent Folin-Ciocâlteu (phosphomolybdowolframic acid).

- Results and discussions

The value obtained from total polyphenols determination is much higher than the standard value from the Romanian Pharmacopoeia X edition for the *Cynarae folium* plant material monography and justifies further research with the determination of the content in other compounds [115].

1.4.4.2.**Determining tannins content**

- Material and method

The dried and pulverized floral tops of the *Lythri herba* plant material was used in this determination and we performed the Folin-Ciocâlteu method according to the European Pharmacopoeia 10.0th edition [106].

The principle of the method: the intensity of the blue coloration of the molybdenum oxides formed by the reduction by polyphenols of the Folin-Ciocâlteu reagent (phosphomolybdowolframic acid) is determined before and after the absorption of the tannins on the skin powder.

- Results and discussions

The value obtained from tannins determination falls within the standards of the European Pharmacopoeia 10.0th edition of at least 5 % tannins for the *Lythri herba* plant material and justifies further research with the determination of other natural compounds of interest [106].

1.4.4.3.**Determining antocyanosides content**

- Material and method

Dried and crushed flower tips of *Lythrum salicaria* L. represent the plant material and the method used for this determination is a colorimetric method provided in the European Pharmacopoeia 10.0th edition [106].

Principle of the method: the intensity of the red coloration of the oxonium salt formed by anthocyanosides in an acid medium is determined. The intensity of the red coloration is directly proportional to the anthocyanoside content expressed in cyanidol 3-glucoside.

- Results and discussions

The value obtained by anthocyanosides falls within the standards of the European Pharmacopoeia 10.0th edition provided in the monograpy of *Myrtilli fructus recens* plant material, a species considered to be the standard for the content of at least 0.3% of anthocyanins expressed in cyanidol 3-glucoside [106].

1.4.4.4.**Determining polyphenolcarboxylic acids content**

- Material and method

Dried and crushed flower tips of *Lythrum salicaria* L. represent the plant material and the method used for this determination is a colorimetric method provided in the European Pharmacopoeia 10.0 edition [106].

Principle of the method: the method is based on the property of polyphenolcarboxylic acids and their derivatives to form nitrosoderivatives with nitric acid, which is spontaneously isomerized to

isonitrosoderivatives (oxymes) which, due to their weak acidity, react with alkaline solutions to form red compounds.

- Results and discussions

The value obtained does not fall within the ranges of the European Pharmacopoeia 10.0th edition provided for the *Plantaginis lanceolatae folium* plant material, considered a standard plant species for a content of at least 1.5% in total o-dihydroxycinnamic acid derivatives expressed in acetoside [106].

1.4.4.5. Determining crude polyholosides content

- Material and method

The working material is represented by the *Lythri herba* fluid aqueous and alcoholic extracts and I used the gravimetric method for this determination.

- Results and discussions

Gravimetric analysis showed a very high content of crude polyholosides, the largest amount being present in the fluid aqueous extract, and the lowest amount was highlighted for the 70 % ethanolic extract.

1.4.5. Determination of antioxidant capacity of the *Lythri herba* fluid aqueous extract

- Material and method

The plant material used is represented by the dried and crushed floral tips of the plant Lythrum salicaria L., from Dobrogea, Romania, and the method used is the DPPH method (1,1'-diphenyl-2-picryl-hydrazyl).

- Results and discussions

The results obtained represent the arithmetic means of three measurements performed; the values obtained when capturing free radicals DPPH show a directly proportional relationship between the antioxidant capacity and the concentration of solutions. The fluid aqueous extract had the scavenger capacity of high DPPH radical, and the IC_{50} value (inhibitory concentration for 50 % of DPPH radicals) of the *Lythri herba* plant material obtained from the *Lythrum salicaria* L. species from Romania falls within the ranges found in the international literature.

1.4.6. Determination of active compounds contents of the *Lythri herba* lyophilized aqueous extract

- Material and method

The floral tips of the *Lythrum salicaria* L. species dried and crushed beforehand represent the plant material used, and the working method for obtaining the lyophilized aqueous extract is represented by

the concentration of the extract with the BUCHI R-215 rotary evaporator, followed by cryophilic drying with CHRIST ALPHA 1-2 B lyophilizer.

The working methods and calculation formulas used for the determination of loss on drying, content in total polyphenols, tannins and crude polyholosides have been set out above in sub-chapters 1.4.1., 1.4.4.1., 1.4.4.2. and 1.4.4.5. To weigh the loss on drying, three measurements were performed (P1, P2, P3), and the final value is the arithmetic mean of the values obtained, at which the standard deviation was calculated.

- Results and discussions

At the end of the lyophilization process, a significant amount of lyophilized aqueous extract of *Lythri herba* was obtained, which was transferred into a sealed glass vial and stored into a desiccator until the following determinations. The results of the determinations did not show significant differences in the active compounds identified from the two extracts; the advantages of stability over time and easy maneuverability, which the lyophilized aqueous extract has, support its use in subsequent determinations and tests.

STUDY 2. CYTOTOXICITY ANALYSIS OF *LYTHRI HERBA* PLANT EXTRACTS

2.1. Working hypothesis

The working hypothesis is that both the aqueous and the alcoholic extract of the *Lythri herba* plant material, from Dobrogea, has no cytotoxic potential. The aim of this study is to capitalize on the results obtained in various biomedical applications.

2.2. Material and method

The working material consisted of aqueous and alcoholic extractive solutions of the *Lythri herba* plant material, and the working method was represented by the BSLA (Brine shrimp lethality assay) test, performed according to the EBPI protocol (Environmental Bio-Detection Products Inc) with modifications regarding the control sample and according to toxicity protocol (Artoxkit M).

2.3. Results and discussions

According to Clarkson's toxicity criterion, non-toxicity is observed for both the aqueous and the alcoholic extract of *Lythri herba* plant material, and the mortality of the nauplii is correlated with the dose (concentration) and with the time of action of the extract on the tested organism. Induced toxicity values, expressed by lethal concentration (LC_{50}), were below the toxicity levels, according to Clarkson's toxicity criterion. The results of direct observations on larvae exposed to different concentrations of the evaluated extracts highlight the differences in manifestation at the cytological level. The highlighted

effect (in the case of BSLA the quantified effect is mortality) indicates the interaction between cellular mechanisms and the complex composition of the extract.

STUDY 3. APPLICATIONS OF LYOPHILIZED AQUEOUS EXTRACT - OBTAINING MUCOADHESIVE MEMBRANES BY COMBINATIONS OF *LYTHRI HERBA* EXTRACT WITH CHITOSAN BIOPOLYMER

3.1. Working hypothesis

The working hypothesis of this study was that membranes of standard chitosan 1 % solubilized in acetic acid and lactic acid combined with aqueous extract of *Lythrum salicaria* L. could have biological properties, which would allow their use in the practice of dentistry.

3.2. Material and method

The lyophilized aqueous extract of the *Lythri herba* plant material and the chitosan biopolymer were used as working materials. We used an adaptation of the working method of Al-Dhubiab et al (2016) [190] with the calculation formula of Nair et al (2013) [191] to highlight the degree of hydration of membranes and epifluorescence microscopy (Optika Microscopes Italy, Series B-350, model B-353LD2) to highlight the arrangement of the extract in the chitosan matrix.

3.3. Results and discussions

Membranes with mucoadhesive properties were for the first time obtained from the combinations of standard chitosan 1% solubilized in acetic acid and lactic acid with the lyophilized aqueous extract of different concentrations of the *Lythri herba* plant material. The degree of hydration (%) of both types of membranes highlighted their ability to maintain constant hydration, stability and flexibility over time. Epifluorescence microscopic examination confirmed the presence of chitosan in membranes, as it has the ability to emit auto-fluorescence at specific wavelengths and gave clear details regarding the structural uniformity of membranes. The lack of uniformity in the membrane structure obtained by solubilizing chitosan in 1 % lactic acid in which the *Lythri herba* aqueous extract was incorporated led to the decision to continue the research only on the standard chitosan membrane solubilized in 1% acetic acid.

STUDY 4. STUDY ON ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES FOR *LYTHRI HERBA* LYOPHILIZED AQUEOUS EXTRACT AND MEMBRANES

4.1. Working hypothesis

The working hypothesis of this study consisted in demonstrating the antibacterial and antifungal properties of the lyophilized aqueous extract and the membranes obtained from chitosan and *Lythri herba* aqueous extract.

4.2. Material and method

The bacteriological study proposed in the doctoral thesis was divided into two stages using as an working method an adaptation of the diffusimetric method. In the first stage, the aqueous lyophilized extract of the *Lythri herba* plant material was tested on lyophilized, stabilized and viable reference bacterial and fungal strains of the American Type of Culture Collection (ATCC). The second stage of the study consisted in testing the lyophilized aqueous extract and its membranes with the chitosan biopolymer on bacterial strains isolated from pathological products taken from the oral cavity, pharyngeal cavity and external auditory canal (softened dentin from tooth decay, purulent secretion from post-extraction alveolitis, otic secretion, gingival fluid taken from a wearer of fixed prosthetic works).

4.3. Results and discussions

The results of the first stage of the study showed that the 5% aqueous extract of the *Lythri herba* plant material showed antibacterial properties against the bacterial species *Enterococcus sp*., *Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa* and antifungal activity for both strains tested, *Candida albicans* and *Candida paralopsis*. In the second stage of the study, the results demonstrated moderate antibacterial activity of the 5 % aqueous extract for *Streptococcus mutans*, high antibacterial and antifungal activity for *Staphylococcus aureus* and *Candida albicans* and total resistance for *Escherichia coli.* Thermostation at 37 ° C for 24 hours resulted in dehydration of the chitosan membranes and the possibility was created that the active principles would not diffuse across the membrane into the culture medium; this observation is supported by the fact that the 5 % aqueous extract of the *Lythri herba* plant material tested separately has a demonstrated antibacterial effect.

STUDY 5. GENOTOXICITY EVALUATION OF THE *LYTHRI HERBA* AQUEOUS EXTRACT BY THE SOS-CHROMO TEST METHOD

5.1. Working hypothesis

The working hypothesis is that the *Lythrum salicaria* L. aqueous extract does not show genotoxicity. Like the cytotoxicity studies, the genotoxicity test on the aqueous extract obtained from the flower tips of the *Lythrum salicaria* L. species and combined with chitosan solutions were performed for the first time.

5.2. Material and method

The SOS Chromo test kit developed by EBPI (Environmental Bio-Detection Products Inc), standard chitosan solutions, oligochitosan (chitosan with small polymer chains), *Lythri herba* plant extract of different concentrations and combinations between chitosan and extract and UV-VIS spectrophotometer Jasco V-630 were used to assess genotoxicity.

5.3. Results and discussions

The results were evaluated quantitatively (directly or indirectly) using spectrometry, as they have a higher safety profile than the qualitative evaluation. The results obtained for the aqueous extractive solutions of the *Lythri herba* plant material, as well as for the combinations between them with chitosan demonstrated the lack of genotoxicity by induction factor values below 1.5.

STUDY 6. HEMOLYTIC ACTIVITY EVALUATION OF STANDARD CHITOSAN MEMBRANES WITH *LYTHRI HERBA* AQUEOUS EXTRACT

6.1.Working hypothesis

The working hypothesis of this chapter is that membranes made by combining 1 % standard chitosan in acetic acid with aqueous *Lythri herba* extract do not produce hemolysis. The aim of the study is to demonstrate the absence of the hemolytic property of standard chitosan membranes solubilized in acetic acid by incorporating the aqueous extract of *Lythrum salicaria* L.

6.2.Material and method

To evaluate the hemolytic activity were used: the Biomaterial Hemolytic Assay test kit developed by EBPI (Environmental Bio-Detection Products Inc) and membranes made of 1 % standard chitosan with the aqueous extract of the *Lythri herba* plant material (0.5, 1, 2 , 3 and 4 g / L). The principle of the method is based on the release of hemoglobin, at the direct contact between the biomaterial and the erythrocyte suspension, which can be measured spectrophotometrically.

6.3.Results and discussions

The hemolytic effect of membranes composed of 1 % standard chitosan in acetic acid and *Lythri herba* extract is inversely proportional to the increase in the concentration of the extract in the combination. The weakest hemolytic effect has the membrane obtained from the combination of the chitosan biopolymer with the 4 g/L lyophilized aqueous extract of the *Lythri herba* plant material.

GENERAL CONCLUSIONS

1. The macroscopic examinations performed could confirm that the plant used in our study was *Lythrum salicaria* L., and the identity of the *Lythri herba* plant material was thus established.
2. The microscopic examinations carried out in sections brought other details regarding the structure of the *Lythrum salicaria* L. plant from Dobrogea, besides those described in the specialized literature.
3. Microscopic characterization of the *Lythri herba* powder revealed elements specific to the aerial part, namely covering trichomes, anomocytic stomata, fragments of lacunar tissue with calcium oxalate clusters and numerous spherical pollen grains.
4. The qualitative chemical study revealed by specific identification reactions the existence of tannins and anthocyanosides, and the Thin Layer Chromatography (TLC) method revealed pharmacologically active compounds such as gallic acid, rutin, vitexin and hyperoside.
5. The results of this study demonstrate that there are no significant differences in the active compounds identified in the two extracts, and the advantages of stability over time and easy maneuverability of the lyophilized aqueous extract support its use in subsequent determinations and tests.
6. The difference between water-soluble and alcohol-soluble active principles is insignificant, which justifies the use of water as the main solvent for obtaining future extractive solutions.
7. The presence of total polyphenols in high concentration explains the antioxidant properties, and the presence of secondary metabolites confirmed in current research strengthen the importance of *Lythrum salicaria* L. from Romania in phytotherapy.
8. Evaluation of the cytotoxicity of *Lythri herba* extracts using the BSLA test demonstrated the absence of cytotoxic effects at 24 hours of both extracts, and the observed microscopic cytological changes correlated with the exposure time and the concentration of *Lythrum salicaria* L. plant extracts.
9. Knowing the aggressiveness of the synthetic solvents used in the extraction methods of active compounds from plants, we directed the research to the principle of "green technology", using water as the main solvent and to choosing a biopolymer for possible medical applications.
10. The results of the present research led to the obtaining membranes from *Lythri herba* aqueous plant extract and 1 % standard chitosan solubilized in both acetic acid and lactic acid for the first time.
11. Epifluorescence microscopic characterization of membranes identifies the chitosan biopolymer by its auto-fluorescent capacity and clearly highlights the details of the different arrangement by encapsulation of the *Lythri herba* aqueous plant extract by the chitosan matrix.

12. The study performed on chitosan membranes solubilized in acetic acid and lactic acid in which the aqueous extract of *Lythri herba* incorporated showed a similar and constant hydrating capacity.
13. The lack of uniformity in the structure of the membrane obtained by solubilizing 1 % standard chitosan in lactic acid and incorporating *Lythri herba* aqueous extract, visible both to the naked eye and microscopically, led to the decision to continue the research only on 1 % standard chitosan solubilized in acetic acid membrane.
14. The results of the bacteriological study demonstrated the existence of the antibacterial effect of the aqueous extract of *Lythri herba* on the reference strains from the group of *Gram-positive* cocci, *Gram-negative* bacilli and *Candida* strains.
15. The 5 % *Lythri herba* aqueous extract has antibacterial and antifungal effects comparable to the antibiotics and antifungals used in current practice.
16. The highest level of sensitivity of the 5 % *Lythri herba* aqueous extract proved to be on a bacterial species with established resistance, namely *Pseudomonas aeruginosa* and on *Candida paralopsis.*
17. The 5 % *Lythri herba* aqueous extract shown to have a moderate antibacterial effect on the cariogenic bacterial flora represented in this study by *Streptococcus mutans.*
18. Inhibitory effects on bacterial and fungal species specific to the oral cavity produced by the 5 % *Lythri herba* aqueous extract meet the limitation of the expansion of the phenomenon of resistance to antibiotics and antifungals, supporting the prospect of using this extract as a basis for obtaining pharmaceutical products use in the practice of dentistry.
19. The aqueous extractive solutions of the *Lythri herba* plant material and the aqueous extract solutions combined with standard chitosan have no genotoxic effects, and these results support the possibility of using these extracts in therapeutic practice.
20. The hemolytic effect of membranes composed of 1 % standard chitosan in acetic acid and *Lythri herba* extract is inversely proportional to the increase of the concentration of the extract in the combination, the membrane with the highest concentration of extract has no hemolytic properties is recommended as an alternative in dentistry.

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