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PHD THESIS SUMMARY

ADVANCED PHYSICAL, HISTOLOGICAL AND BIOCHEMICAL STUDIES IN CUTANEOUS MYCOSES

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CONTENT

ADVANCED PHYSICAL, HISTOLOGICAL AND BIOCHEMICAL STUDIES IN CUTANEOUS MYCOSES

THE OBJECTIVES AND PURPOSE OF THE WORK.....11
PART I. STAGE OF KNOWLEDGE….....12
CHAPTER 1. EMBRYOLOGY, ANATOMY, HISTOLOGY AND FUNCTIONS OF THE SKIN.....12
1.1 Embryological skin organ.....12
1.2 Anatomy of the skin.....13
1.3 Histology of the skin..... .14
1.3.1. Epidermis.....14
1.3.2 Dermo-epidermal junction..... 17
1.3.3 Dermis.....18
1.3.4 The hypodermis.....19
1.4 Vascularization of the skin.....20
1.5 Innervation of the skin.....20
1.6 Appendages of the skin.....22
1.6.1 Sweat glands.....22
1.6.2 Sebaceous glands.....23
1.6.3 Mammary glands.....23
1.6.4 The hair.....23
1.6.5 The neils.....24
1.7 Skin functions….....24
1.7.1 Keratinogenesis….....24
1.7.2 Melanogenesis.....25
1.7.3 The formation of the hydrolipidic film.....25
1.7.4 Secretory function..... 25
1.7.5 Pilogenesis.....26
1.7.6 Thermoregulation function.....26
1.7.7 Neuroexteroceptor function.....27
1.7.8. Neurovasomotor function..... …….28
1.7.9 Metabolic function..... 28
1.7.10 Resistance function, elasticity and plasticity.....29
1.7.11 Endocrine function..... 29
1.7.12 Psychosocial function.....29
1.7.13 Immunological function..... 29
1.7. 13.1The innate immune system 31
1.7.13.2 Adaptive immunity.....31
CHAPTER 2. CUTANEOUS MYCOSES ……35
2.1 General characterization of fungal species involved in mycoses……35
2.2 Classification of mycoses..... .42
2.2.1 Superficial mycoses.....42
2.2.2 Deep mycoses (systemic)..... 43
CHAPTER 3. DIAGNOSIS AND TREATMENT OF MYCOSES…….45

3.1 Diagnosis of infections caused by dermatophytes and yeasts……..…..45
3.2. Treatment of mycoses…….....63
3.2.1 Pharmacological bases……..…..64
3.2.2 The main classes of antifungal substances…….…….....67
3.2.3 Transmission electron microscopy (TEM)……...78
PART II. PERSONAL CONTRIBUTIONS
INTRODUCTION 81
CHAPTER 4. MATERIAL AND METHODS..... 82
4.1 Epidemiological study…….82
4.2 Evaluation of the antifungal efficiency of some natural extracts and some natural/synthetic compounds……82
CHAPTER 5 RESULTS AND DISCUSSION…….……...…….....87
5.1 Retrospective Epidemiological Study on Risk Factors and Variations in Clinical and Paraclinical Parameters in Patients with Mycosis, Regarding the Favoring and Determining Factors of Fungal Infections **(Moroianu O-N.,** Popescu N-D., Petcu L.C, Gurgaș L., Roșoiu N., 2019)……...…….....88
5.2 Experimental Research on the Effectiveness of Natural and Synthetic Substances on Strains of Yeasts and Filamentous Fungi Involved in Human Mycoses…….....108
5.2.1 Experimental Study on the Inhibitory Effect of Some Substances Applied in Various Dilutions on *Candida Albicans* Cultures **(Moroianu O-N,** Popescu N-D & Rosoiu N., 2019)..... 108
5.2.2 Comparative Study of the Action of Some Natural Substances on *Candida Albicans* and *Aspergillus*)**(O-N Moroianu,** Popescu N-D & Roșoiu N.,2020)……....114
5.2.3 Study on the Action of Some Natural Products on *Candida Albicans* Cultures **(Olimpia-Nicoleta Moroianu**, Nelu-Doru Popescu , Alina Raluca Ursu, Natalia Rosoiu, 2022)..... 124
5.2.4. Study of the Action of Some Chemical and Natural Substances on *Candida Albicans* cultures **(Olimpia-Nicoleta Moroianu**, Nelu-Doru Popescu, Alina Raluca Ursu, L. Gurgaș, N. Rosoiu, 2022)..... 146
5.2.5 Study on the Action of 9 Natural Products and the Colloidal Cu Solution on *Candida Albicans* Cultures **(Olimpia-Nicoleta Moroianu**, Nelu Doru Popescu, Alina Raluca Ursu Tony Laurențiu Hangan, Natalia Rosoiu, 2022) 169
5.2.6 Study with 15 Natural Substances and Colloidal Platinum Solution on *Candida Albicans* **(O.-N. Moroianu**, N.-D. Popescu, A. R. Ursu, N. Rosoiu, 2022).....184
5.2.7 Changes Revealed by Transmission Electron Microscopy (TEM) in Cultures of *Candida Albicans* Inoculated with Oregano Oil **(Olimpia-Nicoleta Moroianu**, Nelu-Doru Popescu, Constanța Stefanov, Nicolae Dobrin, Natalia Rosoiu, 2022)..... 208
5.2.8 Study on the Internal and External Factors Involved in the Pathogenesis of Conditions Caused by Yeasts of the Genus and Species Candida **(Moroianu Olimpia-Nicoleta**, Popescu Nelu-Doru, Roșoiu Natalia, Olgun Azis, 2023) 216
General conclusions..... 227
SELECTIVE BIBLIOGRAPHY..... 236
Scientific Papers Published During the Doctoral Inthernship……..……...255
Papers Presented at National and International Scientific Events and Published in the Form of an Abstract.....…….……256
Participation in National and International Scientific Congresses.....257

OBJECTIVES AND PURPOSE OF THE WORK

The paper aims to study the etiopathogenesis of skin-mucosal candidiasis, as well as the evaluation of new prophylactic and therapeutic strategies for these conditions.

In Part I. The State of Knowledge, the present paper aims at an update and a systematization of the information related to the etiopathogenesis of skin-mucosal candidiasis.

In Part II. Personal Contributions, the paper presents the materials, methods and results obtained in 9 studies, through which this thesis contributes to the development of new useful strategies in the prophylaxis and therapy of mycoses.

In some cases I have used active substances (oils, tinctures or other products) in different concentrations (10%, 20%, 30% etc.) for a favorable effect.

In the paper, we also aimed to formulate observations on the changes in the cultures of *Candida albicans* and *Aspergillus spp.*, as a result of the sequential intervention with several natural and synthetic substances under conditions of constant temperature and varied time intervals (24 h, 48 h, 72 h, 96 h and 120 h).

The main objectives of the work consisted in:

1. Carrying out a retrospective study aimed at the analysis and correlation of clinical, paraclinical and epidemiological parameters in a significant sample of patients diagnosed with mycoses;

2. Evaluation of the fungicidal or fungistatic action of some natural and synthetic substances, as potential new, useful strategies in the prophylaxis and treatment of candidiasis.

PART II. PERSONAL CONTRIBUTIONS

INTRODUCTION

Skin diseases in general, and ringworm in particular, have a great impact on people's mood.

These diseases can cause special problems in the way people relate because they are visible.

As Commel said:

"The skin organ represents the monumental facade of the human body."

To better understand mycoses, we conducted several studies. Some were aimed at finding remedies in such conditions, and others indicated collateral diseases in such cases, etc.

I looked for natural remedies and more, in the inhibition of *Candida albicans*, as well as other mycoses such as Aspergillosis.

We investigated biochemically and hematologically two batches of cases: candida/mycosis. We made comparative graphs regarding the living conditions (rural/urban), sex, age, in a comparative study between the two groups, regarding the hospitalized patients during the specified/analyzed period.

We obtained optical microscopy and TEM images, and finally drew specific conclusions.

The studies were carried out at several famous medical laboratories in Constanța: "Provita 2000 Medical Center", "Iowemed-Medicover Medical Center", Clinical

Department of Dermatovenerology within the Emergency County Clinical Hospital "Sf. Apostol Andrei" and the Electron Microscopy Laboratory of the Faculty of Medicine in Constanța.

CHAPTER 4. MATERIAL AND METHODS

For the realization of the doctoral thesis I used various materials and working methods, corresponding to different studies in research.

4.1. Epidemiological study - A number of 65 observation sheets of patients diagnosed with different forms of "candidosis" and a number of 40 observation sheets of patients diagnosed with different forms of "other mycoses".

The main passport data were entered into an SPSS 23 statistical program, obtaining comparative graphs related to living conditions (rural/urban), sex, age, biochemical and hematological investigations.

4.2. Evaluation of the antifungal efficiency of some natural extracts and some natural/synthetic compounds

A study was conducted to determine the action of plant extracts on mycelial cultures of fungi such as *Candida albicans* and *Aspergillus.*

We have conducted experimental studies using plant extracts (oils and tinctures).

I took samples from a person who had Aspergillus cellular detritus (at the auricles), then mixed them with certain dilutions of essential oils or tinctures and applied them to Petri dishes with Saboraud culture medium. I placed these plates in a thermostat at 37ºC for 24 h.

The substances were applied to cultures of Candida and cultures of *Aspergillus* (taken from the pinnae).

The plates were incubated for 24 h at the thermostat (Moroianu et al., 2019).

The substances used in the study had concentrations of 10% and 20%.

A research study sought to highlight the antifungal action of some natural substances on *Candida albicans* cultures. The observations were made using different concentrations of the substances, which acted under constant temperature conditions. At different time intervals, we investigated the inhibition of candida development, by using essential oils such as: tea tree (*Malaleuca alternifolia*), oregano (*Origanum Vulgare*), black cumin (*Bunium Persicum*), coriander (*Coriandrum Sativum*), etc., marigold tincture *(Calendulae Officinalis*) and a capsule of graviola (*Annona Muricata*), over a period of 48 h; for 72 hours I used essential oils, namely: sage (*Salvia Officinalis*), mint (*Folium menthae/ Mentha piperita)*, geranium (*Geranium Cinereum*), aloe (*Aloe Vera),* thyme (*Satureja Hortensis*), tincture of propolis, tincture of plantain (*Plantago Major*), chamomile tincture (*Flores Chamomillae*), colloidal solutions (of super-concentrated Ag ions, of Au and Ag ions), Apa Doamnei, Bitter (from 50 plants with Ganoderma - *Ganoderma Lucidum*-, respectively drops Swedish), apple cider vinegar and wine vinegar 9% concentration.

In one study we used natural and chemical substances.

We used the Sabouraud standard culture medium, on which we seeded samples of *Candida albicans*, from a calibrated assortment (Moroianu O-N et al., 2018), called ATCC. We put the plates in the thermostat, at the standard temperature of 37ºC (Buiuc D., Neguț, M., 2008).

I used for the experiment:

a. Apple cider, apple cider vinegar, iodized salt and citric acid.
b. Two solutions: sodium bicarbonate and iodine tincture

c. Acetic acid samples: I. 1 ml acetic acid + 5 ml SF
II. 2 ml acetic acid + 5 ml SF
III. 3 ml acetic acid + 5 ml SF
IV. 4 ml acetic acid + 5 ml SF

d. Magnesium oil, wormwood oil, Siberian cedar oil, cedar oil – 200 µl (substance); I added 800 µl physiological serum.

e. Ginger oil (*Zingiber officinale*), lemon oil (*Citrus limon*), frankincense oil (*Thymanea),* neem oil (*Azadirachta indica).*

The chemical compounds that were analyzed in the study were prepared in different dilutions.

We then analyzed the size of the diameter of the zones of inhibition and lysis of mycelial colonies at different time intervals (at 24 hours, 48 hours, 72 hours (Moroianu O-N et al., 2018) and at, 96 h).

There were two studies of 120 h in which we also obtained images with the help of the optical microscope. We prepared the substances analyzed in the study, in different dilutions; then, we analyzed the size of the diameter of the zones of inhibition and lysis of the mycelial colony at different time intervals (at 24 hours, at 48 hours and 72 hours, 96 h. and 120 h. respectively).

For this, we used the Sabouraud standard culture medium, on which we seeded samples of *Candida albicans*, from a calibrated assortment (Moroianu O-N et al., 2018), called ATCC.

I put the plates in the thermostat, at the standard temperature of 37ºC

We carried out several experiments during 96 hours, using both natural products (essential oils, tinctures, etc.) and chemical products (ex: sodium bicarbonate, citric acid, iodized salt), in order to be able to follow the action of chemical compounds on crops of *Candida albicans.*

We used a staining method (with methylene blue 3% and gentian violet 3%) to highlight the inhibition/or not of candida, under the action of the different products in the study.

The sequence of operations for the examination of a colored smear in order to identify bacteria:

A smear is made, that is, the biological material is spread in a very thin layer on a glass slide;

It is fixed in a low flame (sometimes with methyl alcohol) then,

The coloring is started according to the following method.

Staining with methylene blue 3% (it is the simplest method):

• the smear made on the slide from the pathological product and then fixed to the flame, is covered with the 3% methylene blue solution;

• then wash with tap water;

• after drying it is examined under a microscope.

With the help of this method, extracellular bacterial germs, intracellular germs and encapsulated germs can be highlighted (Alexandru M et al., 2020).

Similarly, we proceeded with gentian violet 3% in another study, where we colored the slides, which highlighted the inhibition of candida development over a certain period.

Both optical microscopy experiments were conducted over a period of 5 days.

A particular case was performed on *Candida albicans*, collected from a 26-year-old patient who presented to the medical office with a condition in the vaginal area. In this experiment we used a candida identification system called Candifast.

For transmission electron microscopy (TEM) we took 2 samples from the Sabouroud ATCC medium, seeded with *Candida albicans* samples; the environment was initially treated with essential oil of oregano (*Origanum vulgare*); I inserted the plate into the thermostat, at the standard temperature of 37ºC; this was previously held for 72 h at the thermostat to be able to investigate the antifungal effect of oregano oil over time (Moroianu O-N et al., 2018).

After these 72 h, in which *Candida albicans* was inhibited by oregano oil, we sectioned the necessary TEM material.

The samples taken were further processed appropriately so that they could be viewed and analyzed under the transmission electron microscope.

Figure 15. *The Tecnai T12 electronic microscope produced by FEI, which is located at the Faculty of Medicine of the "Ovidius" University in Constanta*

The samples from the experimental variants were processed by the modified JASTROW method and analyzed from an ultrastructural point of view by transmission electron microscopy by going through more many stages of work. Thin sections were double stained with uranyl acetate and lead acetate, after which they were examined with a Tecnai T12 Microscope produced by FEI, which belongs to the Faculty of Medicine of the "Ovidius" University of Constanta since 1998. Materials and methods used are described in detail in “Part II. Personal Contributions" of the PhD Thesis to Experiments.

CHAPTER 5. RESULTS AND DISCUSSION

In this paper, we studied different aspects of the etiopathogenesis, treatment and prophylaxis of mycoses, with a particular focus on *Candida albicans*, by carrying out a retrospective epidemiological study in hospitalized patients with mycoses and evaluating some alternative, natural and chemical remedies, in combating mycoses, in main cause of

Candida albicans infections, given the prevalence of this yeast from young ages to the elderly, due to low immunity and internal and external factors.

The retrospective study was carried out on a group of 105 patients diagnosed with various mycoses (65 with candidiasis and 40 with other types of mycoses), hospitalized between May 2017 and February 2018 in the "Sfântul Apostol Andrei" County Emergency Clinical Hospital in Constanta (department dermatology).

The relevant demographic and clinical data were statistically analyzed using the statistical program SPSS 23, allowing the evaluation of the correlations of different factors such as living environment (rural/urban), sex, age, biochemical and hematological parameters with fungal infections.

We compared the action of some natural products in different concentrations (10%, 20%, 30% and 40%, etc.) on cultures of *Candida albicans* and *Aspergillus sp.*

The observations were made using different concentrations of active substances, under conditions of constant temperature and varied time intervals, respectively 24 h, 48 h, 72 h, 96 h and 120 h.

Thus, in the present paper we evaluated the antifungal effect of some essential oils such as: geranium (*Geranim robertianum),* tea tree (*Melaleuca alternifolia)*, oregano *(Origanum vulgare)*, black cumin (*Bunium persicum*), cloves (*Syzygium aromaticum*), coriander *(Coriandrum Sativum*), ginger (*Zingiber officinale)*, lemon (*Citrus limon),* sea buckthorn oil for internal use (*Hippophae rhamnoides),* cold-pressed mustard oil, etc., tinctures: calendula (*Calendulae officinalis),* iodine 2% , of mouse tail (*Achillea milefolium),* propolis, burdock (*Arctium lappa),* artichoke *(Cynara scolymus),* licorice, i.e. roots of *Glycyrrhiza glabra,* etc. and some capsules, such as: graviola (*Annona muricata)* for a period of 48 h, turmeric & pepper (*Curcuma longa & Piper nigrum)* for 120 h, etc.), colloidal solutions (of superconcentrated Ag ions, Au & Ag , Pt, Cu), as well as other commercial products (vinegar of various types and concentrations, bitters, etc.).

5.1 Retrospective Epidemiological Study on Risk Factors and Variations in Clinical and Paraclinical Parameters in Patients with Mycoses, Regarding the Favoring and Determining Factors of Fungal Infections (Moroianu O-N., Popescu N-D., Petcu L.C, Gurgas L., Rosoiu N., 2019)

The study aims to highlight the differences in the main statistical parameters between two groups of patients diagnosed with mycoses, while also tracking the frequency of association with various other conditions. A number of 65 observation sheets of patients diagnosed with various forms of "candidiasis" and a number of 40 observation sheets of patients diagnosed with various forms of "other mycoses" were selected. The main passport data were entered into an SPSS 23 statistical program, obtaining comparative graphs related to living conditions (rural/urban), sex, age, biochemical and hematological investigations. Important data were also obtained by comparing the association with other diagnoses specified in the medical documentation.

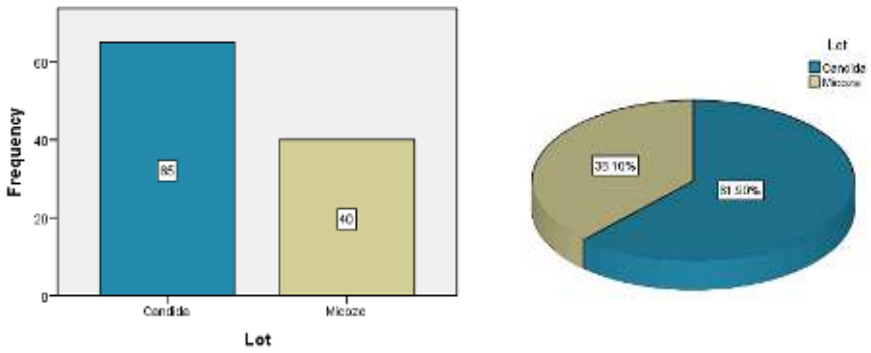


Figure 16. *Column (left) and Pie (right) representation of the distribution of patients by group (Candida/Mycoses) 105 patients were enrolled in the study, 65 with Candida (61.9%) and 40 with Mycoses (38.1%)*

From a statistical point of view, we can say that the two batches are NOT numerically balanced, that is, the two categories do NOT appear with equal probability ($p = 0.015 < \alpha = 0.05$ - One sample CHI-Square Test).

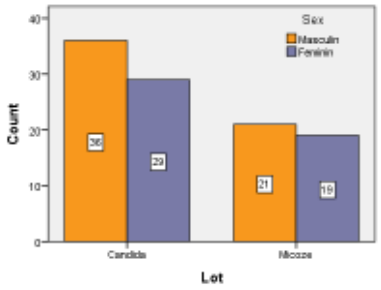


Figure 17. *Column representation of the distribution of patients from the Candida/Mycosis groups according to gender Regarding the distribution of patients from the Candida/Mycosis groups according to gender, we note that, out of a total of 105 patients, 57 (54.3.6%) are men, of which 36 (34.3%) have candida, and 21 (20%) have mycoses, and 48 (45.7%) are women, of which 29 (27.6%) have candida and 19 (18.1%) have mycoses*

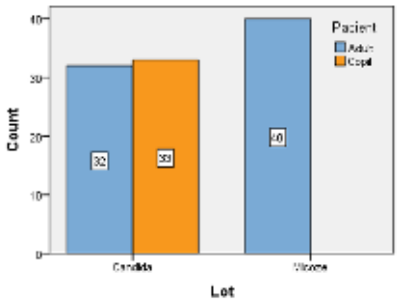


Figure 19. *Representation - Column of the distribution of patients from the Candida/Mycosis groups according to patient (adult/child) In the group of patients with Mycosis, only adults aged between 24 and 87 years were enrolled with an average age of 64.36 years and a standard deviation of 12.83 years*

In the group of patients with Candida, both adults and children were enrolled. The adults were aged between 24 and 92 years with an average age of 58.84 and a standard deviation of 19.12 years. The children were aged between 0 and 9 years with an average

age of 2.18 and a standard deviation of 2.16 years. Between the average hemoglobin/erythrocyte concentration (CHEM) values corresponding to the two candida/mycosis groups (Mcandida = 34.04 [g/dL] and Mmycosis = 33.05 [g/dL]) it is found that there are statistically significant differences: t = 2.868 ; df = 103; Mdiff = 0.990 [g/dL]; p = 0.005 < α = 0.05; 95% confidence interval of the difference between the mean values = (0.305, 1.675) [g/dL]. Between the average ALT values corresponding to the two candida/mycoses groups (Mcandida = 18.78 [U/L] and Mmycoses = 18.62 [U/L]) it is found that there are NO statistically significant differences: t = 0.074; df = 103; Mdiff = 0.156 [U/L]; p = 0.942 > α = 0.05; 95% confidence interval of the difference between the mean values = (-4.054, 4.366) [U/L]. Between the average AST values corresponding to the two candida/mycoses groups (Mcandida = 28.70 [U/L] and Mmycoses = 22.52 [U/L]) it is found that there are statistically significant differences: t = 2.539; df = 103; Mdiff = 6.173 [U/L]; p = 0.013 < α = 0.05; 95% confidence interval of the difference between the mean values = (1.351, 10.995) [U/L].

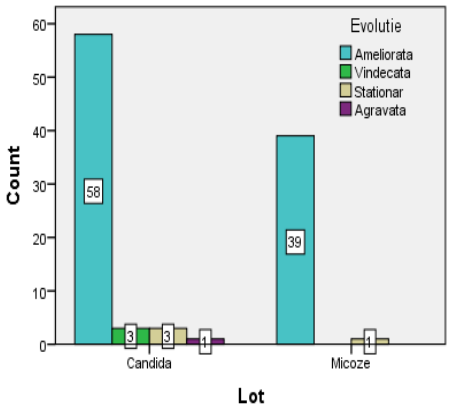


Figure 38. *Representation Column of the distribution of patients from the Candida/Mycoses groups according to evolution*

Table 6. *Distribution of patients in the Candida/Mycoses groups according to evolution*

	Candida (n = 65)		**Mycoses (n = 40)**				
	ni	**%**	**ni**	**%**	**Chi-squared**	**p**	**Differences**
Improved	58	89.23	39	97.50	1.374	0.2410	No
Cured	3	4.62	0	0.00	0.603	0.4375	No
Stationary	3	4.62	1	2.50	0.0006	0.9791	No
Aggraveted	1	1.54	0	0.00	0.0602	0.8061	No

CONCLUSIONS

The prevalence of the male sex is observed in both groups (34.3%, respectively 21%; compared to 27.6%, respectively 18.1% in the female sex). In both groups, the prevalence of coming from the urban environment is observed (52.4%, respectively 27.6%; compared to 9.5%, respectively 10.5% from the rural environment). Regarding age, in the group of "candidiasis", the majority were children (33% compared to adults 32%), and in the group of "other mycoses", no child was registered. Regarding the biochemical and hematological investigations, I notice significant differences in the average values between the two

groups, as follows: in the "candidiasis" group, the average values of lymphocytes (17.64 versus 11.92 103/uL), platelets (310 .03 vs. 247.07 103/uL), AST (28.70 vs. 22.53 U/I); in the "other mycoses" group, the mean values of eosinophils (1282 versus 675 10^3/uL), serum glucose (104.95 versus 95.64 mg/dl), neutrophils (35.75 versus 29.90 103 /uL), urea (40.24 versus 29.97 mg/dl). Comparing these values, it can be concluded that the changes in biological constants in the case of other mycoses are more important, more serious, than those recorded in candidiasis. Regarding the association with other conditions, it is clearly observed that the group of patients diagnosed with "other mycoses" associates more conditions that may be directly or indirectly related to those mycoses than the group with "candidiasis", as follows: venous insufficiency (40% compared to 1.5 %), hypertension (37% vs. 13.8%), type 2 diabetes (17.5% vs. 7.7%), chronic ischemic heart disease (12.5% vs. 6.2%, obesity (17.5% compared to 3.1%). On the other hand, there is an increase in the association of pneumonia cases in the "candidiasis" group (18.5%), compared to the "other mycoses" group (2.5%). This the last observation can be explained as a consequence of the antimicrobial effect of antibiotics on the saprophytic microbial flora, protective against the development of candida albicans. The association of other mycoses with the conditions exemplified above can be explained by the modification of the immune response to various aggressions that act on the human body once with advancing age.

5.2 Experimental Research on the Effectiveness of Natural and Synthetic Substances on Strains of Yeasts and Filamentous Fungi Involved in Human Mycoses

To test and evaluate the antifungal action of natural extracts and natural/synthetic compounds, we ran a shoulder of 8 studies, with different experiments, using different substances and techniques for each study.

5.2.1 Experimental Study on the Inhibitory Effect of Some Substances Applied in Various Dilutions on *Candida Albicans* Cultures (**Moroianu O-N.,** Popescu N-D., Rosoiu N., 2018)

The study followed the action of external factors on *Candida albicans* cultures. The substances analyzed in the study (sodium bicarbonate, sodium chloride, acetic acid and ethyl alcohol) were prepared in different dilutions and applied by means of absorbent pads, according to the model of how to proceed in the case of antibiograms or fungigrams. Results and discussions We made the dilutions for the substances: sodium bicarbonate, acetic acid, sodium chloride, 90 ºC ethyl alcohol in the following way: - we prepared four dilutions for each substance taken in the study. For sodium bicarbonate ($NaHCO_3$) and table salt (NaCl) I proceeded like this:

For 1000 ml sterile distilled water...10 g sodium bicarbonate, respectively NaCl (1%)

For 500 ml sterile distilled water...1 g sodium bicarbonate, respectively NaCl (0.2 %).

To 250 ml of sterile distilled water...0.5 g of sodium bicarbonate, respectively NaCl (0.1 %).

To 125 ml of sterile distilled water...0.05 g of sodium bicarbonate, respectively NaCl (0.04 %)

For ethyl alcohol CH_3-CH_2-OH of 90 ºC and acetic acid CH_3-COOH 1 M (glacial acetic acid), the dilutions used were:

At 4 ml sterile distilled water...3 ml 90ºC alcohol, respectively 1 M acetic acid

To 3 ml sterile distilled water...2 ml 90ºC alcohol, respectively for acetic acid 1 M

To 2 ml of sterile distilled water... ml of 90ºC alcohol, respectively 1 M acetic acid

To 1 ml of sterile distilled water…0.5 ml of 90ºC alcohol, respectively 1 M acetic acid.

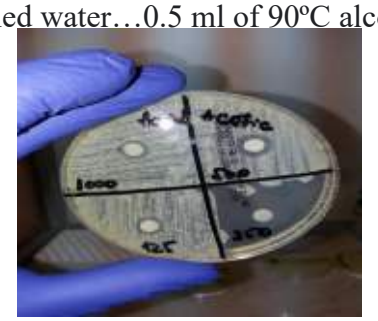


Figure 45. *Petri plate seeded with Candida albicans, on which I applied a washer soaked in glacial acetic acid solution, in four dilutions (concentrations: 25,10%, 23,50%, 28,50%, 28,10%)*

Conclusions

Following these determinations, we found that sodium bicarbonate, ethyl alcohol and sodium chloride did not have the expected inhibitory effect on *Candida albicans* cultures. Only with acetic acid was the inhibitory effect reported (at the concentration of 28,50%). The application of diluted acetic acid, in a concentration of 28.50%, could cause the destruction of *Candida albicans* colonies also in vivo. The study can be extended to many other substances, including plant extracts.

5.2.2 Comparative Study of the Action of Some Natural Substances on *Candida Albicans* and *Aspergillus* (**Olimpia-Nicoleta Moroianu**, Nelu-Doru Popescu, Natalia Rosoiu, 2020)

The study was carried out at the Analysis Laboratory of the "Provita 2000 Medical Center" in Constanța in February 2019 on some fungal cultures.

Experiment no. I

Action of some substances extracted from plants on cultures of *Candida albicans*. The substances subjected to the experimental study were applied to culture plates seeded on Saboraud's medium, with *Candida albicans*.

1. We made the dilutions of 100 µl substance (respective oil)/ 900 µl physiological serum for:

• Essential oils of: Aloe (*Aloe Vera*), eucalyptus (*Eucalyptus Globus*), sage *(Salvia officinalis*), mint (*Mentha piperita*) produced by Aroma Land;

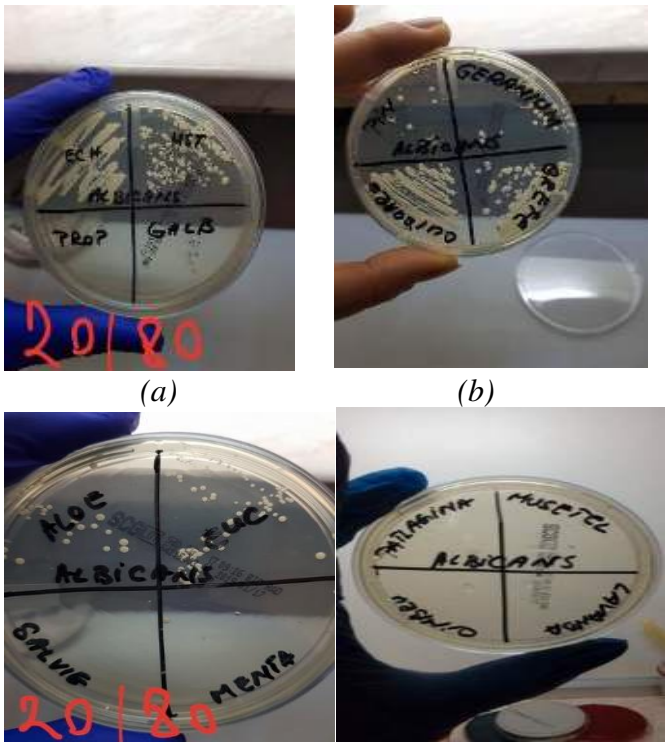
• Tinctures of: echinacea (*Echinacea purpurea*), garlic (*Allium sativum* – garlic bulbs), propolis, calendula (*Calendula officinalis*) from Dacia Plant.

The substances were applied to cultures of Candida / and cultures of *Aspergillus* (taken from the auricles of a patient).

The substances were applied to cultures of Candida/and *Aspergillus* ear mold cultures (taken from pinna). The plates were incubated for 24 h at the thermostat (Moroianu et al., 2019). When applying these substances to cultures for the treatment of *Candida albicans*, with these substances and under these conditions, no changes were observed. I repeated the experiment with the dilutions: 200 µl substance/ 800 µl physiological serum.

I also added the essential oils of: pine (*Pinus Nigra*) and geranium (*Geranium robertianum)*, from Aroma Land, cloves (*Syzygium aromaticum*) from Fares, seeds of grafts (*Citrus Paradisi)* produced by Steaua divina, lavender (*Lavandula Angustifolia*) from Adams, thyme essential oil (*Satureja hortensis L.)* from Solaris, as well as tinctures

of plantain (*Plantago lanceolata folium* – Plantain leaves) and chamomile (*Chamomillae flos* – chamomile flowers) (both tinctures are produced by Hofigal).



(a) *(b)*

(c) *(d)*

Figure 49 (a, b, c, d). Culture plates inoculated with candida albicans on which sage, aloe, eucalyptus, mint, pine, geranium, clove, grafting seeds, thyme, lavender oils were applied in a concentration of 20% and tinctures of echinacea, garlic, propolis, calendula, plantain, chamomile

After 24 hours of thermosetting, the following were observed:

• On the plates where solutions in 10% concentration were applied, no action was noted;

• The plates where solutions in 20% concentration have been applied show sensitivity.

I found the following:

• pine oil and geranium oil determined the partial destruction (about 70%) of the mycelial colonies;

• the oil from seeds of grafts and that of cloves did not produce any change;

• lavender, sage, mint and thyme (essential) oils determined the total destruction of mycelial colonies;

• tincture of plantain, chamomile, calendula and propolis caused the total destruction of mycelial colonies as well.

Candida is sensitive to plantain, chamomile, calendula, propolis (tinctures) and thyme, lavender, sage and mint (oils).

Experiment no. II

The action of some plant extracts on *Aspergillus* cultures In order to highlight the action of some plant extracts on *Aspergillus* cultures, we took samples from a person with *Aspergillus* cellular detritus (at the auricles), after which we mixed them with certain

dilutions of essential oils or tinctures and applied them to Petri dishes with Saboraud culture medium. I put these plates in a thermostat at a temperature of 37ºC for 24 h.

1. I used the following substances:

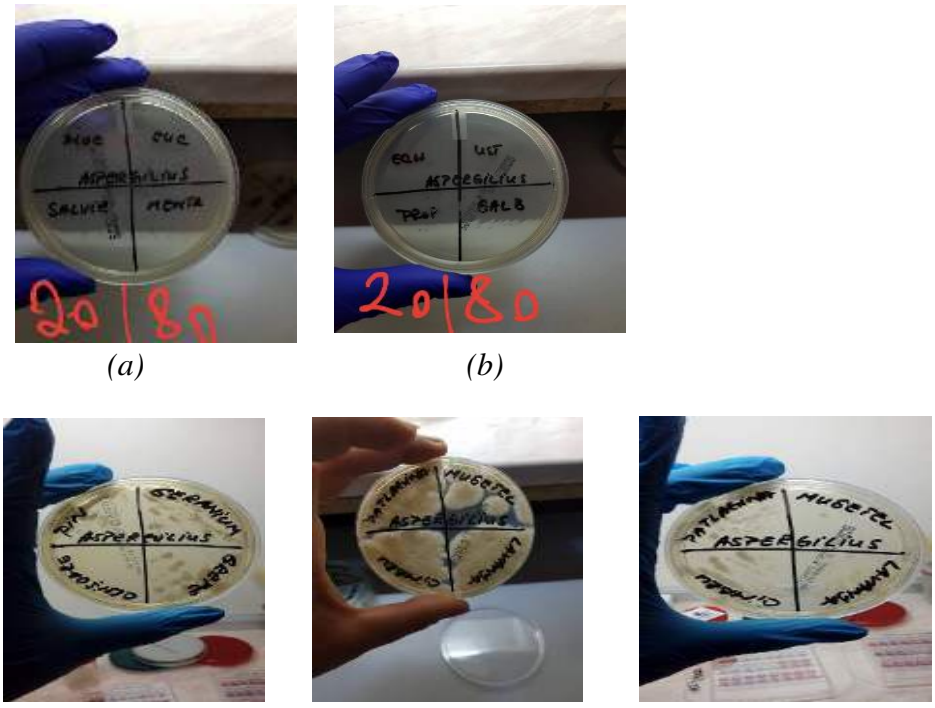
- Aloe (*Aloe Vera*), eucalyptus (*Eucalyptus Globus*), sage (*Salvia officinalis*), mint *(Mentha piperita*) – essential oils 10% (per 100 µl oil + 900 µl physiological serum)
- echinacea (*Echinacea angustifolia, Echinacea purpurea*), garlic (*Alium sativum*), propolis, calendula *(C. Officinalis*) – tinctures

When applying these substances to the cultures for the treatment of *Aspergillus,* no changes were observed.

2. The dilutions of 200 µl substance / 800 µl physiological serum for the previously used substances, to which I added other substances, i.e.:

• essential oils of: pine (*Pinus nigra*), geranium (*Geranium robertianum*), cloves (*Syzygium aromaticum*), grafts (grapefruit seeds), aloe (*Aloe vera)*, eucalyptus (*Eucalyptus globulus*), sage *(Salvia officinalis*), mint (*Mentha piperita*), thyme (*Satureja hortensis L.*), lavender (*Lavandula Angustifolia*), ginger.

• tinctures of: plantain (*Plantago lanceolata folium)*, chamomile (*Chamomillae flos)*, echinacea, garlic (*Alium sativum*), propolis, calendula (*C. Officinalis)*, applied to culture media (Moroianu O-N et al., etc.).



(a) *(b)*

(c) *(d)* *(e)*

Figure 52 (a, b, c, d, e) *Plates inoculated with Aspergillus on which oils in 20% concentration or tinctures were applied Eucalyptus oil, Aloe, sage, mint, cloves, as well as garlic tinctures, propolis, calendula and echinacea demonstrated 100/100 fungicidal action on Aspergillus colonies after a 24-hour incubation*

Discussions

I found that in the case of applying some oils in a concentration of 20%, namely: clove, aloe, sage, eucalyptus, mint, as well as some tinctures: propolis, echinacea, garlic,

marigolds, a fungicidal action occurs on *Aspergillus* colonies, after 24 h of incubation. This effect was not evident after applying the oil of: thyme, geranium, pine, grafts, ginger lavender or the tinctures of: chamomile and plantain.

Conclusions

The results of the two experiments demonstrate that in the therapy of cutaneous or mucous mycoses with *Aspergillus,* the local application of some products extracted from plants, marketed in the form of oils, in a concentration of 20%, or of some tinctures, in their native state, can be subjected to study.

5.2.3 Study on the Action of Some Natural Products on *Candida Albicans* Cultures (**Olimpia-Nicoleta Moroianu,** Nelu-Doru Popescu, Alina Raluca Ursu, Natalia Rosoiu, 2022)

In the present study we proposed a series of experiments, some of which were carried out over 48 hours and others over 72 hours using natural products (essential oils, tinctures, capsules, bitters, etc.) to highlight the action of the substances on *Candida albicans* cultures.

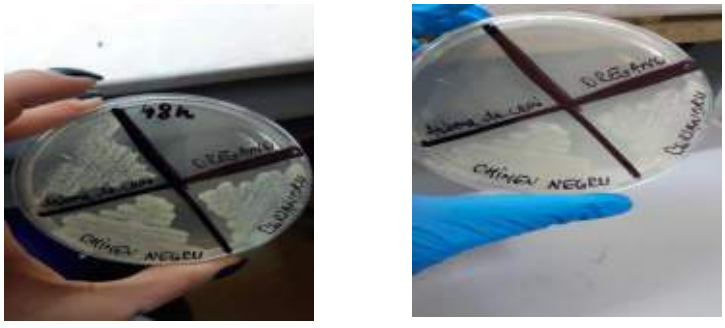
Results

We followed the action on candida of some essential oils such as: tea tree, oregano, black cumin, coriander, rosemary, juniper, marigold tincture and a graviola capsule over a period of 48 hours; also, for the 72 h I used essential oils (sage, mint, geranium, aloe vera, thyme), tinctures (of calendula, propolis, plantain and chamomile), graviola capsules, colloidal solutions (of super concentrated Ag ions, of Au and Ag ions), Lady's Water, Bitter (from 50 plants with ganoderma, respectively Swedish drops), apple vinegar and wine vinegar 9%.

I.48 h experiments

We prepared dilutions of 200 µl substance/800 µl saline.

In this experiment I used tea tree essential oil, oregano, black cumin, coriander, rosemary, juniper and calendula tincture. I also dissolved 1 capsule of graviola (pure extract) in 2 ml of saline. One graviola vegetable capsule contains 5:1 extract of graviola fruit (Annona muricata) - graviola powder - 200 mg. To all this I added 2-3 colonies of *Candida spp.* Mix, leave at the thermostat for about an hour, then from the emulsion obtained, sow it on the Sabouroud plate and put it in the thermostat for 24 hours, after which the result is read. Candida is sensitive to oregano oil and tea tree oil.



(a) *(b)*

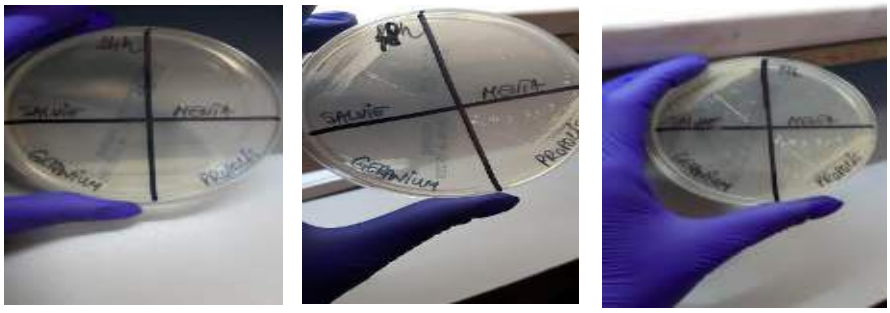
Figure 53 (a, b) *Culture plates seeded with Candida albicans on which oils of: tea tree, oregano, black cumin and coriander were applied at a concentration of 20% at 24 h and 48 The plates were left for a further 24 hours at the thermostat*

At 48 h the result was read: candida was sensitive only to oregano oil.

II**. 72 h experiments**

a. Candida plates with sage oil, peppermint oil, geranium oil and propolis tincture were kept for 72 h at the thermostat. Dilutions of 200 µl substance/800 µl physiological serum were made for the essential oils, and the propolis tincture was used in the product concentration.

After 24 hours, all 4 substances had an inhibitory effect on candida cultures.



(a) *(b)* *(c)*

Figure 55 (a, b, c) *Culture plates seeded with Candida albicans on which sage, mint, geranium oils were applied in a concentration of 20%, as well as propolis tincture at 24h, 48h and 72h*

At 48h it was re-reading and found that only geranium oil and a little peppermint oil were inhibitory, and also after 72 hours of seeding.

b. I used lavender oil, eucalyptus oil, plantain tincture, as well as chamomile tincture. In the first 24 h, lavender and eucalyptus managed to eliminate candida, but after 48 h and 72 h, respectively, candida reappeared.

c. In the experiment we had marigold tincture, aloe vera essential oil and graviola (2 capsules of pure extract). We made dilutions of 200 µl substance / 800 µl physiological serum; the tincture was used directly from the bottle and the graviola capsules were dissolved in 4 ml saline.

d. On culture media with Candida I put thyme (*Satureja hortensis L.*) essential oil, super concentrated Ag^+ silver ion solution (colloidal Ag 30 ppm), Au and Ag ion solution with nanometer sized particles in structured and distilled water (15 ppm, water distilled and structured). I took 5 ml of Ag ion solution (super concentrated) and approx. 5 ml of Au and Ag ion solution (4 puffs each) exactly as they were in the bottles (without other dilutions). A colony of *C. albicans* was dissolved in the stock solutions and then seeded on Sabouraud medium and left at the thermostat for 72 h. For the thyme oil, I took 200 µl of substance and added 800 µl of physiological serum. With thyme oil, silver ions (Ag^+) and gold and silver ions, the sensitivity was seen from the beginning and later, i.e. 48 h and 72 h, respectively.

e. Lady's Water (therapeutic water rich in Ca and Mg)

In 2 ml of product called "Lady's water" I put a colony of *Candida albicans* until a concentration of 0.5 McFarland was formed. From this I sowed on Sabouraud medium. After 3 days I could see that the result is null; the product used was ineffective.

f. In 2 ml of substance (Bitter from 50 plants produced by Dacia Plant, Swedish drops from BANO and apple vinegar) I dissolved 2-3 colonies of candida; also, the 2 Para Fight pills from Coral, were dissolved in 4 ml of physiological serum, I added 2-3 candida colonies;

I put them on a plate and left them in the thermostat for 3 days to read the result. After the first 24 hours, it could be observed that the bitters obtained from 50 plants, as well as the Swedish drops, had an inhibitory effect on candida cultures. After 72 hours from the start of the experiment, the two types of bitters, Swedish drops and bitters from 50 plants with ganoderma (Romanian product made by Dacia Plant) obviously inhibited candida cultures.
g. Wine vinegar of concentration 9% I dissolved a colony of *Candida albicans* in physiological serum until the McFarland concentration was reached. We made 3 dilutions of commercially available wine vinegar.
Dilution 1/2 I mixed 0.5 ml of the dilution made initially with 0.5 ml of vinegar. Dilution 1/4 I took 0.25 ml of the dilution obtained initially over which I added 0.75 ml of vinegar. Dilution 1/8 To 0.125 ml of the dilution I added 0.875 of vinegar from the wine. All the dilutions thus obtained were applied to Sabouraud medium seeded with *Candida albicans* and were thermostated at a temperature of 37°C. After 24 hours, an inhibitory action could be observed at the last dilution (1/8), but after 42 h, respectively 72 hours, the result is null; candida is back.

Discussions

The substances that were analyzed in the study were prepared in different dilutions and applied by means of absorbent pads as in the case of fungigrams or antibiograms. We then analyzed the size of the diameter of the zones of inhibition and lysis of the mycelial colony at different time intervals (at 24 hours, at 48 hours and 72 hours) (Moroianu O. - N. et al., 2018). For 24 h, lavender, eucalyptus, sage and propolis tincture oils had an inhibitory effect on *Candida albicans*. The peppermint oil worked for a little more than 24 h, but the oregano, thyme and geranium oils, the 50-herb bitters with ganoderma, as well as the swedish drops, the super concentrated Ag solution and the solution with Ag and Au ions. In the case of experiments carried out during 48 h, an inhibitory action of tea tree and oregano oils was observed for the first 24 h compared to black cumin and coriander oil present on the same plate, later, after 48 h, only oregano oil with inhibitory action. Rosemary oil, juniper oil, calendula tincture and capsule containing graviola (1 dissolved capsule) did not work. For the following experiments (of 72 h), after 24 h of seeding, sage, mint, geranium and propolis tincture oils removed candida, at 48 h and 72 h only geranium and mint oils are slightly effective against of sage oil and propolis tincture. Lavender oil is effective for 24 hours compared to eucalyptus oil, plantain and chamomile tinctures.
Concentrated Ag solution, Ag and Au ion solution with nanometer-sized particles and thyme essential oil help remove mycosis; the product called "Lady's water" is ineffective in treating candida. The bitter from 50 plants with ganoderma, the Swedish drops and less apple cider vinegar inhibited candida in the first 24 hours, compared to the "parafight" product (from Coral); then only the bitter from 50 plants with ganoderma (Romanian bitter from Dacia Plant) and the Swedish drops produced by BANO maintained their ability to inhibit candida. For wine vinegar, the 1/8 dilution helped to stop the development of candida for the first 24 h, after which candida grew in the 4 dilutions proposed for analysis. We can combine thyme essential oil with lavender, geranium oil with sage and mint, propolis tincture with chamomile, aloe vera oil with tea tree oil or possibly add mint oil.
Certainly, wine vinegar consumed frequently would help in stopping the multiplication of candida or used in baths or by washing the infested place with wine vinegar solution, respectively 1/8 dilution as we found in the study. Colloidal Ag and Ag and Au ion

solutions were very effective, as well as 50 herb bitters and Swedish drops, which means we can confidently use them to treat *Candida albicans.*

Conclusions

At the end of the study, we noticed an inhibitory action, persistent after 48 and 72 hours on *Candida albicans* cultures, determined by the essential oils of oregano (*Origanum vulgare*), thyme (*Satureja hortensis L.),* geranium (*Geranium robertianum*), the bitters of 50 ganoderma plants, swedish drops, super-concentrated Ag solution and Ag and Au ion solution.

In the case of mint oils, eucalyptus (*Eucalyptus globulus*), sage (*Salvia officinalis),* lavender *(Lavandula angustifolia*), propolis tincture, apple cider vinegar and 9% wine vinegar solution (1/8 dilution) the antifungal effect was for a short period of time, the colonies regenerating. For this reason, it would be advisable that the treatment with these substances be repeated at certain time intervals, to successfully combat *Candida albicans*.

5.2.4 Study of the Action of Some Chemical and Natural Substances on *Candida Albicans* Cultures (**Olimpia-Nicoleta Moroianu**, Natalia Rosoiu, Alina Raluca Ursu, 2022)

In the present study, we carried out several experiments during 96 hours, using both natural products (essential oils, tinctures, etc.), as well as chemical (sodium bicarbonate, citric acid, iodized salt), in order to monitor the action of chemical compounds on *Candida albicans* cultures.

Results

We investigated the action on candida of some essential oils of: ginger (*Zingiber Officinale),* lemon *(Citrus Limon*) (from Hypericum Impex SRL), frankincense *(Thymanea*) and neem (*Azadirachata Indica*), cedar (*Cedrus)* and Siberian cedar (*Pinus Sibrica)*, wormwood (*Artemisia Absinthium)*, magnesium oil, tincture of iodine 2%, solutions with sodium bicarbonate, apple cider, apple vinegar, citric acid, as well as four solutions of acetic acid 1M, over a period of 96 hours. In the first phase, I made the necessary dilutions, after which I added 2-3 colonies of *Candida spp*. I mixed and left them at the thermostat for about an hour. Later, from the emulsion obtained, I seeded on the Sabouroud plate and introduced the samples to the thermostat for 24 h, 48 h...96 h, reading the result for each case.

Figure 67 (a) *Petri dishes seeded with Candida albicans with the chemical compounds used in the study (2% iodine tincture, sodium bicarbonate solutions, apple cider, apple cider vinegar, acid citric as well as the four solutions of 1M acetic acid, oils of: ginger, lemon, frankincense and neem, cedar and Siberian cedar, wormwood, magnesium) in a concentration of 20 %)*

Experiment of 96 h (4 days)

a. In the first experiment I used apple cider, apple cider vinegar, iodized salt and citric acid. To prepare the citric acid solution, I dissolved 5 g of the substance (citric acid) in 20 ml physiological serum. Apple cider and apple cider vinegar were used as they were commercially available. The iodized salt solution was prepared from 5 g of iodized salt and 20 ml of saline.

(a) *(b)*

Figure 68 (a, b) *Culture plate seeded with Candida albicans and apple cider, apple vinegar, citric acid and iodized salt at 24 h (a) and 48 h (b)*

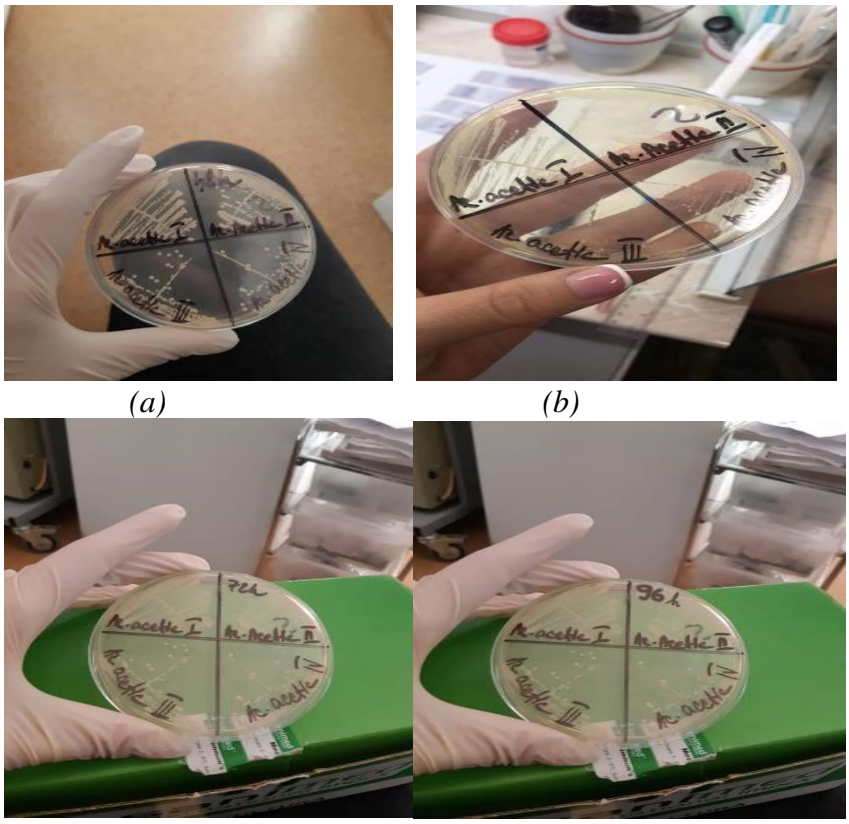
For apple cider and apple vinegar we observed sensitivity in the first 24 h after sowing. After 48 h candida reappears in the case of the 4 substances used in the study; there is sensitivity to apple cider vinegar at 48 h and after, but not completely.

b. Sodium bicarbonate: 5 g/ 20 ml physiological serum, respectively 10 g/ 20 ml physiological serum. Tincture of iodine 2% was used in its native state. For sodium bicarbonate solutions, no action appears against *Candida albicans* colonies during the first 4 days of incubation. The tincture of iodine proved very active on the Candida colonies during the 4 days.

c. Acetic acid 1M For the experiment with acetic acid I prepared in the first phase 4 solutions/samples of acetic acid 1 M (see **Table 8**).

Table 8. Composition of microbiologically analyzed acetic acid samples (used in the study)

No. crt	Samples	Acetic acid1M (ml)	Physiological serum (ml)	Volumetric ratio (acietic acid/ PS)
1.	I	1	5	1/ 5
2.	II	2	5	2/5
3.	III	3	5	3/5
4.	IV	4	5	4/5



(a) *(b)* *(c)* *(d)*

Figure 73 *Culture plate seeded with Candida albicans to which the four solutions of 1M acetic acid were applied at 24 h (a), 48 h, 72 h and 96 h, respectively*

24 h after Candida seeding a certain sensitivity is observed for solutions/samples II, III and even IV of 1 M acetic acid, but not enough. The acetic acid used for the 48h, 72h and 96h, specifically samples III and IV has a degree of fungicidal action, but *Candida albicans* does not disappear permanently.

d. Siberian cedar oil (crude cold-extracted seed oil - for internal use), cedar essential oil, magnesium oil, wormwood oil -200 µl oil /800 µl physiological serum After the 24 h, to the oil cedar essential appears a degree of sensitivity, unlike Siberian cedar oil for internal use. The four substances used in the experiment: Siberian cedar oil, cedar oil, wormwood oil and magnesium oil were inactive on *Candida albicans*, for the 4 days of the experimental study.

e. Ginger, lemon, frankincense and neem oil-200 ml oil/800 ml physiological serum In the case of: ginger, frankincense and lemon oils, a fungicidal action occurs in the first 24 hours. After 48 h, the action is maintained only with the ginger oil, a little with the lemon oil and a little with the frankincense oil. With ginger oil, candida did not reappear 72 h after seeding; also, with ginger essential oil, fungicidal action is also observed after 96 h, compared to neem oil, respectively, frankincense oil and lemon oil. Lemon oil and frankincense oil show a certain fungicidal action from the beginning, until the end of the 96 h; however, candida continued to re-emerge in a very small amount with these substances 2 days after seeding.

Discussions

The dilutions of 200 µl substance/800 µl physiological serum were made for the essential oils, and the applied iodine tincture had a concentration of 2%. Apple cider and apple vinegar were sampled as they were commercially available. Iodized salt, sodium bicarbonate and citric acid were used in solutions (in liquid state). For acetic acid CH_3-COOH, 1M, we made 4 different samples for the proposed study (see **Table 8).** These substances were either in the form of tinctures, or in the form of essential oils or other commercially available solutions. Over the solutions I added some colonies (2, 3) of *Candida Spp.* Apple cider and apple vinegar applied in their native state show fungicidal action in the first 24 h, compared to the iodized salt solution and the citric acid solution used in the study.

With tincture of iodine, an obvious fungicidal action is observed against *Candida albicans* and less with apple cider vinegar after 4 days after inoculation with *Candida albicans*. The sodium bicarbonate solutions used did not show fungicidal action against *Candida albicans*; proved ineffective at the concentrations used. In contrast, iodine tincture maintained its antifungal effect for the 4 days. Ginger, frankincense and lemon oil show a degree of fungicidal action in the first 24 hours compared to neem oil, as well as 48 hours after inoculation with *Candida albicans*. Also, after 72 h and 96 h, sensitivity appears to ginger oil, frankincense and a little to lemon oil compared to neem oil, which was not effective against candida. It was observed that the four substances used in the experiment: Siberian cedar oil, cedar oil, wormwood oil and magnesium oil, were ineffective on *Candida albicans*, during the 4 days of the study, with the exception of cedar oil, which for 24 h showed fungicidal action against candida. Oil of lemon and frankincense showed some sensitivity from the beginning until the completion of the 96 h, but candida continued to reappear with these substances at the concentrations used. Apple cider vinegar used in food can be a prophylactic, but also curative remedy in the treatment of Candida.

Candida albicans shows sensitivity for the first 24 h to the ginger, frankincense and lemon oils and even more, for the 96 h from seeding to the ginger oil, for dilutions of 200 µl substance/800 µl physiological serum. For this reason, the mentioned substances can have prophylactic and curative indications on *Candida albicans*. Tincture of iodine proved its antifungal effect for the 96 h of research.

Conclusions

For the oils we used 200 µl substance (the respective oil)/ 800 µl physiological serum to which we added a few colonies (2, 3) of *Candida spp*. For the apple cider vinegar, in the first 24 h sensitivity of the Candida colonies appears, compared to apple cider, iodized salt solution and citric acid. After 24 hours, a certain sensitivity of Candida colonies to apple cider vinegar is observed, up to 96 hours of evaluation, but not completely; Candida

reappears. The 2 baking soda solutions we used in the study were found to be inactive in treating Candida. For the 4 days there is some sensitivity to samples III and IV of 1 M acetic acid, but not enough to fight candida. A higher concentration of acetic acid solutions (acetic acid which is commonly called vinegar) could favorably influence the treatment of *Candida albicans.* A marked sensitivity of Candida colonies is observed for ginger oil and tincture of iodine, and a little for apple cider vinegar, after the 4 days of the study. Candida is totally sensitive to iodine tincture and ginger oil. The results of the experiments reveal the fact that to treat mycoses of the skin and mucous membranes, we can use plant extracts (tinctures or oils) that can be applied locally. Thus, ginger oil is recommended, which showed a maximum fungicidal effect on *Candida albicans* colonies after 96 hours of incubation. The recommended concentration is 20%, in daily applications, until a total healing of the lesions. Also, frankincense and lemon oils cause sensitivity of Candida colonies in the first 24 hours and, subsequently, to a lesser extent after 48 hours from seeding, up to 96 hours. We can consider that frankincense and lemon oil used in higher concentrations would give a good anifungic result or possibly by mixing them with other fungicidal substances. *Candida albicans* is sensitive to apple cider vinegar, especially for the first 48 hours, and to a lesser extent thereafter. It could be used more often in food, with a prophylactic purpose. Tincture of iodine and ginger oil showed their antifungal role in treating candida for the 4 days of the study.

5.2.5 Study on the Action of 9 Natural Products and the Colloidal Solution of Cu on *Candida Albicans* Cultures (**Olimpia-Nicoleta Moroianu**, Nelu Doru Popescu, Alina Raluca Ursu, Tony Laurențiu Hangan, Natalia Rosoiu, 2022)

In the present study we performed several experiments during 120 hours, using both natural products (essential oils, tinctures, etc.) and chemical ones (colloidal solution of Cu) in order to discover substances with anifungic action on *Candida albicans* cultures. The work highlights the action of 9 natural substances and the colloidal Cu solution on *Candida albicans* cultures. The products under study are:

• the oils of: white musk, hemp (*Canabis sativa)*, opium (*Lachryma papaveris*), cinnamon (*Cinnamomum verum*), geranium (*Geranium robertianum*), and sandalwood (*Santalum austrocaledonicum*);

• sweet wood tincture (Little boy), (*Glycyrrhiza glabra roots*), thyme tincture (*Thymus vulgaris)*;

• colloidal copper solution.

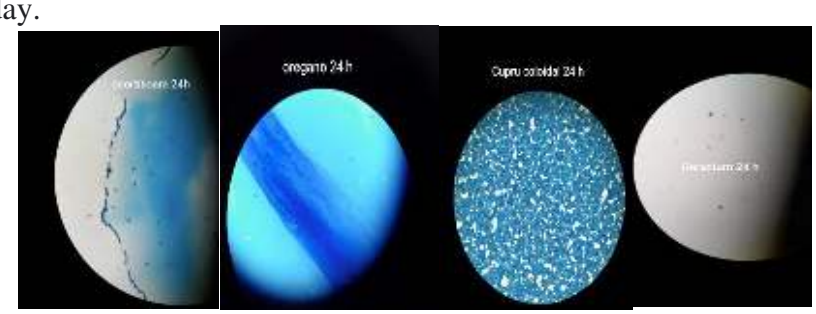
Subsequently, for the natural substances that inhibited candida, we sampled colony portions from the plates for examination under the optical microscope; for coloring I used methylene blue 3%. After drying I examined under a microscope.

Results

5-day experiment I used 800 µl saline + 200 µl essential oil.

I seeded on Sabouraud medium. We thermostated the cultures for 120h, at a temperature of 37°C. We took readings and interpretations at 24 h, 48 h, 72 h, 96 h and 120 h. After 24 h of inoculation *Candida albicans* cultures show sensitivity to cinnamon oil compared to white musk oil and hemp oil. The colloidal copper solution also shows fungicidal action 24 hours after seeding, compared to the oils: opium, thyme and licorice tincture. Oregano oil inhibited *Candida albicans* cultures 24 hours after application compared to sandalwood oil. 24 h after the application, I made slides stained with methylene blue, which I analyzed under the optical microscope, with a 100x objective. We

visualized the slides under the optical microscope for the substances that showed sensitivity on the first day.



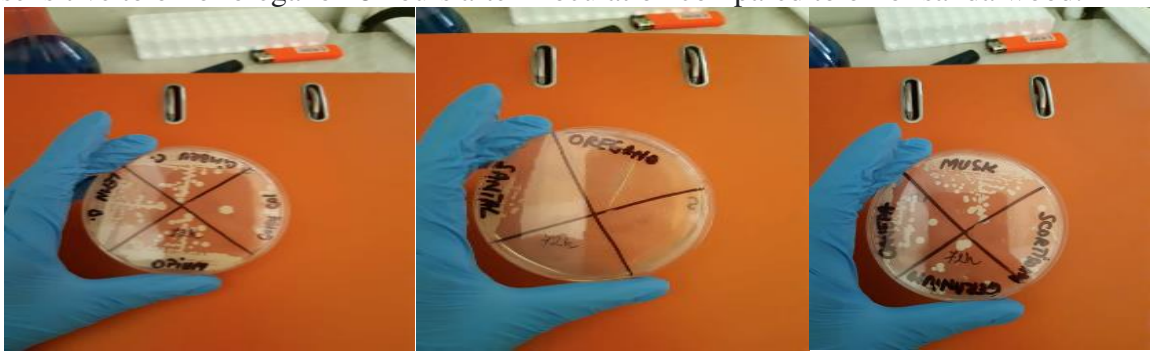
(a) *(b)* *(c)* *(d)*

Figure 78. *Mycelial cultures to which cinnamon, oregano, geranium oils were applied, respectively colloidal copper solution after thermostating for 24 hours, examined under the optical microscope with a x40 objective*

A reduction of the mycelia is observed in the first 24 hours in the case of the oils of geranium and oregano oil versus colloidal copper solution and cinnamon oil; in colloidal copper solution and cinnamon oil the micelles do not shrink.

In cinnamon and geranium, sensitivity can be observed 48 h after seeding compared to the oils of: white musk and hemp.

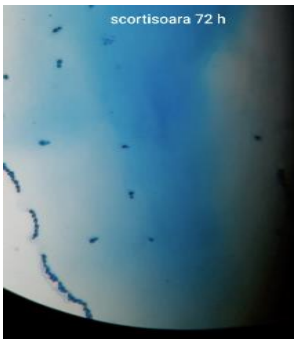
The 4 substances: opium oil, colloidal Cu solution, licorice tincture and cultivated thyme oil do not inhibit the development of candida 48 hours after seeding. *Candida albicans* is sensitive to oil of oregano 48 hours after inoculation compared to oil of sandalwood.



(a) *(b)* *(c)*

Figure 81(a, b, c). *Culture plates inoculated with Candida albicans to which white musk, cinnamon, geranium, hemp, opium, cultivated thyme, oregano and sandalwood oils were applied at a concentration of 20%, as well as licorice tincture, respectively solution colloidal copper at 72 h*

After 72 h from seeding, of all the substances used in the study, oregano oil inhibited the development of *Candida albicans* and, to a lesser extent, the colloidal Cu solution. Thus, we followed the two plates with the respective substances under the microscope.



(a) (b)

Figure 45(a, b) *Mycelial cultures to which oregano and cinnamon oils were applied, after thermostating for 72 h, examined under the optical microscope with a 100 X objective*

We could observe under the microscope the decrease in the number of mycelia in the case of oregano oil and its increase for the oil of cinnamon after 3 days of thermosetting. After 96 h from inoculation with *Candida albicans*, of all the substances used in the study, only oregano oil inhibited the development of Candida. After 5 days of inoculation with Candida albicans only oregano oil shows anifungic action (see figure above).

Discussions

After 24 h and 72 h from seeding, we made slides stained with methylene blue, which we analyzed under the optical microscope, with the objective of 40x. We found the following:

• The colloidal copper solution partially inhibited mycotic colonies in the first 24 hours, then we noticed the growth of *Candida albicans* in the following hours on the culture medium, both macroscopically and microscopically.

• Cinnamon oil partially inhibited the culture in the first 24 h, culture that reappeared on the medium after 48 h.

• Geranium oil inhibited the growth of the culture for 24 h, *Candida albicans* then growing on the medium after 48 h.

• The oils of sandalwood, white musk, opium, as well as licorice or cold wood tincture were not active in the treatment of candida during the 5 days, the candida colonies growing.

• Oregano oil determined the total inhibition of the culture, observing under the microscope as well as on the medium the lack of *Candida albicans* colonies until the end of the 120 h.

Conclusions

During the 120 h it can be observed that only the oregano oil and maintained the inhibition capacity compared to the other oils: white musk, cinnamon, geranium, hemp, opium, cultivated thyme (*Thymus vulgaris*) and sandalwood in a concentration of 20%, as well as licorice tincture (*Glycyrrhiza glabra* roots), respectively copper colloidal solution. It can be observed that the essential oil from cultivated thyme is less effective than the essential oil of thyme used in another experiment. Thus, the essential oil of cultivated thyme cannot stop the development of candida even in the first 24 h compared to the essential oil of thyme that was effective during the 3 days analyzed in the previous study. It is also observed in the case of the cinnamon oil, even the geranium oil and the colloidal copper solution that during the 120 h, the candida remained at the same level, which leads us to the idea that the cinnamon oils and geranium, as well as colloidal copper solution, used in higher concentrations, successfully inhibits the development of candida.

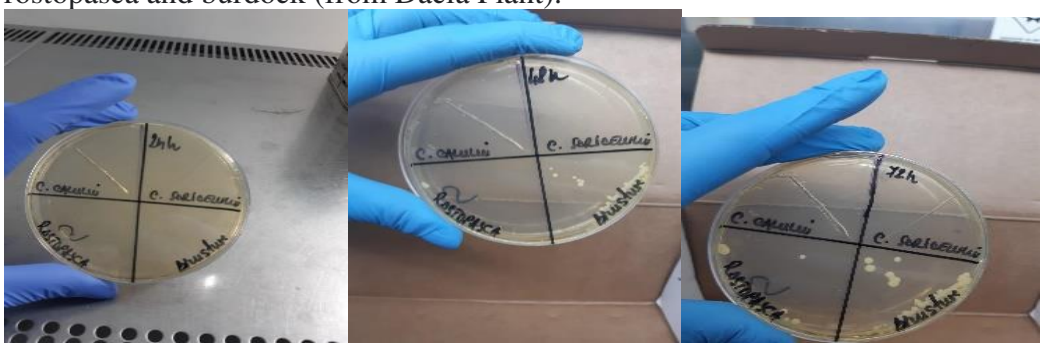
5.2.6 Study With 15 Natural Substances and Colloidal Platinum Solution on *Candida Albicans* (**Olimpia-Nicoleta Moroianu**, Nelu-Doru Popescu, Natalia Rosoiu; in progress of publication in a well-rated journal)

In this study (during 5 days) we used oils: white sea buckthorn oil for internal use *(Hippophae rhamnoides*), cold-pressed mustard oil, Biomicin forte A 15- natural essential oils (product composed of sunflower oil, thyme essential oil - *Thymi aetheroleum*, clove essential oil – *Caryophylli floris aetheroleum*) and, tinctures of: horsetail (*Equisetum arvense*), burdock (*Lappa arctium*), artichoke (*Cynara scolymus),* horseradish (*Chelidonium majus),* mouse tail (*Achillea milefolium*), hot pepper *(Capsicum annuum*), juice, certain products such as syrup - BIOSEPT A 13 – syrup of medicinal plants with honey, vitamin C and propolis, juice of chokeberry (15% concentration, lemon juice, grape pulp juice minimum 20%), capsules of 100% natural substances - turmeric 250 & pepper (pills from PRONATURAL) and colloidal solution of Pt.To highlight the action of natural products on *Candida albicans* cultures, we used Sabouraud's standard culture medium, on which we seeded samples of *Candida albicans*, from a standardized assortment, called ATCC 60193.

Results and discussions

5-day experiment In the first phase of the experiment I prepared the necessary substances; we dissolved the turmeric & pepper pill in SF, the BIOMYCIN FORTE A15 capsule (soft capsule) was prepared for the study and the sea buckthorn oil was dissolved in SF; I cut the capsule; one capsule of Biomycin forte A15 (contains 250 mg of oils); I crushed/crushed the turmeric & pepper pill (which weighs 250 mg). The tinctures were used in their normal state. Also, chokeberry juice and colloidal platinum solution were used in their existing state. Later I added a few colonies of *Candida spp*. (about 2-3). I made readings and interpretations at 24 h, 48 h, 72 h, 96 h and 120 h.

a. I used tinctures of: horse tail (from Dacia Plant), mouse tail (produced by Hofigal), rostopasca and burdock (from Dacia Plant).



(a) *(b)* *(c)*

(d) *(e)*

***Figure 86 (a, b, c, d, e**) Culture plates inoculated with Candida albicans on which different tinctures (of horsetail, mousetail, yarrow and burdock) were applied at 24 h, 48 h, 72 h, 96 h and 120 h*

After 24 h from seeding, the tinctures of: horsetail and mousetail had antifungal action in the treatment of candida, unlike the tinctures of horsetail and burdock.

48 h after sowing, the result was the same. For the substances with an inhibitory effect, we made colored slides at 48 h, 72 h, 96 h and 120 h after seeding, which we put under an optical microscope with an immersion objective / x100 clarity for in-depth viewing. Staining was done with gentian violet 3%.

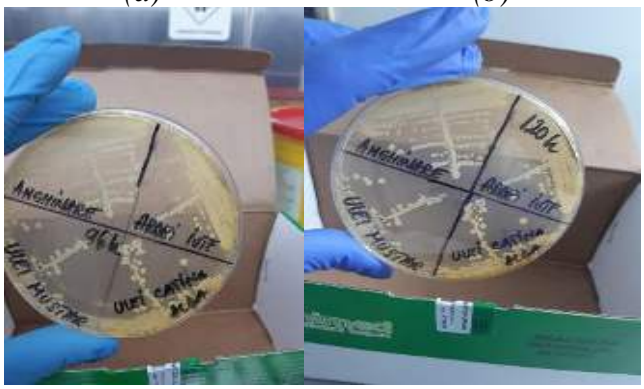
(a) *(b)* *(g)* *(h)*

***Figure 88** Mycelial cultures on which we put horsetail and mousetail tinctures after thermostating for 48 h (a, b) and 120 h (g, h) examined under the optical microscope with a x100 objective*

During the 5 days of the study the two tinctures of: horsetail (from Dacia Plant) and mousetail (produced by Hofigal) maintained their antifungal capacity *against Candida albicans*, and the tinctures of: rosehip and burdock did not show an inhibitory effect during this period. The microscopic appearance of the Candida cultures was slightly different for the substances in the study: tincture of mouse tail and tincture of horse tail; we visualized filaments characteristic of *Candida albicans* and a mycelial shrinkage in these cases.

b. I used tinctures of: artichoke (from Dacia Plant) and hot peppers (*Capsicum annuum,* fruit, 10% - from Faunus Plant), cold-pressed mustard oil (from Herba Sana) and sea buckthorn oil (produced by Hypericum Impex S.R.L.), 100% natural product. For the two oils we achieved a concentration of 30% by dissolving 300 µl substance /700 µl SF.

(a) *(b)* *(c)*



(d) *(e)*

Figure 89. *Culture plates seeded with Candida albicans on which mustard and sea buckthorn oils were applied in a concentration of 30% and tinctures of: artichoke and hot pepper at 24 h (a), 48 h (b), 72 h (c), 96 h (d) and 120 h (e)*

24 h after seeding, the hot pepper tincture had an antifungal effect. 48 hours after sowing, all 4 substances: tinctures (artichoke and hot pepper), mustard oil and sea buckthorn oil have no antifungal action. Also, at 72 h, 96 h and 120 h, the 4 substances cannot destroy *Candida albicans.*

For the hot pepper tincture, I did not make any more colored slides because candida reappeared 48 hours after sowing.

c. Platinum colloidal solution, aronia juice, turmeric & pepper (pills) - product from PRO NATURAL and the product BIOSEPT A13 – syrup. The aronia juice is actually a combination of aronia juice 15%, lemon juice, grape pulp juice minimum 20%; it was used directly from the bottle, as was the colloidal platinum solution (produced by Aqua Nano 480ml, 10 ppm). I obtained the solution of Turmeric & Piper by dissolving a pill from this product, i.e. powder from the root of Curcuma/Turmeric (*Curcuma longa*) 200 mg & powder from the fruits of black pepper – *Piper nigrum* 50 mg; thus, I dissolved the powder from a pill in 4 ml of physiological serum. BIOSEPT-A 13 syrup with honey, vitamin C and propolis from Fares was used in its existing state; it consists of several substances, such as: minimum 64% bee honey, aqueous extracts of: echinacea (*Echinaceae herba*), thyme (*Serpylli herba),* sorghum (*Origani Herba)*, stone lichen (*Lichen Islandicus*), cinnamon (*Cinnamomi cortex*), ginger rhizomes (*Zingiberis rhizoma),* olive leaves (*Oleae folium*), cloves *(Caryophylli flos*), dry extract of *Astragalus* membranaceus – min. 35%, propolis hydroglyceric extract 1.2%; vitamin C 1%; essential oils of: geranium (*Pelargoni*

aetheroleum), lemon (*Citri aetheroleum*), cinnamon *(Cinnamomi aetheroleum*), evening primrose (*Melissae aetheroleum*) - minimum 0.045% in variable proportion. During the 5 days of the study, only the product turmeric & pepper inhibited the growth of the *Candida albicans* culture from the first day compared to the colloidal solution of Pt, chokeberry juice and the product BIOSEPT A13 – syrup with honey, vitamin C and propolis from Fares and a maintained this capacity until the end. We made slides for the optical microscope in the case of the product with turmeric& pepper at 48 h, 72 h and 120 h.

(a) *(b)* *(c)* *(d)*

Figure 91. *Mycelial cultures on which we applied turmeric 250 & pepper lozenge (dissolved in SF) after thermostating for 48 h (a), 72 h (b), 96 h (c) and 120 h (d) examined under the optical microscope with objective x 100*

For the product with turmeric & pepper we noticed a decrease in mycelia under the optical microscope.

d. BIOMYCIN FORTE A15, produced by Fares, which is available in the form of capsules; it consists of sunflower oils, thyme essential oil - *Thymi aetheroleum*, clove essential oil - *Caryophylli floris aetheroleum*. The product was used as such; I cut the capsule with oils, then added 3 colonies of *Candida albicans*. From the first 24 h *Candida albicans* increased; 2-3 colonies appeared on the plate with BIOMYCIN forte A15. During the 120 h of study, *Candida albicans* grew in the presence of the natural product Biomicin forte A15, which is why we no longer made plates for the optical microscope.

Conclusions

During the 5 days of the experiment, we found that the tinctures of horsetail (from Dacia Plant) and mouse tail (produced by Hofigal), unlike the tinctures of: wort and burdock (from Dacia Plant), inhibited the development of *Candida albicans* from the first day until the last day of the experiment. Hot pepper tincture (from Faunus Plant) had an antifungal effect for 24 h compared to artichoke tincture (from Dacia Plant), cold-pressed mustard oil (from Herba Sana) and sea buckthorn oil (produced by Hypericum Impex S.R.L.) 100/% natural. During the 5 days of the experiment, artichoke tincture (from Dacia Plant), cold-pressed mustard oil (from Herba Sana) and sea buckthorn oil (produced by Hypericum Impex S.R.L.) could not stop the development of *Candida albicans.* In the case of hot pepper tincture, we can propose the use of this product several times or in combination with other anifungic substances, possibly, for preventive purposes, we consume hot pepper at the table. Turmeric 250 & pepper lozenge (product from PRO NATURAL) versus colloidal platinum solution, chokeberry juice (actually a combination of chokeberry juice 15%, lemon juice, grape pulp juice minimum 20%) and the BIOSEPT product A13 - syrup with medicinal plants, honey, vitamin C and propolis from Fares had an antifungal effect during the 120 h of study. BIOMYCIN FORTE A15, produced by Fares (in the form of capsules and consisting of sunflower oils, essential oil of thyme - *Thymi aetheroleum*, essential oil of cloves - *Caryophylli floris aetheroleum*) has no antifungal action in the treatment of candida for the 120 h of evaluation.

Also, on the plates colored with gentian violet and the products that inhibit the development of candida (tinctures of: horsetail (from Dacia Plant) and mousetail (produced by Hofigal), as well as turmeric & pepper 250 capsules could be observed under the microscope optical reduction of the number of candida mycelia.

In conclusion, the 3 substances under study: tinctures of: horsetail, mousetail and turmeric & pepper capsules can be used successfully to stop the development of *Candida albicans* and to a lesser extent or, possibly, repeatedly for a lasting effect, we can also use the hot pepper tincture either separately or in combination with other natural substances known as antifungal.

5.7 Changes Revealed by Transmission Electron Microscopy (TEM) in Cultures of *Candida Albicans* Inoculated with Oregano Oil (**Olimpia-Nicoleta Moroianu**, Nelu-Doru Popescu, Constanta Stefanov, Nicolae Dobrin, Natalia Rosoiu, 2022)

For transmission electron microscopy (TEM) we took 2 samples from the Sabouroud medium seeded with *Candida albicans* samples, from a calibrated assortment, called ATCC; the medium was initially treated with oregano essential oil; I inserted the plate into the thermostat, at the standard temperature of 37ºC; this was kept for 72 h at the thermostat (Moroianu O-N et al., 2018), (Moroianu O. et al., 2022) (Moroianu O. - N., 2022). After these 72 h in which *Candida albicans* was also inhibited by oregano oil *(Origanum vulgare)*, an oil that contains carvacrol (Nostro A., Papalia T., 2012) and thymol (Guarda A. et al., 2011), we sectioned the necessary TEM material. The sections fines were double-stained with uranyl acetate and lead acetate, after which they were examined under a Tecnai T12 Microscope produced by FEI, which is located at the Faculty of Medicine of the "Ovidius" University of Constanta.

Discussions and results

Evaluation of the sections Overall characterization was performed using photomicrographs taken at sizes of x 2900 - x 30000. From these we selected a few representative images and subsequently interpreted what we obtained.

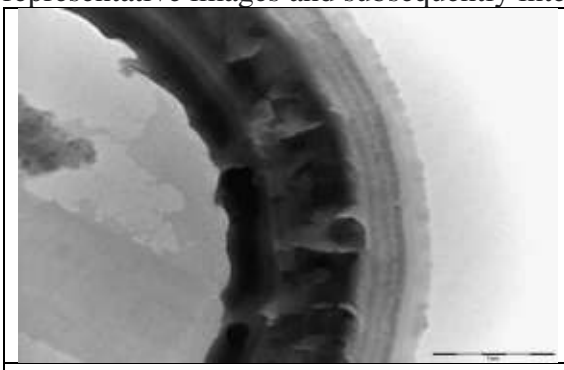


Figure 107. *Photomicrograph size x 23000, Candida albicans culture inoculated with oregano essential oil. Dissociation between the cell wall and the membrane can be observed*

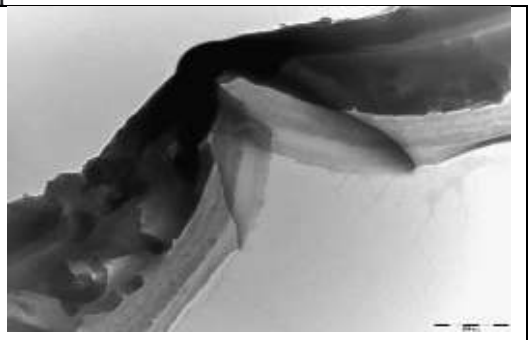


Figure 108. *Photomicrograph size x 30000, Candida albicans culture inoculated with oregano essential oil. Dissociation between the cell wall and the membrane can also be observed*

Conclusions

Oregano essential oil can be successfully used as an antifungal for conditions caused by *Candida albicans*. This antifungal works by dissociating the cell wall and the candida membrane thanks to the thymol and carvacrol in the composition. The antifungal action of oregano essential oil is irreversible and has a residual effect.

V.2.8 Study on the Internal and External factors Involved in the Phathogenesis of Diseases Determinated by *Candida Genus* and Species (Moroianu Olimpia-Nicoleta, Popescu Nelu-Doru, Gurgaș Leonard, Rosoiu Natalia, Olgun Azis, 2023)

The study was conducted between August 1-31, 2017 at the "Iowemed-Medicover Medical Center" on a 26-year-old patient who presented to the medical office with a condition in the vaginal area. Samples were taken.

Discussions and results

Patient M.C., 26 years old, complained of discomfort in the vaginal area, specifically itching, itching and unpleasant odor. *Candida albicans* was isolated in the vaginal discharge. We put samples for analysis on petri dishes that were left for 72 h at a thermostat at 37 °C, to make observations. In the present study we used a candida identification system called Candifast.

Figure 111. *Candifast Identification System*

Candifast is an identification kit based on fermentation tests and determining the presence of urease. Minerals: copper and zinc have a role in boosting immunity and increasing fertility (Vandeputte P. et al., 2012). This test can identify several Candida species, more precisely 8. This test can specify whether the tested strain is part of the genera: *Geotrichum or Rodothorula, Trichosporon,* but without being able to specify the genus or species. Candifast also contains a sensitivity test kit. The following strains of Candida can be identified: *Candida albicans, Candida tropicalis, Candida glabrata, Candida krusei*. The identification kit is based on: Assimilation tests; Enzyme tests; Morphological tests. Following laboratory determinations/tests, *Candida albicans* species was identified in the patient in question. There is clear evidence showing a direct relationship between the consumption of antibiotics and the resistance developed by microbial agents (Chirila S., Alexiu S.A., 2018). It has been proposed to normalize the vaginal pH by alkalinizing the vaginal mucosa, i.e. frequent washes with sodium bicarbonate solutions, administration of essential vitamins, etc.

After a period of 14 days, the MC patient returned to the laboratory, where we found complete healing following laboratory analyses.

Conclusions

The occurrence of candidiasis can be prevented by:

- alkalizing techniques of integumentary and mucous surfaces (for example with solutions containing sodium bicarbonate);
- maintaining the ecological balance with other microorganisms, by using eubiotics and vitamin complexes;
- ensuring the appropriate immunological status from a functional point of view, by ensuring hours of physical and mental rest, and a balanced dietary intake of nutritional principles and essential vitamins;
- avoiding excesses of general antibiotic therapy.

General conclusions

Regarding the "Retrospective epidemiological study on risk factors and variations of clinical and paraclinical parameters in patients with mycoses, on the factors favoring and determining fungal infections" we determined several aspects.

In both groups, the prevalence of the male sex is observed (34.3%, respectively 21%; compared to 27.6%, respectively 18.1% for the female sex).

In both groups, the prevalence of coming from the urban environment is observed (52.4%, respectively 27.6%; compared to 9.5%, respectively 10.5% from the rural environment). Regarding age, in the group of "candidiasis", the majority were children (33% compared to adults 32%), and in the group of "other mycoses", not a single child was registered. Regarding the biochemical and hematological investigations, we noticed significant differences in the average values between the two groups, as follows: in the "candidiasis" group, the average values of lymphocytes are higher (17.64 versus 11.92 103/μL), platelets (310.03 vs. 247.07 103/μL), AST (28.70 vs. 22.53 U/I); in the "other mycoses" group, the mean values of eosinophils (1282 versus 675 10^3/μL), serum glucose (104.95 versus 95.64 mg/dl), neutrophils (35.75 versus 29.90 103 /μL), urea (40.24 versus 29.97 mg/dl).

By comparing these values, it can be concluded that the changes in biological constants in the case of other mycoses are more important, more serious, than those recorded in candidiasis.

Regarding the association with other conditions, it is clearly observed that the group of patients diagnosed with "other mycoses" associates more conditions that may be directly or indirectly related to those mycoses than the group with "candidiasis"; these are: venous insufficiency (40% versus 1.5%), hypertension (37% versus 13.8%), type 2 diabetes (17.5% versus 7.7%), chronic ischemic heart disease (12.5 % vs. 6.2%, obesity (17.5% vs. 3.1%).
We noted the increase in the association of pneumonia cases in the "candidiasis" group (18.5%), compared to the "other mycoses" group (2.5%). This last observation can be explained as a consequence of the antimicrobial effect of antibiotics on the saprophytic microbial flora, protective against the development of *Candida albicans*. The association of other mycoses with the conditions exemplified above can be explained by the modification of the immune response to various aggressions that act on the human body with advancing age.

Regarding "Experimental research on the effectiveness of natural and synthetic substances on the strains of yeasts and filamentous fungi involved in human mycoses" the following conclusions can be distinguished for each individual study:

Study no. 1

Following these determinations, we found that sodium bicarbonate, ethyl alcohol and sodium chloride did not show the expected inhibitory effect on *Candida albicans* cultures. Only with acetic acid was the inhibitory effect reported (at a concentration of 28.50%).

The application of diluted acetic acid, in a concentration of 28.50%, causes the destruction of Candida albicans colonies both in vitro and in vivo.

Study no. 2

Through the two experiments (with Candida and *Aspergillus,* respectively) within the study, it is evident that in the therapy of mycoses of the skin and mucous membranes, the local application of products extracted from plants, sold in the form of oils or tinctures, can be recommended.

The recommended products are:

a. for Aspergillus

• the oil of: eucalyptus, aloe (*Aloe Vera)*, sage (*Salvia officinalis)*, mint (*Mentha piperita*), cloves (*Syzygium aromaticum*), as well as garlic tinctures, propolis, calendula (*Calendula officinalis*.) and echinacea (*Echinacea angustifolia*); demonstrated after a 24-hour incubation that they have a 100/100 fungicidal action on *Aspergillus* colonies.

b. for *Candida albicans*

• tinctures of: calendula (*Calendula officinalis)* and propolis, plantain (*Plantago lanceolata)*, chamomile (*Chamomillae flos);*

• the oils of: sage (*Salvia officinalis*), mint (*Mentha piperita)*, thyme (*Satureja hortensis L.)* and lavender (*Lavandula Angustifolia).*

The recommended concentration is 20%, in daily applications, until complete healing.

Study no. 3

At the end of the study, we noticed an inhibitory action on *Candida albicans* cultures, determined by the essential oils: of oregano (*Origanum vulgaris*), thyme (*Thymus vulgaris*), geranium (*Geranium roberrtianum)*, bitters from 50 plants with ganoderma, Swedish drops, superconcentrated Ag solution and Ag and Au ion solution.

In the case of mint oils, eucalyptus, sage, lavender, propolis tincture, apple cider vinegar and 9% wine vinegar solution (with 1/8 dilution), the antifungal effect was for a short period of time, the colonies regenerating it; thus, it would be advisable that the treatment with these substances be repeated at certain time intervals, to successfully combat *Candida albicans*.

Favorable results in the inhibition or lysis of *Candida albicans* cultures were obtained following experiments 48 h and 72 h after application, respectively:

• essential oil of oregano (*Origanum vulgare)*;
• geranium essential oil (*Geranium robertianum*);
• thyme essential oil (*Satureja hortensis L.);*
• bitters from 50 plants and with ganoderma;
• Swedish drops;
• superconcentrated colloidal solution with Ag;
• the colloidal solution with Ag and Au ions.

The products used in the study can be applied repeatedly in order to obtain a lasting cure.

Study no. 4

The concentrations of the oils used in the studio were 20%.

For apple cider vinegar, sensitivity of Candida colonies appears in the first 24 h compared to apple cider, iodized salt solution and citric acid. After 24 h, a certain sensitivity of Candida colonies to apple cider vinegar is observed, up to 96 h of evaluation, but not totally; *Candida albicans* reappears. The 2 sodium bicarbonate solutions we used in the study proved inactive in treating Candida during the 4 days of the experiment.

Similarly, for the 4 days some sensitivity is observed to samples III and IV of 1M acetic acid, but not enough to combat candida. The results of the experiments demonstrate the fact that to treat mycoses of the skin and mucous membranes, we can use plant extracts (tinctures or oils) that can be applied locally. The recommended concentration is 20%, in daily applications, until a total healing of the lesions.

Also, frankincense and lemon oils cause sensitivity of Candida colonies in the first 24 hours and, subsequently, to a lesser extent after 48 hours from seeding, up to 96 hours. We can consider that frankincense and lemon oil used in higher concentrations would give a good anifungic result or possibly by mixing them with other fungicidal substances.

Lemon oil (*Citrus limon*) and Frankincense oil (*Thymanea)* can be used repeatedly/alternately with other antifungal substances.

Candida albicans is sensitive to apple cider vinegar, especially for the first 48 hours, and to a lesser extent thereafter. It could be used more often in food, with a prophylactic purpose.

Tincture of iodine 2% and ginger oil (*Zingiber officinale)* demonstrated their antifungal role in treating candida during the 4 days of the study.

Study no. 5

For the 120-h study with substances used to combat candida, it can be seen that only oregano oil maintained its inhibitory capacity, compared to the other oils: white musk, cinnamon, geranium, hemp, opium, and sandalwood in concentration of 20%, as well as licorice tincture (*Glycyrrhiza glabra* roots), cultivated thyme tincture (*Thymus vulgaris*) or colloidal copper solution. It can be observed that the action of the tincture from cultivated thyme is less effective than that of the essential oil of thyme, used in another experiment. Thus, the tincture of cultivated thyme cannot stop the development of candida even in the first 24 h, compared to the essential oil of thyme, which was effective during the 3 days analyzed in the previous study.

It is also observed in the case of the cinnamon oil, even the geranium oil and the colloidal copper solution, that during the 120 h, the candida remained at the same level, which proves that the cinnamon oils (*Cinnamomum verum*) and geranium (*Geranium robertianum*), as well as colloidal copper solution, used in higher concentrations, could successfully inhibit the development of candida.

Study no. 6

The substances used in the study (essential oils) were applied in higher concentrations of 30%. During the 5 days of the experiment, we found that the tinctures of horsetail and mouse tail, unlike the tinctures of: rostopasca and burdock, inhibited the development of *Candida albicans* from the first day until the last day of the experiment. BIOMYCIN FORTE A15 has no antifungal action in the treatment of candida for the 120 h of evaluation.

Also, on the plates colored with gentian violet and the products that inhibit the development of candida (tinctures of: horse tail and mouse tail, as well as turmeric & pepper capsules),

the decrease in the number of candida mycelia could be observed under the optical microscope.

In conclusion, for the 5 days of the experiment, 3 substances stood out: horsetail tincture (*Equisetum arvense*), mousetail tincture (*Achillea milefolium*), as well as turmeric & pepper capsules (*Curcuma longa & Piper nigrum)* these can be used successfully to stop the development of *Candida albicans* and to a lesser extent or, possibly, by repeated application, for a lasting effect, you can also use the hot pepper tincture, either separately or in combination with other natural substances known as antifungals.

Study no. 7

Oregano essential oil can be used successfully as an antifungal for conditions caused by *Candida albicans*; this antifungal works by dissociating the cell wall and the candida membrane, thanks to the synergistic action of thymol and, respectively, carvacrol, from the composition of oregano essential oil, and has a residual, at the same time irreversible, effect in fighting candida.

Study no. 8

In order to prevent the occurrence of candidiasis, you can intervene by:

• techniques for alkalizing the integumentary and mucous surfaces (for example with solutions containing sodium bicarbonate);

• maintaining the ecological balance with other microorganisms, by using eubiotics and vitamin complexes;

• physical and mental rest;

• avoiding any excesses of general antibiotic therapy.

Following the laboratory determinations, the patient under study was proposed to normalize the vaginal pH/ alkalinize the vaginal mucosa (respectively frequent washes with 2% sodium bicarbonate solutions), the administration of essential vitamins, as well as to avoid the use of antibiotics throughout the treatment. After returning to the laboratory approximately 14 days later, we found complete healing of the patient M.C. following laboratory analyses.

As a result of research studies, numerous natural and synthetic substances have been identified capable of combating infections often considered difficult to cure, such as *Candida albicans* and *Aspergillosis.* There are multiple possibilities to use these substances, either individually or in specific combinations, to achieve a rapid antifungal effect. The concentration of these substances plays a crucial role; it has been observed that at higher concentrations (20%, 30%, etc.), whether they are essential oils or other therapeutic products available in the market, a high yield is obtained in the treatment of mycoses.

Thus, numerous products (oils, tinctures, therapeutic products, etc.) have been identified that are effective in the case of recurrent infections with Candida or other fungi.

Following the analysis of the results obtained in the two studies, the following findings and conclusions can be formulated:

• By comparing the values of the two groups of candidiasis/"other mycoses" from the retrospective epidemiological study, it can be concluded that the changes in biological constants in the case of other mycoses are more important, more serious, than those recorded in candidiasis;

• Regarding the association with other conditions, it is clearly observed that the group of patients diagnosed with "other mycoses" associates more conditions that may be directly or indirectly related to those mycoses than the group with "candidiasis"; these are: venous

insufficiency (40% versus 1.5%), hypertension (37% versus 13.8%), type 2 diabetes (17.5% versus 7.7%), chronic ischemic heart disease (12.5 % vs. 6.2%, obesity (17.5% vs. 3.1%);
• We noted the increase in the association of pneumonia cases in the "candidiasis" group (18.5%), compared to the "other mycoses" group (2.5%); this observation can be explained as a consequence of the antimicrobial effect of antibiotics on the saprophytic microbial flora, protective against the development of *Candida albicans.*

a. Recommended products for *Aspergillus:*
• The essential oils of eucalyptus (Eucalyptus globulus), aloe (Aloe Vera), sage (Salvia officinalis), mint (Mentha piperita), cloves (Syzygium aromaticum) in a concentration of 20%, as well as tinctures of garlic, propolis, calendula (Calendula officinalis) and echinacea (Echinacea purpurea) demonstrated, after a 24-hour incubation, a 100% fungicidal action on Aspergillus colonies.
b. Recommended products for *Candida albicans:*
• The application of diluted 1M acetic acid, in a concentration of 28.50%, causes the destruction of *Candida albicans* colonies both in vitro and in vivo;
• The oils of: sage (*Salvia officinalis*), mint (*Mentha piperita*) and lavender (*Lavandula Angustifolia)* which inhibited candida for 24 hours and, up to 72 hours, only partially;
• Thyme essential oil *(Satureja hortensis L.)* had an anifungic effect for 3 days, in contrast to the tincture of cultivated thyme, which did not stop the development of candida even 24 hours out of the 120-hour study days;
• The bitter from 50 plants with ganoderma and Swedish drops (they had an antifungal effect for 72 h);
• The superconcentrated colloidal solution with Ag ions, the colloidal solution with Ag ions and Au inhibited the development of candida during the 3 days of the study;
• Tincture of iodine 2% and ginger oil (*Zingiber officinale)* demonstrated their antifungal role in the treatment of candida during the 4 days of the study;
• Lemon oil *(Citrus limon*) and frankincense oil (*Thymanea)*, cinnamon oil (*Cinnamomum verum/C. Zeylanicum)* can be used repeatedly/alternately with other antifungal substances; they inhibited the development of candida for 24 h and, subsequently, to a lesser extent after 48 h from seeding, up to 96 h;
• Apple cider vinegar with antifungal effect for 24 hours and partially up to 96 hours;
• Hot pepper oil (*Capsicum annuum*) and apple cider for 24 hours out of the 96 hours of study;
• The essential oil of oregano (*Origanum vulgare)* – showed an antifungal effect for 120 h;
• geranium essential oil inhibited the development of candida for 24 h and partially up to 48 h out of 120 h of study;
• Geranium essential oil (*Geranium robertianum)* and colloidal copper solution kept Candida at the same level throughout the 120 hours, demonstrating that using these products in higher concentrations could successfully inhibit Candida growth; geranium oil (*Geranium robertianum)* and colloidal copper solution during the 120 h kept candida at the same level, which proves that the use of these products in higher concentrations could successfully inhibit the development of candida;
• Horsetail tincture (*Equisetum arvense)*, mousetail tincture (*Achillea millefolium)* and turmeric & pepper capsules (*Curcuma longa & Piper nigrum)* inhibited the development of *Candida albicans* during the 120 hours;

• The essential oil of oregano (*Origanum vulgare)* has a residual effect, at the same time irreversible, in combating candida (TEM in this sense);
• Horsetail tincture (*Equisetum arvense)*, mousetail tincture (*Achillea milefolium)*, as well as turmeric & pepper capsules (*Curcuma longa & Piper nigrum)* they inhibited the development of *Candida albicans* during the 120 hours;
• The recommended concentration for essential oils and other products is 20% or even higher, in daily applications, until complete healing
• All pictures from the studies carried out are original.

In conclusion, in various types of mycoses, the substances act differently; the study can be extended to include more natural substances and can be continued compared to other mycelial cultures. It is very important to keep our body healthy because in certain situations, when the body is weakened, all kinds of fungal infections can easily set in.
The results of the evaluated studies can be applied as an alternative in the fight against mycoses, complementary to the usual drug treatments.

The originality of the conducted study consists in expanding the scope of the state of knowledge through biomedical current affairs in cutaneous mycoses.

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