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PHD THESIS SUMMARY

ADVANCED PHYSICAL, HISTOLOGICAL AND BIOCHEMICAL STUDIES IN CUTANEOUS MYCOSES

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CUPRINS

ADVANCED PHYSICAL, HISTOLOGICAL AND BIOCHEMICAL STUDIES IN CUTANEOUS MYCOSES

Key words: candida Albicans, Aspergillosis, Sabouroud medium, optical microscopy, TEM

OBJECTIVES AND PURPOSE OF THE WORK

In the present paper we have studied several aspects. On the one hand, we followed the differences in the therapeutic results in a case report with hospitalized mycoses, by analyzing the observation sheets from the "St. Andrei" County Hospital in Constanta (Dermatology department), on the other hand, we looked for different remedies (natural and chemical) compared to the classic ones, in combating mycoses and, mainly, Candida albicans, which is frequently encountered from young ages to the elderly, both with low immunity, and/or internal and external use factors. We investigated 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". The patients were hospitalized in "Saint Andrei" Hospital in Constanta (Dermatology department), between May 2017 and February 2018. 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, in a candida/other mycoses comparative study, referring to hospitalized patients during the specified/analyzed period. Important data were also obtained by comparing the association with other diagnoses specified in the medical documentation. We compared the action of some natural products in different concentrations on candida and Aspergilus during 24 h.

Also, the work aims to highlight the action of some natural substances, and not only, on Candida albicans cultures. The studies related to the work in the case of fighting candida were carried out during 24 h, 48 h, 72 h, 96 h or 120 h, etc. The observations were made using different concentrations of the substances that acted under constant temperature conditions and different time intervals. Thus, in the present work we investigated the inhibition of the development of candida through the use of essential oils such as: geranium, tea tree, oregano, black cumin, coriander, ginger, lemon, white sea buckthorn oil for internal use or Hippophae rhamnoides, pressed mustard oil cold, etc., tinctures (of calendula, iodine 2%, mouse tail, propolis, burdock, artichoke, licorice, or bittersweet, i.e. roots of Glycyrrhiza glabra, etc.) and some capsules (for lexample, graviola for a period of 48 of h; turmeric & pepper for 120 h etc), colloidal solutions (of super concentrated Ag ions, Au & Ag, Pt, Cu), as well as other commercial products (vinegar of several types and in different concentrations/dilutions , bitter etc). In some cases I have used substances (oils or other products) in a concentration of 10%, 20% or even 30% for a favorable effect. In the present study, we aimed to formulate observations on the changes in Candida albicans cultures, as a result of the sequenced intervention with several natural and chemical products.

The main objectives of the work consisted in: 1. Analyzing the action of selected plant substances on two mycelial species, aiming at the selection of natural substances for the treatment of mycoses such as Candidiasis and Aspergillosis. 2. Comparison of passport data in the case of patients with mycoses, respectively other mycoses from the "Sf. Andrei" (Department of Dermatology) from Constanța during the analyzed period. 3. Highlighting the in vitro mycolytic action of 1M acetic acid on Candida albicans cultures;

also highlighting the lack of action of different concentrations of sodium bicarbonate, sodium chloride and alcohol on the same types of cultures.
4. Indication of the action of 9 natural substances (essential oils, tinctures, etc.) and the Cu colloidal solution on Candida Albicans cultures during 5 days.
5. Study of Candida albicans in the presence of 15 natural substances and the colloidal solution of Pt.
6. Formulation of observations on the changes in Candida albicans cultures, as a result of the sequenced intervention with several natural products.
7. Visualization and examination under the optical microscope of slides with Candida albicans, on which we applied various substances and obtained an antifungal effect and staining (with methylene blue 3% and gentian violet 3%) at the "Center Medical Provita 2000" Clinic ” in the periods concerned.
8. Capturing images in the section and analyzing the electron-microscopic structure of the Candida albicans culture treated with oregano essential oil; In this case, we used Candida albicans ATCC 60193 cultures previously treated with oregano essential oil, which was initially left for 72 h at the thermostat, in order to be inhibited by the oregano essential oil; taking samples from this medium and treating them accordingly so that they can be visualized, analyzed and interpreted under the transmission electron microscope (TEM).
9. Combating the internal and external factors underlying candida infestation/disease, as a result of a particular case supported at the "Iowemed-Medicover Medical Center" in Constanța between August 1-31, 2017. The paper aims to systematize information about the etiopathogenesis skin-mucosal candidiasis, with the aim of highlighting the most effective prophylactic and therapeutic indications of these conditions.

PART II. PERSONAL CONTRIBUTIONS

INTRODUCTION

In general, skin diseases, and in an extraordinary way, mycoses, have a special impact on the patient's state of mind. 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". In order to better understand mycoses, we conducted several studies. Some were intended to find remedies in such diseases, and others indicated collateral diseases in such cases, etc. I looked for natural remedies and, not only, in the inhibition of Candida albicacans, as well as other mycoses such as Aspergillosis. We investigated biochemically and hematologically two batches of candida/mycoses obtaining comparative graphs regarding living conditions (rural/urban), sex, age, biochemical and hematological investigations, in a comparative study of candida/other mycoses, referring to hospitalized patients during the specified period / analyzed. We captured optical microscopy and TEM images and finally drew the characteristic conclusions. The studies were carried out at several famous medical laboratories in Constanta: "Provita 2000 Medical Center", "Iowemed-Medicover Medical Center", Clinical Department of Dermatovenerology within the Emergency County Hospital "St. Apostol Andrei" and the Electron Microscopy Laboratory of the Faculty of Medicine in Constanta.

CHAPTER 4. MATERIAL AND METHODS

To achieve the characteristic theme of the doctoral thesis, I used various materials and working methods because I had different studies in research.

1. Thus, 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 were selected for the comparative statistical study regarding candida/mycoses 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.

2. Another study was conducted to treat mycoses such as Candidiasis and Aspergillosis. To determine the action of plant extracts on mycelial cultures, we conducted experimental studies using plant extracts (oils and tinctures).

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

• The substances were applied to cultures of Candida/and Aspergillus ear mold cultures (taken from the auricular pavilions). 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%.

3. Another 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 that acted under constant temperature conditions and different time intervals; we researched the inhibition of candida development by using essential oils such as: tea tree, oregano, black cumin, coriander, etc., marigold tincture and a graviola capsule over a period of 48 hours; for the 72 h I used essential oils, namely: sage, mint, geranium, aloe vera, thyme, tinctures (such as: calendula tincture, propolis, plantain and chamomile), graviola capsules, colloidal solutions (of ions of Ag super concentrated, of Au and Ag ions), Apa Doamnei, Bitter (from 50 plants with ganoderma, respectively Swedish drops), apple vinegar and wine vinegar 9% concentration.

4. In another 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 introduced the plates to the thermostat, at the standard temperature of 37ºC (Buiuc D., Neguț, M., 2008).

I used for the 4 day experiment:

a. Apple cider, apple cider vinegar, iodized salt and citric acid.

b. 2 sodium bicarbonate solutions 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 - 200µl (substance); I added 800 µl physiological serum.

e. Ginger oil, lemon oil, frankincense oil, neem oil. 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 the mycelial colonies at different time intervals (at 24 hours, 48 hours, 72 hours (Moroianu O-N et al, 2018) and at, 96 h).
5. 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 (12), 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 variety (Moroianu O-N et al, 2018), called ATCC. We introduced the plates to the thermostat, at the standard temperature of 37 ºC.
In the present study, 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 one study), in order to be able to follow the action of the compounds chemicals on Candida albicans cultures. We used a staining method (with methylene blue 3% and gentian violet 3%) to highlight the inhibition/or not of candida in the presence of the different products under study, natural and not only. The sequence 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 weak flame (sometimes with methyl alcohol) then
• The coloring is started according to the following method. Staining with 3% methylene blue 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 stained slides that inhibited the development of candida over a certain period. Both optical microscopy experiments were performed over a period of 5 days.
6. A particular case was performed on a patient with Candida albicans. The study was conducted on a 26-year-old patient who presented to the medical office with a condition in the vaginal area. Patient M.C. , aged 26, complained of discomfort in the vaginal area, specifically itching, itching and unpleasant smell; samples were taken from the patient in question. In this experiment we used a candida identification system called Candifast.
7. 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 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 also inhibited by oregano oil, we sectioned the necessary TEM material. The samples taken

were properly treated further so that they could be visualized and analyzed under the transmission electron microscope.

Figure 1 *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

5.1 EXPERIMENTAL STUDY ON THE INHIBITORY EFFECT OF SOME SUBSTANCES APPLIED IN VARIOUS DILUTIONS ON CANDIDA ALBICANS CULTURES (**MOROIANU O-N.,** POPESCU N-D., ROŞOIU 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 9 *Petri plate seeded with Candida Albicans, on which I applied a washer soaked in glacial acetic acid solution, in four dilutions (concentrations: 44%, 41, 18%, 34, 43% and, likewise, 34, 43%)*

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 33.43%, approximately 33.4%). The application of diluted acetic acid, in a concentration of 33.43%, 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 COMPARATIVE STUDY OF THE ACTION OF SOME NATURAL SUBSTANCES ON CANDIDA ALBICANS AND ASPERGILLUS (**Olimpia-Nicoleta MOROIANU**, Nelu-Doru POPESCU, Natalia ROSOIU, 2020)

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: a. Oils of: Aloe, eucalyptus, sage, mint

b. Tinctures: Echinacea, garlic, propolis, marigold

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 oils of pine, geranium, cloves, seeds of grafts, as well as tinctures of plantain and chamomile.

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

Figure 13 (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.

The dilutions of 200 µl substance/800 µl physiological serum for:

• 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 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, eucalyptus, sage, mint – essential oils

• echinacea, garlic, propolis, calendula – tinctures I made the dilutions of 100 µl substance (respective oil)/ 900 µl saline. 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.:

- oils of: pine, geranium, cloves, grafts (grapefruit seeds), Aloe, eucalyptus, sage, Mint;

- tinctures of: thyme, lavender, plantain, chamomile, echinacea, garlic, propolis, calendula applied to culture media (Moroianu O-N et al, etc.).

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

Figure 16 (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

After this period, I found that in the case of some oils (specifically those of clove, aloe, sage, eucalyptus and echinacea), as well as in the case of some tinctures, for example the tincture of propolis, chamomile, garlic and calendula among those proposed in the study that a sensitivity occurs, which means we can treat Aspergillus naturally. Aspergillus shows sensitivity to dilutions of 200 µl substance/800 µl physiological serum for eucalyptus oil, sage, mint, echinacea and garlic tincture, calendula and propolis in the 24 h.

Conclusions

The results of the two experiments demonstrate that in the therapy of cutaneous mycoses and mucous membranes, the local application of products extracted from plants, sold in the form of oils or tinctures, can be recommended. Recommended products are: marigold and propolis tinctures; sage and peppermint oils. The recommended concentration is 20%, in daily applications, until complete healing.

5.3 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 physiological serum. In this experiment we used essential oil of tea tree, oregano, black cumin, coriander, rosemary, juniper and yellow tincture (fig.1 and fig.2). I

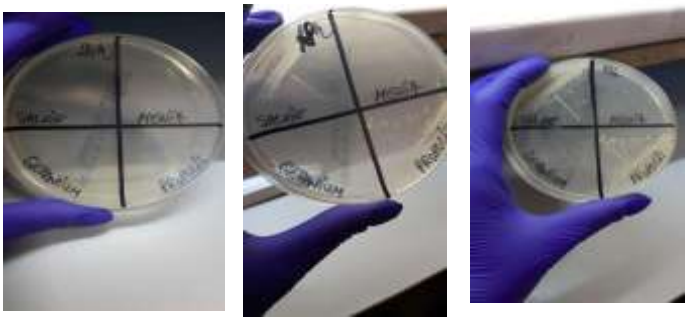
also dissolved 1 capsule of graviola (pure extract) in 2 ml of saline. A graviola vegetable capsule contains 5:1 extract of graviola fruit (Annona muricata) - graviola powder - 200 mg. To all this I added 2-3 colonies of Candida spp. Mix, leave at the thermostat for about an hour, then from the obtained emulsion it is seeded on the Sabouroud plate and placed in the thermostat for 24 h, after which the result is read. Candida is sensitive to oregano oil and tea tree oil.

Figure 17 (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 directly from the bottle. After 24 hours, all 4 substances had an inhibitory effect on candida cultures.



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

Figure 19 (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 and 48h*

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 the candida plates I put thyme 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 noted an inhibitory action on Candida albicans cultures determined by the essential oils of oregano, thyme, geranium, bitters from 50 medicinal plants with ganoderma, Swedish drops, the superconcentrated solution of Ag and the solution with Ag and Au ions . 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, for this reason, 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, respectivelyoregano essential oil;
- geranium essential oil;
- thyme essential oil;
- bitters from 50 medicinal plants with ganoderma; -swedish drops;
- the superconcentrated colloidal solution with Ag;
- colloidal solution with Ag and Au ions.

One can repeatedly use the above mentioned products in order to obtain a lasting cure.

5.4 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

In this study we investigated the action on candida of some essential oils of: ginger, lemon, frankincense and neem, cedar and Siberian cedar, wormwood, magnesium, tincture of iodine, sodium bicarbonate solutions, cedar apples, apple cider vinegar, citric acid, as well as four solutions of 1M acetic acid, 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 31 (a, b, c, d, e, f, g) *Petri dishes seeded with Candida albicans with the chemical compounds used in the study (1% 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 32(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 V.1).

Table V.1. 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 37 *Culture plate seeded with Candida albicans to which the four solutions of 1M acetic acid were applied at 24 h (a, b), 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 CH3-COOH, 1 M, we made 4 different samples for the proposed study (see Table IV.1). 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.

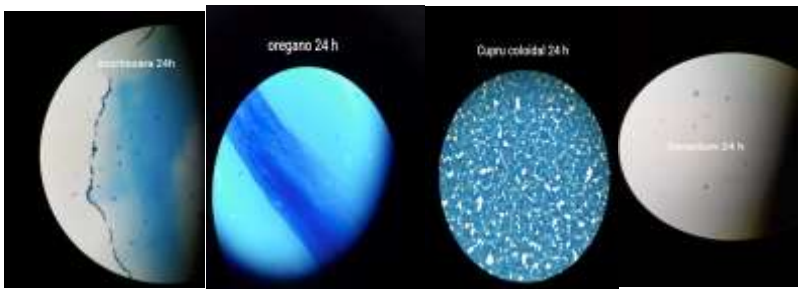
5.5 Study on the action of 9 natural products and the colloidal solution of Cu on Candida albicans cultures (**Olimpia-Nicoleta Moroianu**, Nelu Doru Popescu, Alina Raluca Ursu, Tony Laurențiu Hangan, Natalia Rosoiu, 2022)

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. 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.

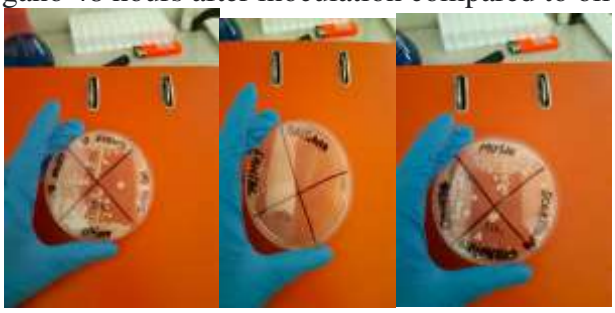


(a) *(b)* *(c)* *(d)*

Figure 42 *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 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 44 *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.

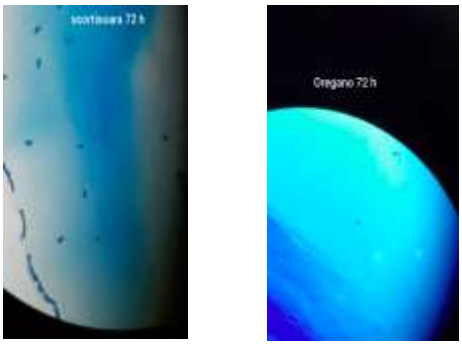


Figure 45 *Mycelial cultures to which oregano and cinnamon oils were applied, after thermostating for 72 h, examined under the optical microscope with a 100 X objective*
We could observe under the microscope the decrease in the number of mycelia in the case of oregano oil and its increase for the oil 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, could successfully inhibit the development of candida.

5.6 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, burdock, artichoke, horsetail, mouse tail, hot pepper, 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. 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.

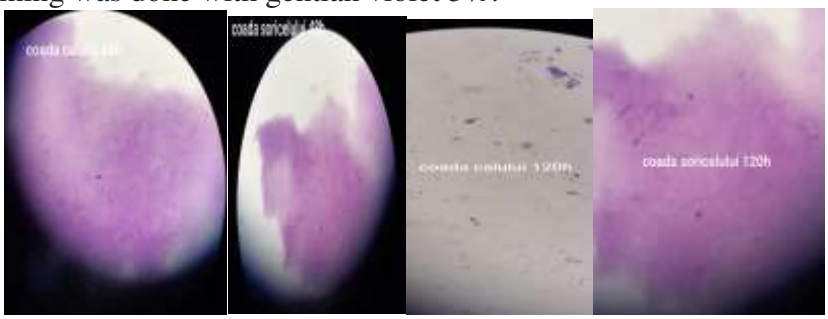
a. I used tinctures of: horse tail (from Dacia Plant), mouse tail (produced by Hofigal), rostopasca and burdock (from Dacia Plant).

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

***Figure 50 (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%.

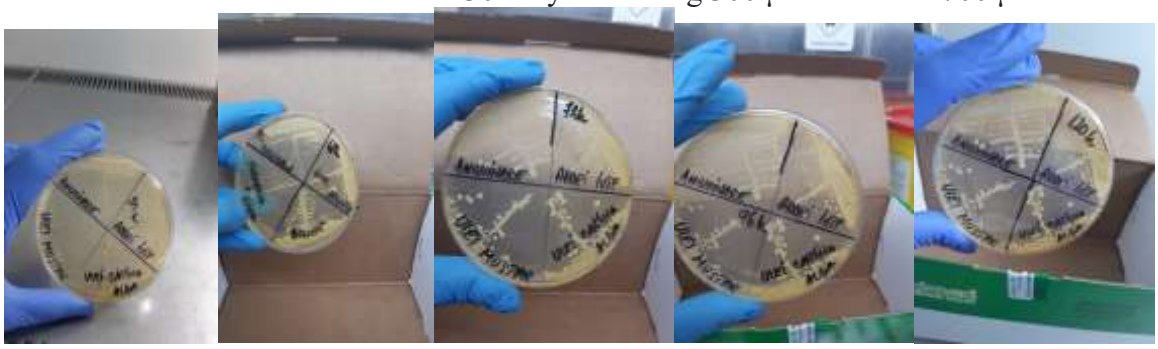


(a) *(b)* *(g)* *(h)*

***Figure 52** 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 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 250 & 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 250 & 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 250 & 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 250 & pepper at 48 h, 72 h and 120 h.

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

Figure 55 *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 (from Dacia Plant), mousetail (produced by Hofigal Plant) and turmeric 250& 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 (from Faunus Plant) either separately or in combination with other natural substances known as

5.7 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)

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

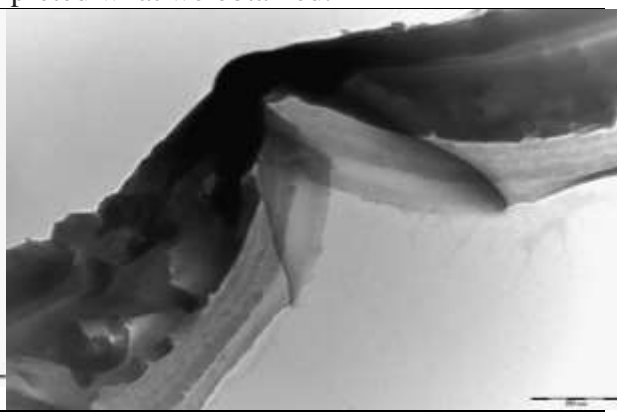


Figure 73 Photomicrograph size x 30000, Candida albicans culture inoculated with oregano essential oil. Dissociation between the cell wall and the membrane can also be observed.

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.8 STUDY ON THE INTERNAL AND EXTERNAL FACTORS INVOLVED IN THE PATHOGENESIS OF DISEASES DETERMINED BY CANDIDA GENUS AND SPECIES (**Moroianu Olimpia-Nicoleta**, Popescu Nelu-Doru, Gurgaș Leonard, Rosoiu Natalia, Olgun Azis; in the process of appearing in a well-rated journal)

The study was conducted on a 26-year-old patient who presented to the medical office with a condition in the vaginal area. Samples were taken. Discussions 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.

(a)

(b)

Figure 78 *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 8 species of Candida and can specify whether the tested strain belongs to the genera Trichosporon, Geotrichum or Rodothorula, 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 SA, 2018). Normalization of the patient's pH, administration of vitamins, etc. was proposed. 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. Following the laboratory determinations, the patient was proposed to normalize the vaginal pH/ alkalinize the vaginal mucosa, i.e. frequent washings with 2% sodium bicarbonate solutions, the administration of essential vitamins, as well as avoiding the use of antibiotics throughout the treatment.

5.9. Preliminary comparative statistical analysis of two groups of patients with mycoses: candidiasis, respectively other mycoses (**MOROIANU O.N.,** POPESCU D.N., PETCU L.C., GURGAȘ 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.

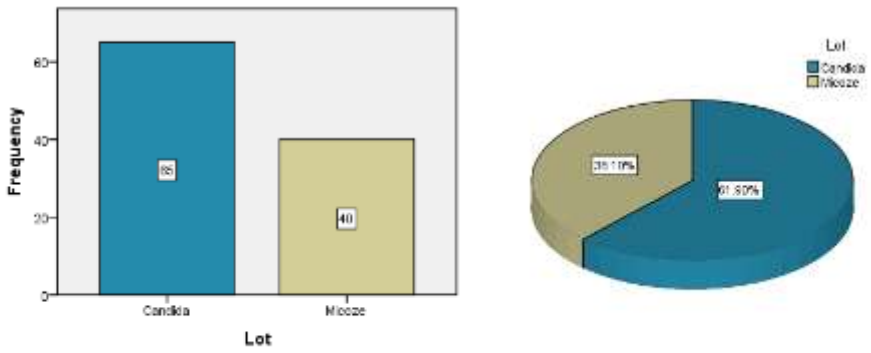


Figure 79 *Column (left) and Pie (right) representation of the distribution of patients by group (Candida/Mycoses) 105 patients were enrolled in the study, 65 with Candida (61.9%) and 40 with Mycoses (38.1%).*

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).

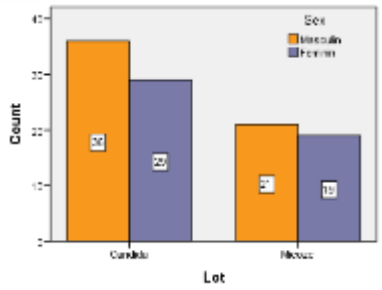


Figure 80 *Column representation of the distribution of patients from the Candida/Mycosis groups according to gender Regarding the distribution of patients from the Candida/Mycosis groups according to gender, we note that, out of a total of 105 patients, 57 (54.3.6%) are men, of which 36 (34.3%) have 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.*

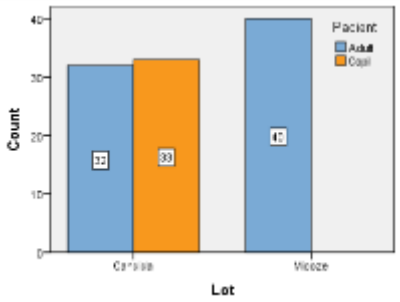


Figure 82 *Representation - Column of the distribution of patients from the Candida/Mycosis groups according to patient (adult/child) In the group of patients with Mycosis, only adults aged between 24 and 87 years were enrolled with an average age of 64.36 years and a standard deviation of 12.83 years.*

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].

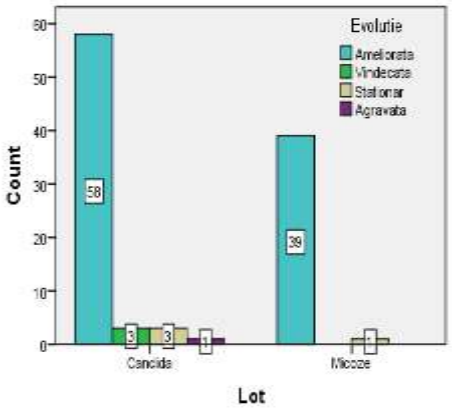


Figure 101 *Representation Column of the distribution of patients from the Candida/Mycoses groups according to evolution*

Table V.3. The chi-square test for comparing two proportions (from independent samples) expressed as a percentage.

	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.

General conclusions

From the experiments carried out and the data obtained we can formulate the following assessments, comments and conclusions.

Experiment no. I

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 for acetic acid was the inhibitory effect reported (at the concentration of 33.43%, about 33.4%). The application of diluted acetic acid, in a concentration of 33.43%, causes the destruction of Candida Albicans colonies both in vitro and in vivo. The study can be extended to many other substances, including plant extracts.

Experiment no. II

Through the two experiments (with Candida and Aspergillus respectively) within the study, it is highlighted that in the therapy of skin and mucous mycoses, the local application of products extracted from plants, sold in the form of oils or tinctures, can be recommended. The recommended products are: - marigold and propolis tinctures; - sage and peppermint oils. The recommended concentration is 20%, in daily applications, until complete healing.

Experiment no. III

At the end of the study, we noted an inhibitory action on Candida albicans cultures, determined by the essential oils: of oregano, thyme, geranium, bitters from 50 plants, ganoderma, Swedish drops, superconcentrated Ag solution and Ag ion solution and Au. In the case of peppermint, eucalyptus, sage, lavender oils, propolis tincture, apple cider vinegar and 9% wine vinegar solution (with 1/8 dilution), the antifungal effect was for a short period of time, colonies regenerating; 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 respectively after the application of: essential oil of oregano, essential oil of geranium, essential oil of thyme, bitters from 50 plants and with ganoderma, Swedish drops, superconcentrated colloidal solution with Ag, colloidal solution with Ag and Au ions. The products used in the study can be used repeatedly to achieve a lasting cure.

Experiment no. IV

For oils I used 200 µl of substance (the respective oil)/800 µl of physiological serum, to which I added a few colonies (2, 3) of Candida spp. For apple vinegar, in the first 24 h sensitivity of Candida colonies appears, compared with 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 and frankincense oil 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. The 2% iodine tincture and ginger oil demonstrated their antifungal role in treating candida during the 4-day study.

Experiment no. V

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, thyme (Thymus vulgaris) and sandalwood in a concentration of 20%, as well as licorice tincture (Glycyrrhiza glabra roots), respectively colloidal copper solution. It can be observed that the action of essential oil from cultivated thyme is less effective than that of 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, 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 and geranium oils, as well as colloidal copper solution, used in higher concentrations, could successfully inhibit candida growth.

Experiment no. VI

The substances used in the study (essential oils) were applied in a higher concentration, of 30%. During the 5 days of the experiment, we found that the tinctures

of horsetail (from Dacia Plant) and mousetail (produced by Hofigal), unlike the tinctures of: rostopasca and burdock (from Dacia Plant) inhibited the development of Candida Albicans from the first day to the last day of the experiment. 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, it could be observed at the optical microscope the decrease in the number of candida mycelia. In conclusion, for the 5 days of the experiment, 3 substances stood out: horsetail tincture (from Dacia Plant), mousetail tincture (produced by Hofigal Plant), as well as turmeric capsules 250 & pepper; these can be used successfully to stop the development of Candida albicans and to a lesser extent or, possibly, repeatedly for a lasting effect, you can also use the hot pepper tincture (from Faunus Plant) either separately or in combination with other natural galsubstances known as antifuns.

Experiment no. VII

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.

Experiment no. VIII

In order to prevent the occurrence of candidiasis, it is possible to intervene through: • 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.

Experiment no. IX

The prevalence of the male sex is observed in both groups (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). In terms of 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 noted 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 average 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 vs. 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 % versus 6.2%, obesity (17.5% versus 3.1%). We noted an 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 different aggressions that act on the human body with aging. Following research studies I have discovered numerous natural substances and not only, capable of combating diseases that are sometimes considered difficult to cure (Candida albicans, Aspergillosis). There are certainly countless possibilities to use substance natural herbs either separately or in certain combinations for a rapid antifungal effect. The concentration of substances is an important factor in these cases; we observed that at higher concentrations of some products (20%, 30%) either essential oils or other therapeutic products or substances existing on the market we obtain a very good yield in curing mycosis.

Thus, I discovered countless products (oils, tinctures, therapeutic products, etc.) able to help in these less pleasant situations. In different mycoses, the substances act differently; the study can be extended with more natural substances. Also, the study 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, achieved by the usual drug treatment.

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4. 3.06.2022 – The scientific event **"Man. Environment. Pollution"** at the "Ovidius" University Constanta within the **Romanian Chemistry Society** in its **9th edition**, where both I and the students from the 9th grade "Carol" Commercial College Constanta received participation diplomas B for the special contribution (I participated with a power point together with 2 students); I mention the fact that I am a member of the Romanian Chemical Society.