



Study on the Action of Natural Products on Candida Albicans Crops

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To cite this article:

Olimpia-Nicoleta Moroianu, Nelu-Doru Popescu, Alina Raluca Ursu, Natalia Rosoiu. Study on the Action of Natural Products on Candida Albicans Crops. *International Journal of Biomedical Engineering and Clinical Science*. Vol. 8, No. 3, 2022, pp. 27-32.

doi: 10.11648/j.ijbecs.20220803.11

Received: June 22, 2022; Accepted: July 9, 2022; Published: August 15, 2022

Abstract: The paper aims to highlight the action of some natural substances on Candida albicans crops. The observations were made using different concentrations of substances that acted under conditions of constant temperature and different time intervals. Thus, in the present study we investigated the inhibition of candida development by using essential oils such as: tea tree, oregano, black cumin, coriander, rosemary, juniper, marigold tincture and a graviola capsule for a period of 48 h; for the 72 h we used essential oils, namely: sage, mint, geranium, aloe vera, thyme, tinctures (such as: tincture of marigold, propolis, plantain and chamomile), graviola capsules, colloidal solutions of Ag ions super concentrate, of Au and Ag ions), Lady's Water, Bitter (from 50 plants with ganoderma, respectively Swedish drops), apple cider vinegar and 9% wine vinegar. For the proposed study we put all these substances on dishes with samples of Candida albicans and let them act for 48 hours or 72 hours to understand what is happening. At the end of the experiments we interpreted the results obtained and issued the necessary conclusions, ie we could know for sure if these substances used in the research study are good or not to be able to eliminate Candida albicans from the human body infected with candida.

Keywords: Candida Albicans, Candida Spp, Culture Medium, Essential Oil, Tincture

1. Introduction

Fungal infections are a serious global problem, accounting for more than 1.6 million deaths annually [1].

One million species of fungi are currently recognised, of which 300 are pathogenic to humans and of these over three-quarters primarily infect the skin and subcutaneous tissues.

Superficial fungal pathogens are the fourth commonest cause of any human disease worldwide. Historically, superficial fungal infections have caused minimal disease in temperate climates, with the most severe outbreaks occurring in the tropics and subtropics. The use of potent immunosuppressant and antimicrobial drugs has increased

the incidence of fungal infective episodes in temperate climates. Currently, there is emerging resistance to antifungal medications and to date no human fungal vaccine exists.

Some fungi live on the skin as part of the normal skin flora while others come into contact with the skin through the environment and animals. Superficial fungal infections attack the epidermis, mucosa, nails and hair, and are divided into two groups: moulds (e.g. dermatophytes) and yeasts (e.g. Candida) [2].

Superficial mycoses are prevalent worldwide. They are often caused by dermatophytes and restricted to the stratum corneum. The host's immune response against infections caused by dermatophytes basically depends on the host's defense against metabolites of the fungi, virulence of the infecting strain or

species and anatomical site of the infection [3]. Infections caused by *Candida* spp. are the most frequent, accounting for 80% of cases of systemic fungal infections [4, 5].

Candidiasis is a condition caused by yeasts of the genus *Candida*, especially by the species *Candida albicans* and, occasionally, by other species.

Candida albicans can cause superficial infections of the mucous membranes and skin and deep infections of the internal organs (endocarditis, meningitis, etc.) under immunosuppressed conditions [6].

This rather plethoric genus (*Candida*) comprises over 200 species. The most important species of medical interest belonging to the genus *Candida* are: *albicans*, *african*, *boidinii*, *catenulata*, *chiropterorum*, *colliculosa*, *curvata*, *ciferrii*, *dubliniensis*, *famata*, *glabrata*, *globosa*, *guilliermondii*, *haemulonii*, *holmii*, *inconspicua*, *intermedia*, *kefyr*, *krusei*, *lambica*, *lipolytica*, *lusitaniae*, *magnolia*, *melibiosica*, *membranaefaciens*, *norvegensis*, *norvegica*, *parapsilosis*, *pelliculosa*, *pulcherrima*, *rugosa*, *maris*, *pararugosa*, *tropicalis*, *usu*, *valida*, *viswanathii*, *zeylanoides* [7]. Candidiasis is a fairly common fungal infection, affecting both women and men and can occur at any age. They can be located either superficially - on the skin and mucous membranes - or deep, systemic or visceral.

Candida albicans is a saprophyte of the digestive and vaginal mucosa, in biological balance with endogenous bacterial flora. Factors that disrupt the specific and non-specific defense mechanisms of the human body are:

1.1. Factors Related to the Terrain of the Individual

1. physiological: pregnancy, age (the two extremes of age);
2. pathological: AIDS, endocrine diseases (diabetes, Cushing's syndrome, Addison's disease, hypothyroidism, hypoparathyroidism), iron deficiency, malignant haematological diseases (Hodgkin's disease), skin lesions with loss of substance, Sjögren's syndrome, imbalance of intestinal and cutaneous flora, liver disease, tuberculosis, visceral neoplasms;
3. local skin factors: moisture, maceration, obesity.

1.2. Iatrogenic Factors

- 1) prolonged antibiotic therapy;
- 2) corticosteroids in general, sometimes for topical use;
- 3) other immunosuppressants [8].

Of such species, *C. parapsilosis* has already been ranked as the second most frequent cause of candidemia in some countries of Southern Europe, Africa, North America and, notably, in a great portion of South America, especially in neonates, transplant recipients and patients with other malignancies such as cancer [9-12].

Candida albicans is the most pathogenic species of *Candida*, and the host-fungus interaction can be seen as a meeting between fungal virulence and host defense mechanisms [13].

Candidiasis usually manifests as superficial cutaneous-mucosal infections, but can also progress to the disseminated form, candidemia [14].

High frequency of antimicrobial resistance to the commonly

tested antibiotics is a concerning alarm. Therefore, effective infection control programs should be established promptly [15].

2. Material and Methods

The study was conducted between February 15 and June 30, 2019 at the Clinic "Provita Medical Center 2000" in Constanța. The aim of this study was to investigate the effectiveness of several products (essential oils, tinctures, capsules, bitters, etc.) in the treatment of *Candida albicans*.

To highlight the action of natural products on *Candida albicans* cultures, we used standard Sabouraud culture medium, on which we seeded samples of *Candida albicans*, from a calibrated assortment called ATCC. I inserted the plates on the thermostat, at the standard temperature of 37°C [16]. In the present study we proposed a series of experiments, some of which were performed over 48 hours and others for 72 hours using natural products (essential oils, tinctures, capsules, etc.) to highlight the action of substances on crops of *Candida albicans*.

3. Results

I watched the action on *Candida* of some essential oils such as: tea tree, oregano, black cumin, coriander, rosemary, juniper, marigold tincture and a graviola capsule for a period of 48 hours; also for the 72 h I used essential oils (sage, mint, geranium, aloe vera, thyme), tinctures (of marigold 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 cider vinegar and 9% wine vinegar.

3.1. 48 h Experiments

We prepared dilutions of 200 µl substance / 800 µl saline. In this experiment we used tea tree essential oil, oregano, black cumin, coriander, rosemary, juniper and marigold tincture (Figure 1 and Figure 2). We also dissolved 1 capsule of graviola (pure extract) in 2 ml of saline. A vegetable capsule of graviola contains 5:1 extract of graviola fruit (*Annona muricata*) - graviola powder-200 mg. To all this we added 2-3 colonies of *Candida* spp. *Candida* is sensitive to oregano oil and tea tree oil.

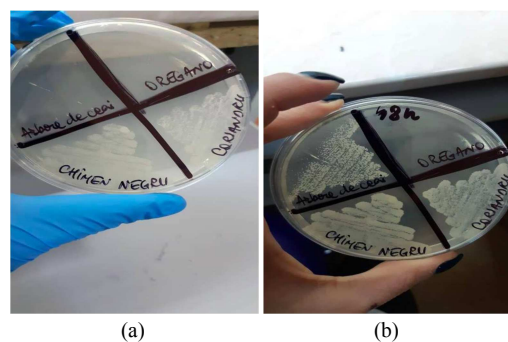


Figure 1. (a, b). Culture plates seeded with *Candida albicans* on which oils of: tea tree, oregano, black cumin and coriander were applied in a concentration of 20% at 24 h and 48 h.

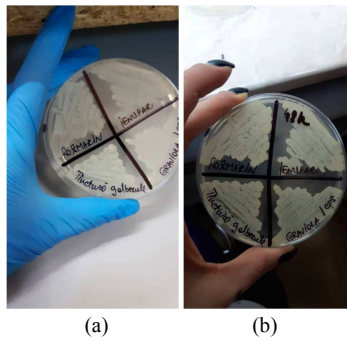


Figure 2. (a, b). *Candida albicans* seed plates on which rosemary and juniper oils were applied in a concentration of 20%, as well as marigold tincture and graviola solution at 24 h and 48 h.

The plates were left on the thermostat for another 24 hours. At 48 h the result was read: candida was sensitive only to oregano oil.

3.2. 72 h Experiments

- a) *Candida* plates with sage oil, peppermint oil, geranium oil and propolis tincture were kept at the thermostat for 72 h.

Dilutions of 200 μ l substance / 800 μ l saline were made for essential oils, and propolis tincture was used directly from the bottle. After 24 hours, all 4 substances had an inhibitory effect on candida cultures.

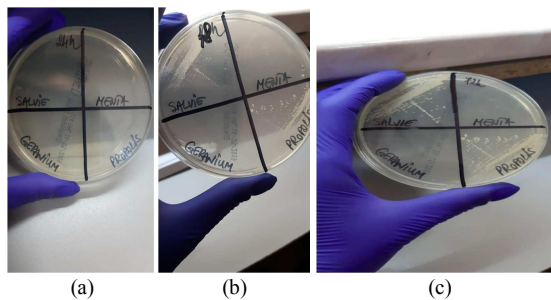


Figure 3. (a, b, c). *Candida albicans* seeded culture plates on which sage, mint, geranium oils were applied in a concentration of 20%, as well as propolis tincture at 24 h, 48 h and 72 h.

At 48 h the reading was done again and I found that only the geranium oil and a little peppermint oil acted as inhibitors, as well as after 72 hours from sowing.

- b) I used lavender oil, eucalyptus oil, plantain tincture, as well as chamomile tincture.

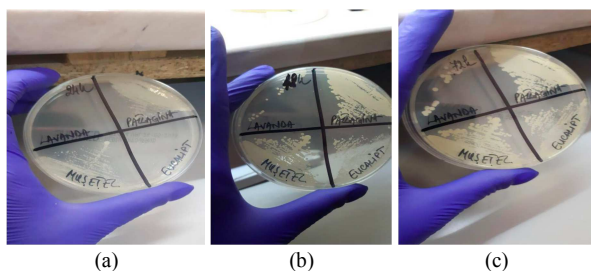


Figure 4. (a, b, c). *Candida albicans* seed plates on which lavender and eucalyptus oils were applied in a concentration of 20%, as well as plantain and chamomile tincture at 24 h, 48 h and 72 h.

In the first 24 hours, lavender and eucalyptus managed to remove the candida, but after 48 and 72 hours, respectively, the 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 μ l substance / 800 μ l saline;

The tincture was used directly from the bottle, and the graviola capsules were dissolved in 4 ml of saline.

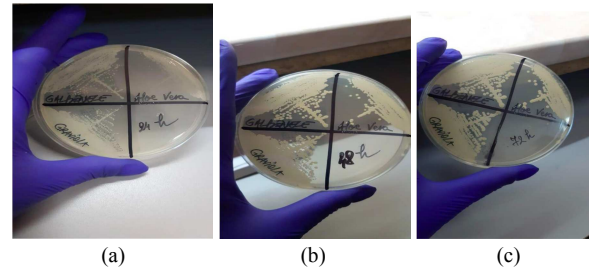


Figure 5. (a, b, c). *Candida albicans* seed culture plates with 20% aloe vera oil and marigold tincture and graviola solution obtained after dissolving the 2 capsules at 24 h, 48 h and 72, respectively h.

- d) I put thyme essential oil on the candida plates, super concentrated Ag + silver ion solution (colloidal Ag 30 ppm), Au and Ag ion solution with nanometric particles in structured and distilled water (15 ppm, water distilled and structured). We took 5 ml of solution of Ag ions (super concentrated) and about 5 ml of solution of Au and Ag ions (4 puffs) fixed as they were in bottles (without other dilutions). A colony of *C. albicans* was dissolved in the solutions taken as such and then seeded in Sabouraud medium and left to the thermostat for 72 hours.



Figure 6. Bottles with colloidal solutions with Ag (colloidal silver spray), respectively spray with Ag and Au.

For thyme oil I took 200 μ l of substance and added 800 μ l of saline. In thyme oil, silver ions (Ag^+) and gold and silver ions, the sensitivity was seen from the beginning and later, ie 48 hours and 72 hours, respectively.

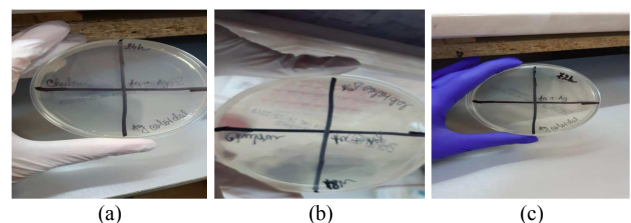


Figure 7. (a, b, c). Crop plates seeded with *Candida albicans* on which 20% thyme oil was applied, concentrated solution of superconcentrated colloidal Ag, solution of Au and Ag with nanometric particles in structured and distilled water at 24 h, 48 h and 72 h.

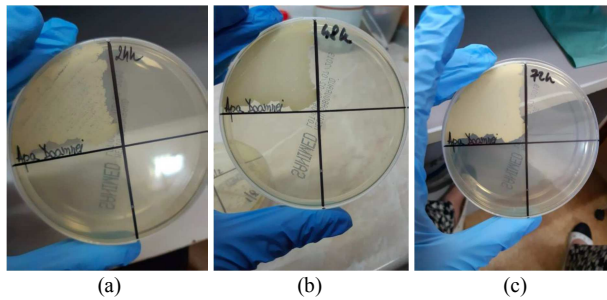


Figure 8. (a, b, c). Culture plates seeded with *Candida albicans* on which the lady's water was applied at 24 h, 48 h and 72 h.

e) Lady's water (therapeutic water riched in Ca and Mg)

From this I sowed Sabouraud in the medium. After 3 days I could see.

From this I sowed Sabouraud in the medium. After 3 days I could see that the result is zero; the product used was ineffective.

In a 2 ml of product called "Lady's water" I put a colony of *Candida Albicans*, until a concentration of 0.5 McFarland was formed.

f) In 2 ml of substance (Bitter of 50 plants produced by Dacia Plant, Swedish drops from BANO and apple cider vinegar) we dissolved 2-3 colonies of candida; also, the 2 Para Fight pills from Coral, were dissolved in 4 ml of saline, we added 2-3 colonies of candida; I put them on a plate and left them on the thermostat for 3 days to read the result.



Figure 9. Cultivated test tubes in Sabouraud medium with *Candida albicans* and Swedish drops, bitter from 50 plants with ganoderma, parafight and apple cider vinegar.

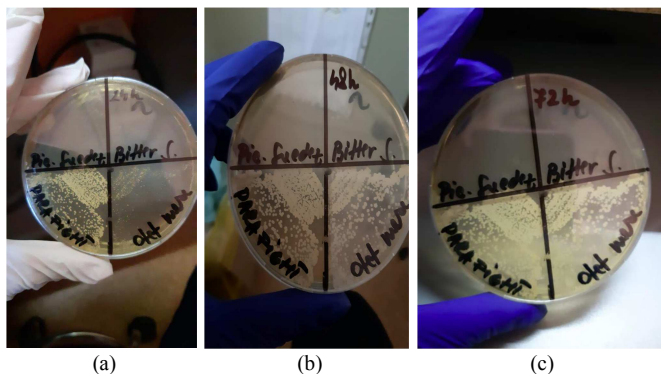


Figure 10. (a, b, c). Culture plates shown with *Candida albicans* on which Swedish drops were applied, bitter from 50 plants with ganoderma (romanian product), Para Fight (from Coral) and apple cider vinegar at 24 h, 48 h and 72 h.

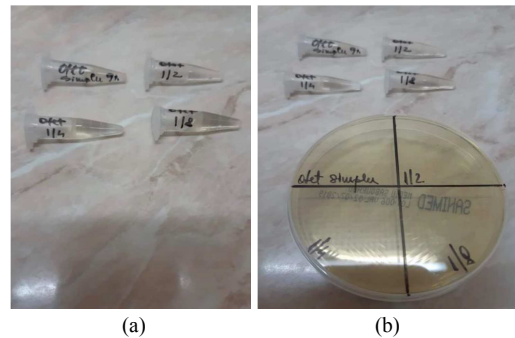


Figure 11. a. Vinegar samples and 1/2, 1/4, 1/8 dilutions of vinegar, b. Petri dish seeded with *Candida albicans*, vinegar and various dilutions of vinegar.

After the first 24 hours, it was observed that the bitter obtained from 50 plants, as well as the Swedish drops had an inhibitory effect on the candida cultures. After 72 hours from the beginning of the experiment, the two types of bitter, Swedish drops and bitter from 50 plants with ganoderma (Romanian product made by Dacia Plant) obviously inhibited the candidiasis cultures.

g) Wine vinegar with a concentration of 9%

We dissolved a colony of *Candida albicans* in saline until the McFarland concentration was reached.

I made 3 dilutions of vinegar made from wine that is commercially available.

Dilution 1/2

I mixed 0.5 ml of the dilution originally made with 0.5 ml of vinegar.

Dilution 1/4

I took 0.25 ml of the dilution originally obtained over which I added 0.75 ml of vinegar.

Dilution 1/8

To 0.125 ml of the dilution I added 0.875 wine vinegar.

All the dilutions thus obtained were applied on Sabouraud medium sown with *Candida Albicans* and were thermostated at 37°C.

After 24 hours, an inhibitory action could be observed at the last dilution (1/8), but after 42 hours, respectively 72 hours, the result is zero; *Candida* has returned.

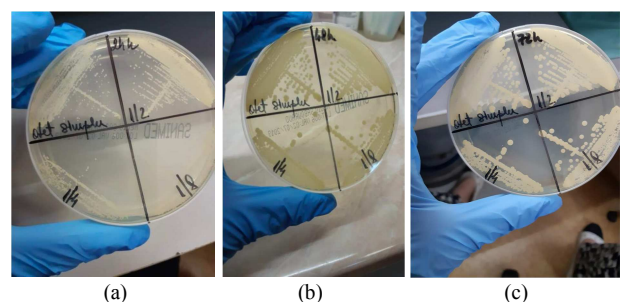


Figure 12. (a, b, c). *Candida albicans* seeded culture plates on which vinegar solutions were applied (in the original form found in the glass, as well as dilutions of 1/2, 1/4, 1/8), thermostated for 24 h, 48 h and 72 h.

4. Discussions

The substances analyzed in the study were prepared in different dilutions and applied by means of absorbent washers

as in the case of fungigrams or antibiograms. These substances were either in the form of tinctures or in the form of essential oils. We then analyzed the size of the diameter of the inhibition and lysis areas of the mycelial colony at different time intervals (24 hours, 48 hours and 72 hours) [16].

For 24 hours, lavender, eucalyptus, sage and propolis tincture had an inhibitory effect on *Candida albicans*. Peppermint oil acted for a little over 24 hours, but the best were the oils of oregano, thyme and geranium, the bitter of 50 plants with ganoderma, as well as the Swedish drops. The super-concentrated solution of Ag, as well as solution with Ag and Au ions.

In the case of the experiments carried out during 48 h, an inhibitory action of tea tree and oregano oils was observed for the first 24 h against the black cumin and coriander oil existing on the same plate, subsequently, at 48 h, it remained only oregano oil with inhibitory action. Rosemary oil, juniper oil, marigold tincture and graviola (1 dissolved capsule) did not work.

For the following experiments (for 72 h), after 24 h after sowing, sage, mint, geranium and propolis tincture oils removed candida, at 48 h and 72 h only geranium and mint oils are less effective than of sage oil and propolis tincture. Lavender oil is effective for 24 hours compared to eucalyptus oil, plantain tincture and chamomile. Within a few hours of applying lavender oil, candida culture reappears.

Bitter from 50 plants with ganoderma, Swedish drops and less apple cider vinegar inhibited candida in the first 24 hours, compared to the product "parafight" (from Coral); further, 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 hours, after which candida increased in the 4 dilutions proposed for analysis.

It is a good idea to use these substances successfully and also in certain combinations. If a tincture contained extracts from two or even three plants it would have a better result in a shorter time. We can combine thyme essential oil with lavender, geranium oil with sage and mint, propolis tincture with chamomile oil, aloe vera oil with tea tree oil or, possibly, add more peppermint oil. Certainly, frequently consumed wine vinegar would help stop the multiplication of candida or used in bathrooms or by washing the place infested with wine vinegar solution, respectively 1/8 dilution as we discovered in the study. The colloidal solutions of Ag and the one with Ag and Au ions were very effective, as well as the bitter from 50 plants and the Swedish drops, which means that we can use them with confidence in the treatment of *Candida albicans*.

5. Conclusions

At the end of the study we noticed an inhibitory action on the cultures of *Candida albicans* determined by the essential oils of oregano, thyme, geranium, bitter from 50 herbs with ganoderma, Swedish drops, superconcentrated solution of Ag

and solution with Ag ions and Au. In the case of peppermint, eucalyptus, sage, lavender, propolis tincture, apple cider vinegar and 9% wine vinegar solution (with 1/8 dilution) the antifungal effect was for a short time, the colonies regenerating, for this reason it would be advisable to repeat the treatment with these substances at certain intervals to successfully control *Candida albicans*.

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

1. oregano essential oil;
2. geranium essential oil;
3. thyme essential oil;
4. bitter from 50 herbs with ganoderma;
5. Swedish drops;
6. colloidal solution superconcentrated with Ag;
7. colloidal solution with Ag and Au ions.

The above mentioned products can be used alternatively and repeatedly in order to obtain a lasting healing.

Abbreviations

h- hour, ATCC - standard culture of *Candida albicans*, µl - microliters, ml-milliliters, McFarland - about 10^5 - 10^6 cells / ml, Au - gold, Ag- silver, Ag + - silver ions, Ca- Calcium, Mg- magnesium.

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