Evaluation of antifungal activity of some essential oils on Candida species

اعداد:

ريام خالد صلاح

لبنى عقيل صادق

أشراف:

د.قاسم فوزي عبد الكريم

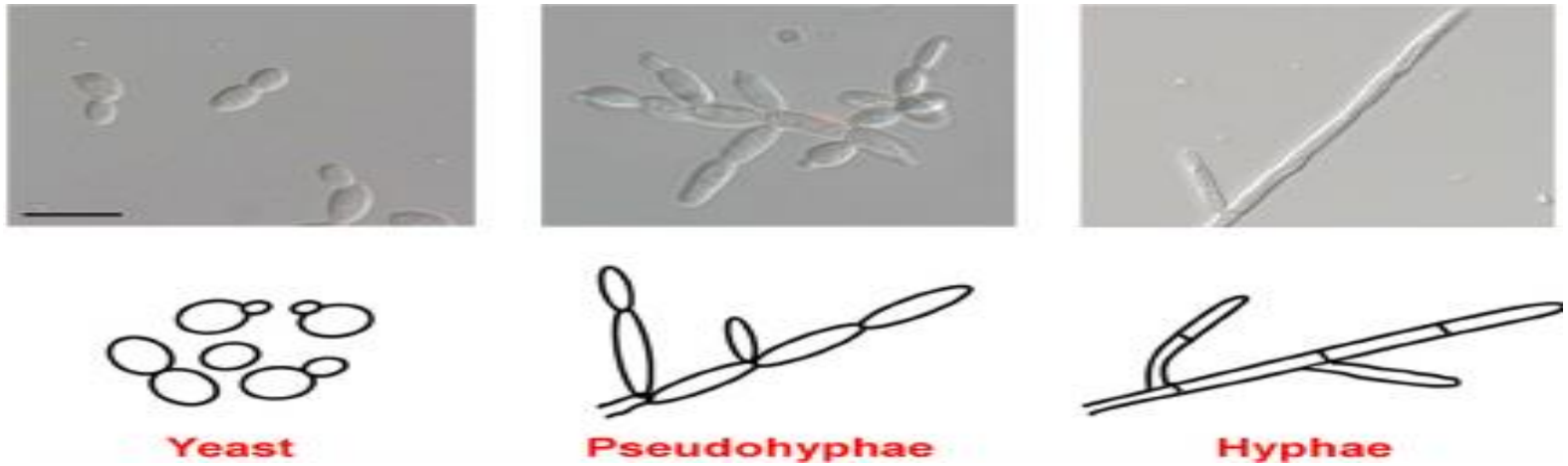
د. زينب صبيح

Introduction

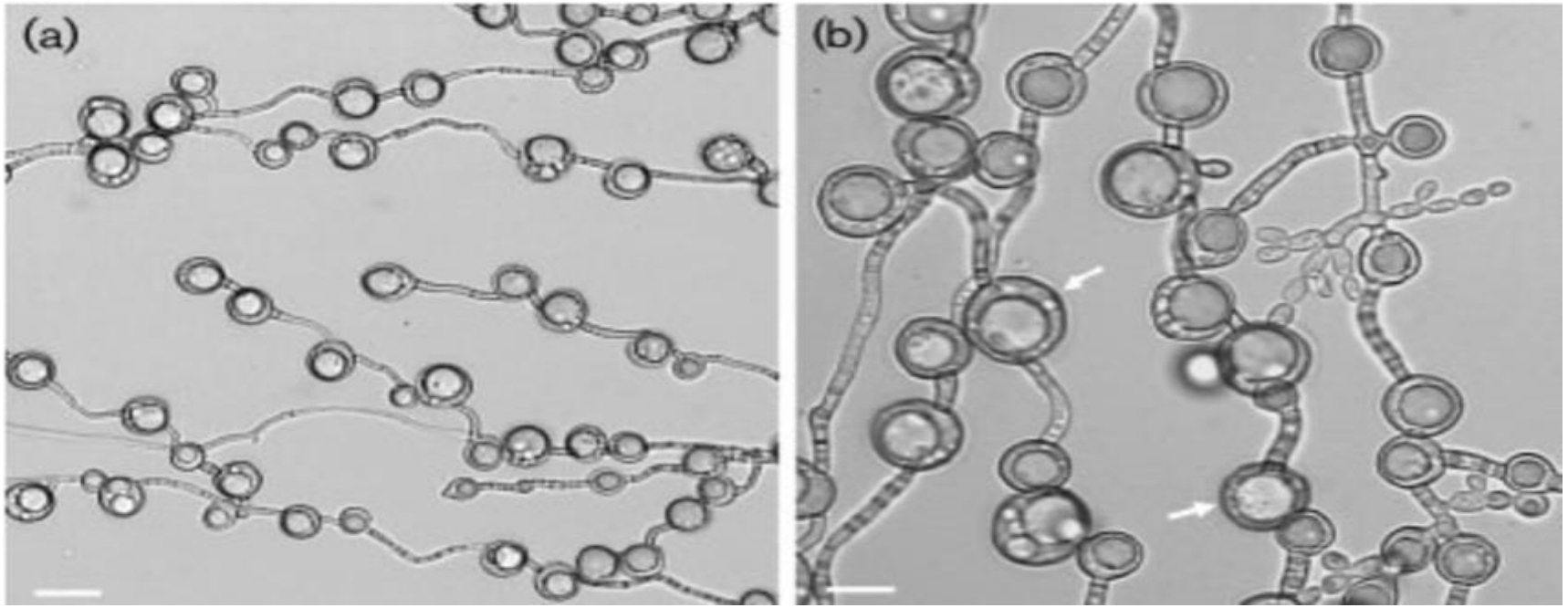
- Fungus is Eukaryotic organisms, have rigid cell walls containing glucans and chitin. Incapable of producing food (heterotrophs

- They are opportunistic especially in:
- immunocompromised patient ex: AIDS, malignancies, lymphomas, leukemia, conditions like diabetes mellitus, TB etc.
- Prolonged use of antibiotics, anticancer drugs & anti-inflammatory drugs Low birth weight neonates .
- Therapeutic procedures eg. organ transplantation, open heart surgery, artificial heart valves, indwelling catheters e.g. urinary & parenteral drug administration.
- Burn patients are the most susabtable to develop type of candida infections like nosocomial bloodstream infections, candidaemia candidiasis ,oral thrush, vaginitis etc.

- Candida can grow in four forms:
- **First**. Under standard conditions with optimal nutrients, yeast grow in log phase as budding cells (blastoconidia), which are spherical to oval in shape and are approximately 2–5 × 3–7 µm in size. It's the harmless form of candida.
- **Second**. certain species, such as can produce a filamentous type of growth, such as true hyphae *in vitro* and *in vivo*, depending on environmental conditions.
- **Third.** More frequently, *C. albicans* also can produce pseudohyphae

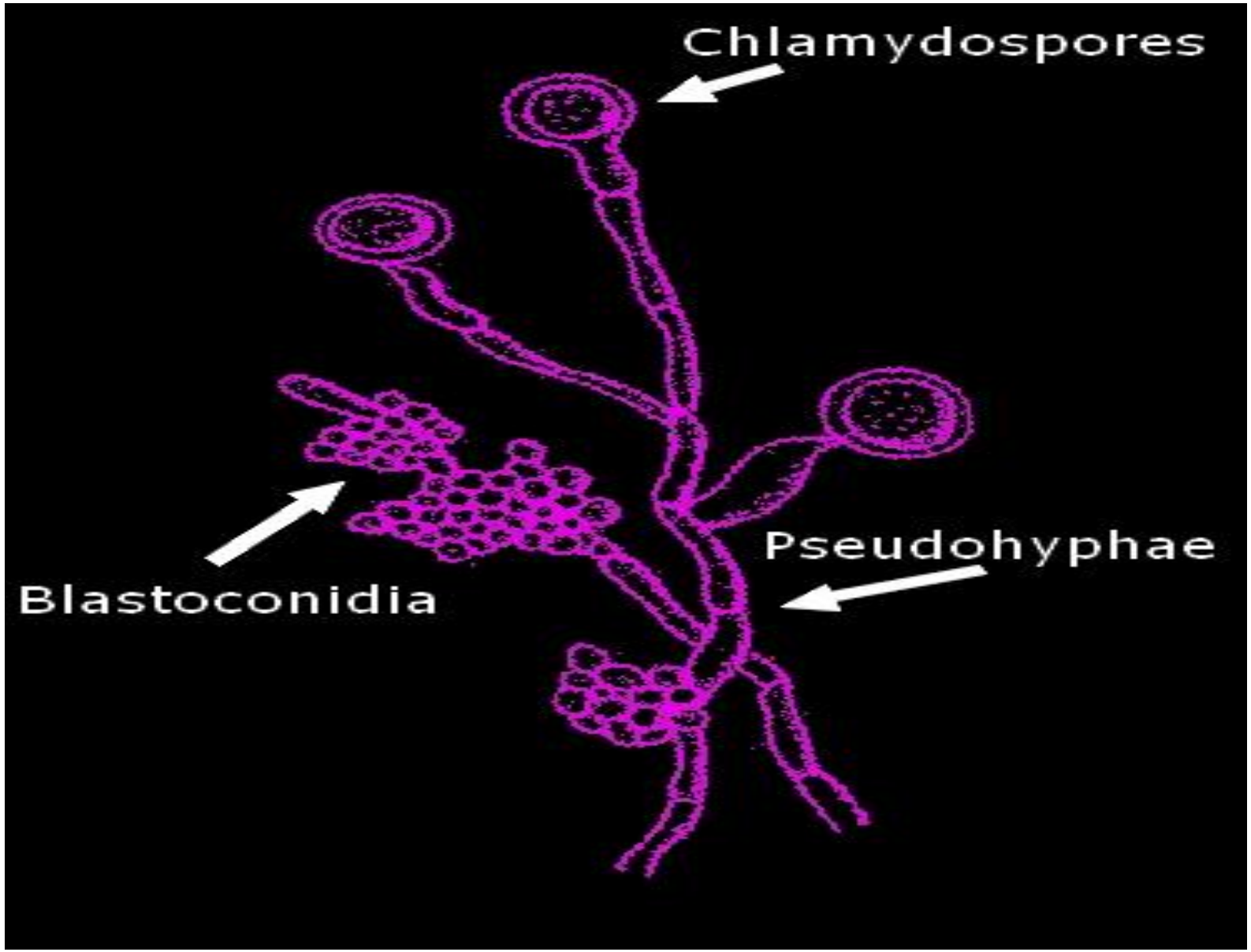


- **Fourth.** only in the recent years that some pseudohyphae converts into spores called Chlmydospores, reasons for spores formations is unknown but some theories said its form when the nutritions are depleted and due to dehydration,
- Air always contains thousands of fungi spores or conidia, in a metabolically inactive reversible rest period which enables them to survive adverse environmental conditions.

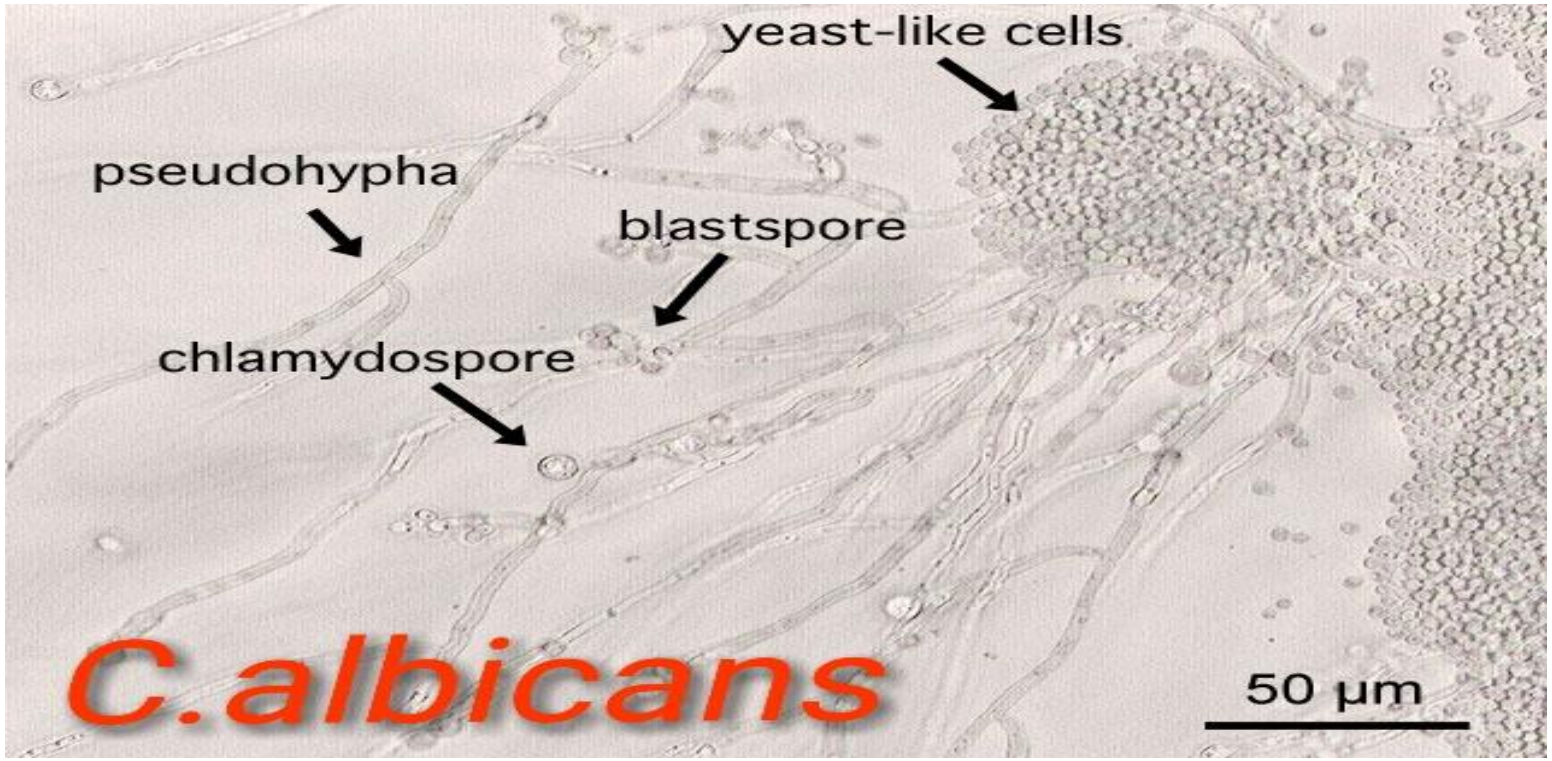


- Transitions between the two phenotypes can be induced in vitro in response to several environmental causes such as pH or temperature, or different compounds such as Nacetylglucosamineor proline. However, perhaps the most critical criterion for pathogenicity is the induction of the mycelial form by serum or macrophages. In addition to the intrinsic biological interest of this dimorphism, its ability to switch between the yeast and the hyphal mode of growth has been implicated in its pathogenicity.

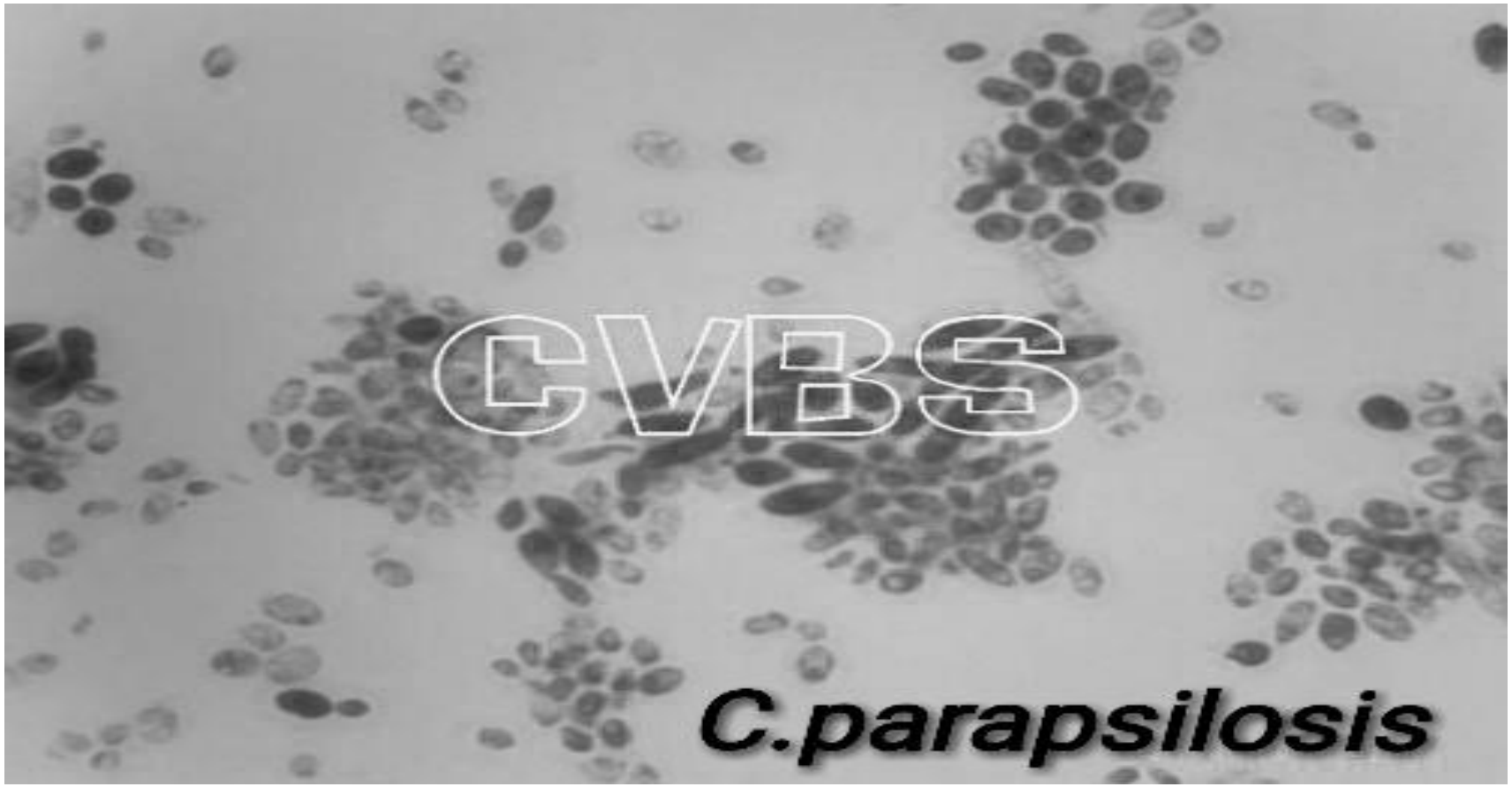
- All species produce blastoconidia singly or in small clusters. Blastoconidia may be round or elongate. Most species produce pseudohyphae which may be long, branched or curved. True hyphae and chlamydospores are produced by strains of some *Candida* spp.


Chlamydospores
Blastoconidia
Pseudohyphae

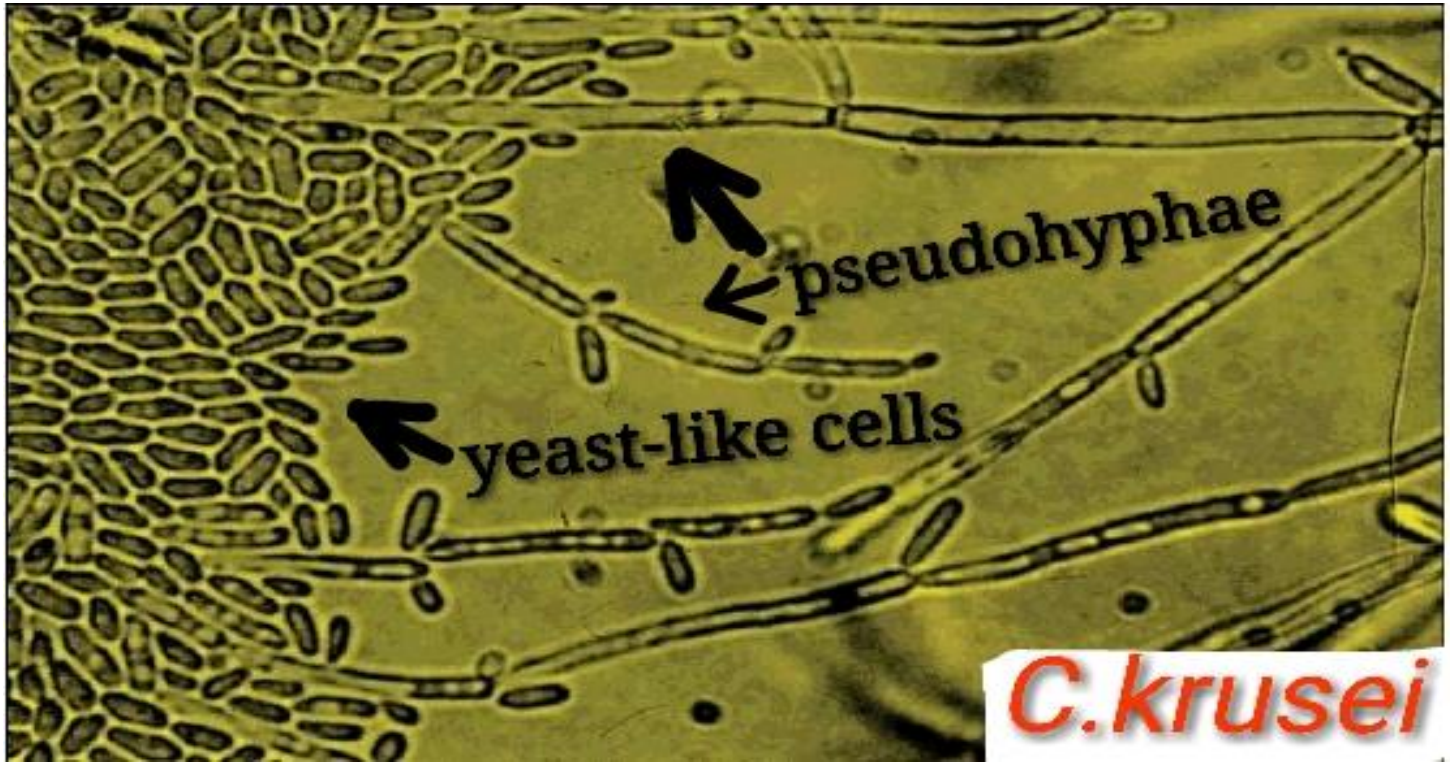
- *Candida albicans* is characterized by its morphological plasticity, called polymorphic. It is capable of vegetative growth *in vitro* and *in vivo* as ovoid budding yeast-like cells. These appear as smaller round 'grape-like' clusters.
- Also grow as branching filamentous called pseudohyphae or parallel-sided hyphal cells.



- *C. parapsilosis* reproduces by budding, ovoid, elliptical and elongated occurring singly, in pairs or short chains or cluster does **not** produce true hyphae, but can generate only pseudohyphae that are characteristically large and curved, and often referred to as ‘giant cells’.



- C.Krusei is found growing as a single cell or as pseudohyphae in the single cell form, the cells of *C. krusei* are round or ovoid and can be easily separated while in the pseudohyphae form the cells are more elongated and attached to neighboring cells
- C.krusei is a yeast species that does **not** produce spores.
-



- For the pathogenicity of *C.albicans*, its ovoid-shaped budding yeast and parallel-walled true hyphae forms are the most important

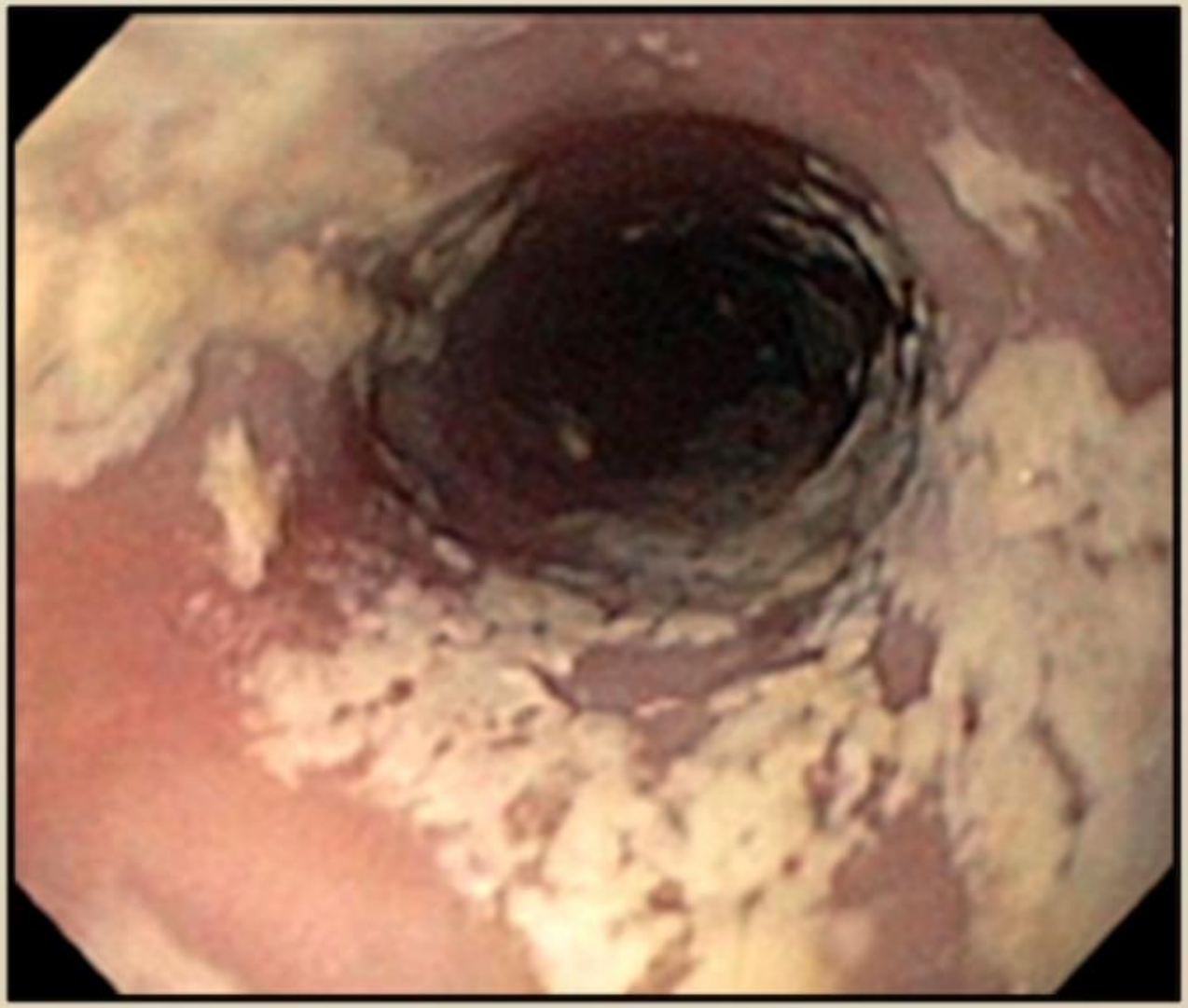
- while the yeast form is believed to be important in the spread of *Candida*.

- There are 3 major type of infections caused by Candida albicans:

-oropharyngeal candidiasis, vulvovaginal (genital) candidiasis, and invasive candidiasis (candidemia). Among the candida spp, C.albicans is the most commonly associated with candidemia

- *C. albicans* infections are usually treatable with fluconazole, while severe infections require amphotericin B

Thrush



Esophageal candidiasis

- C.Krusei is considered a relatively uncommon, emerging opportunistic human pathogen with high mortality that infects mainly cells

- C. krusei is found primarily in patients with haematological malignancies or bone marrow transplant patients who are receiving fluconazole prophylaxis)

- It is inherently resistance to fluconazole and exhibits a reduced susceptibility to other antifungal drugs

- *C. parapsilosis* can cause Endocarditis when patients using prosthetic valves, intravenous drug, or having intravenous parenteral nutrition, abdominal surgery, immunosuppression, treatment with broad-spectrum antibiotics, and previous valvular disease
- *Candida parapsilosis is occasionally encountered in onychomycosis.*
- the treatment is still unknown

- Polyenes and azoles have been the antifungals of choice in the treatment of these fungal infections. However, many problems remain to be solved for most of the available antifungal drugs such as nephro- and hepato-toxicity associated with the use of amphotericin B. A lipid formulation of amphotericin B is less toxic but more costly.

- Azoles, particularly fluconazole, are less toxic after oral or intravenous administration and consequently are often employed. However, azole therapy failures have been observed due to intrinsic resistance in *Candida* spp. such as *C. krusei* and acquired resistance in previously susceptible strains of *C. albicans* due to their continual use in AIDS and cancer patients.

- About 3.6–7.2 % of *C. albicans* isolates from women with vaginitis are resistant to fluconazole and, oropharyngeal candidiasis due to the fluconazole-resistant *Candida* has long been a problem for HIV patients. Since the immunocompromised population is increasing in number, proper medical therapy is needed to overcome problematic drug resistant strains of *C. albicans*

- In developing countries considering the limitations of currently available antifungal drugs regarding toxicity, activity, cost, and emerging resistance, the search for new alternative strategies is justified.

- Herbal medicine has been used successfully for many years. Herbal medicines are a promising source of therapeutics in the pharmacological

- Black seed oil ,ginger and lemon oil show according to other studies antifungl activity and have promising future to prepared pharmaceutical drugs for the resistance species

- Many studies are on to find alternative antifungals whether they are pharmaceutical drugs or just homemade remedies. We'll focus in this research on three species: *C.albicans* , *C.krusei* , *C.parapsilosis* by using these oils

-

9 BLACK SEED OIL BENEFITS

May Prevent and Help Manage Diabetes

Anti-Cancer Activity

Protects Liver

Anti-Inflammatory

Promotes Skin Health

Anti-Bacterial and Anti-Fungal

Promotes Weight Loss

Boosts Immunity

Fights Allergies

- **Ginger**
- (*Zingiber officinale*) is a ***flowering*** plant that use as spice or in flolk medicine.

- The characteristic fragrance and flavor of ginger result from volatial oils that compose 1-3% of the weight of fresh ginger, primarily consisting of zingeron, shogaols and gingerols with gingerol (1-[4'-hydroxy-3'-methoxyphenyl]-5-hydroxy-3-decanone) as the major pungent compound. Zingerone is produced from gingerols during drying, having lower pungency and a spicy-sweet aroma

- The **lemon**, *Citrus limon*
- Citrus essential oils (CEOs) are a mixture of volatile compounds consisting mainly of monoterpene hydrocarbons and are widely used in the food and pharmaceutical industries because of their antifungal activities

Aim of the study

- Evaluate the antifungal activity of extracts of different home remedy materials on Candida spp.
- Produce an effective treatment, medical or non-medical.
- Compare between the old typical treatment with the new ones.
- Resolve the problem of resistance to the present anti-fungal drugs.

MATERIALS AND METHODS

- **Organisms:**

  Isolated *Candida albicans*, *C.krusei*, *and C.parapsilosis*.

- **Tested materials:**

  Fluconazole 50 mg tab, nystatin drops, Lemon oil, ginger, black seed oil

- **Others materials and tools:**

  Distilled water, cooled incubator, autoclave, petri dish, loop, microtubes.

Methodology:

- The most used are, disk diffusion method, agar dilution method, Broth dilution methods and others.

- In our research we'll use the well diffusion method.

1) media:

- In this research, we used the Sabouraud Dextrose Agar (SDA)
- Sabouraud Dextrose Agar (SDA) is used for the isolation, cultivation, and maintenance of non-pathogenic and pathogenic species of **fungi** and **yeasts**.
- The pH is adjusted to approximately 5.6 in order to enhance the growth of fungi, and to slightly inhibit bacterial growth in clinical specimens.

Ingredients of SDA	Gm/L
Mycological peptone (enzymatic digest of casein and animal tissues)	10 gm
Dextrose	40 gm
Agar	15 gm
Distilled water	1000 ml
pH adjust to 5.6 at 25^0 C	

- **Extraction of the antifungal substance from the rude materials:**

- **Lemon oil:** the oil is use as it is.

- **Ginger:** dissolve the powder in ethanol to extract the lipid-soluble antifungal substance.

- **Black seed oil:** the oil is use as it is.

isolation of Candida species fungi:

Isolation of candida species from oral cavity and fingertips of complete denture wearers.

Activation of fungi:

Loopful fungal spores were streaked on SDA plates and incubated at 37° c for 2-3 days.

Screening and evaluation of antifungal activity

Antifungal effect of 3 materials are testing to determine the ZOI of Candida species was performed by well diffusion method

Procedure for preparation of media:

1) SDA agar medium was prepared from commercially available dehydrated base by dissolve a certain amount in a certain volume of D.W, according to the manufacturer instructions.
2) Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3) Sterilization by autoclave at 121° C for 15 minutes.
4) Cool to 45 to 50° C
5) Pour the freshly prepared and cooled medium into the glass or plastic flat bottomed petri dishes till the level, horizontal surface to give uniform depth.

- For processing of specimen, select and streak 3-5 well-isolated colonies from an agar plate culture. The top of each colony was touched with a sterile loop, and the growth was transferred into another plate.

Testing the antifungal effect of each material on each Candida species:

1. Use fluconazole tab by grinding it and dissolved in DW , few Nystatin drops ,lemon oil ,ginger powder and black seed oil.

2. Make holes in each agar and put few drops of each substances.

3. Incubate the plates at 25 – 30° C in an inverted position (agar side up) in cool incubator (high humidity) for 4 days.

4. Examine the result.

Results

Inhibition diameter (mm)			
Antifungal drugs	**C.albicans**	**C.krusei**	**C.parapsilosis**
Nystatin 25 µg/ml	14 mm	14 mm	10 mm
Fluconazole 5 µg/ml	15 mm	15 mm	9 mm


0.5
0.25
S. Para
0.25

- For C.albicans:
- Lemon oil gave an inhibition zone of 13.3 mm.
- Black seed oil gave zone of inhibition of 30 mmm
- Ginger gave zone of inhibition of 2 mm.

- For C.parapilosis
- Lemon oil gave inhibition zone of 30 mm
- Black seed oil gave inhibition zone of 30 mm
- Ginger didn’t give inhibition

- For C.krusei
- Lemon oil gave inhibition zone of 15 mm
- Black seed oil gave inhibition zone of 20 mm
- Ginger gave no growth inhibition

Discussion

- Usually fluconazole shows a diffuse zone of inhibition which is difficult to read and measure. And although it shows some growth inhibition zone in this experience but it was less than the supposed standard zone of inhibition of fluconazole for candida species. 25 µg/ml fluconazole gives zones of inhibition of ≥20 mm at 24 h but all our experience species didn't have a ZOI more than 15 mm although we use a 50 µg/ml concentration and not 25 µg/ml.
- Same with nystatin , our results was less than the standard ZOI of nystatin for Candida spp.
- The Black seed shows a good ZOI for the three spp. Due to its strong antifungal activity. The lemon oil shows a moderate activity and causes growth inhibition of C.albicans and C.parapsilosis but no inhibition of C.krusei due to its high resistance. The ginger has weak antifungal activity and cause growth inhibition of only C.albicans.

- Based on their Minimum fungicidal concentration, effective oils were categorized into three categories. Who exerted fungicidal effect at less than 0.15% concentration of oils, were grouped into the most effective class.
- The oils exhibiting MFCs in the range of 0.16–1.5% concentration were considered moderately effective (Lemon oil).
- Oils which required more than 1.5% concentration, were regarded as less effective (ginger).
- The Fluconazole-resistant species was sensitive to black seed and lemon oil. Results of this study indicate that oils of plant origin may find use as potential anti-Candida agents.

MECHANISMS OF CANDIDAL RESISTANCE TO SYNTHETIC DRUGS

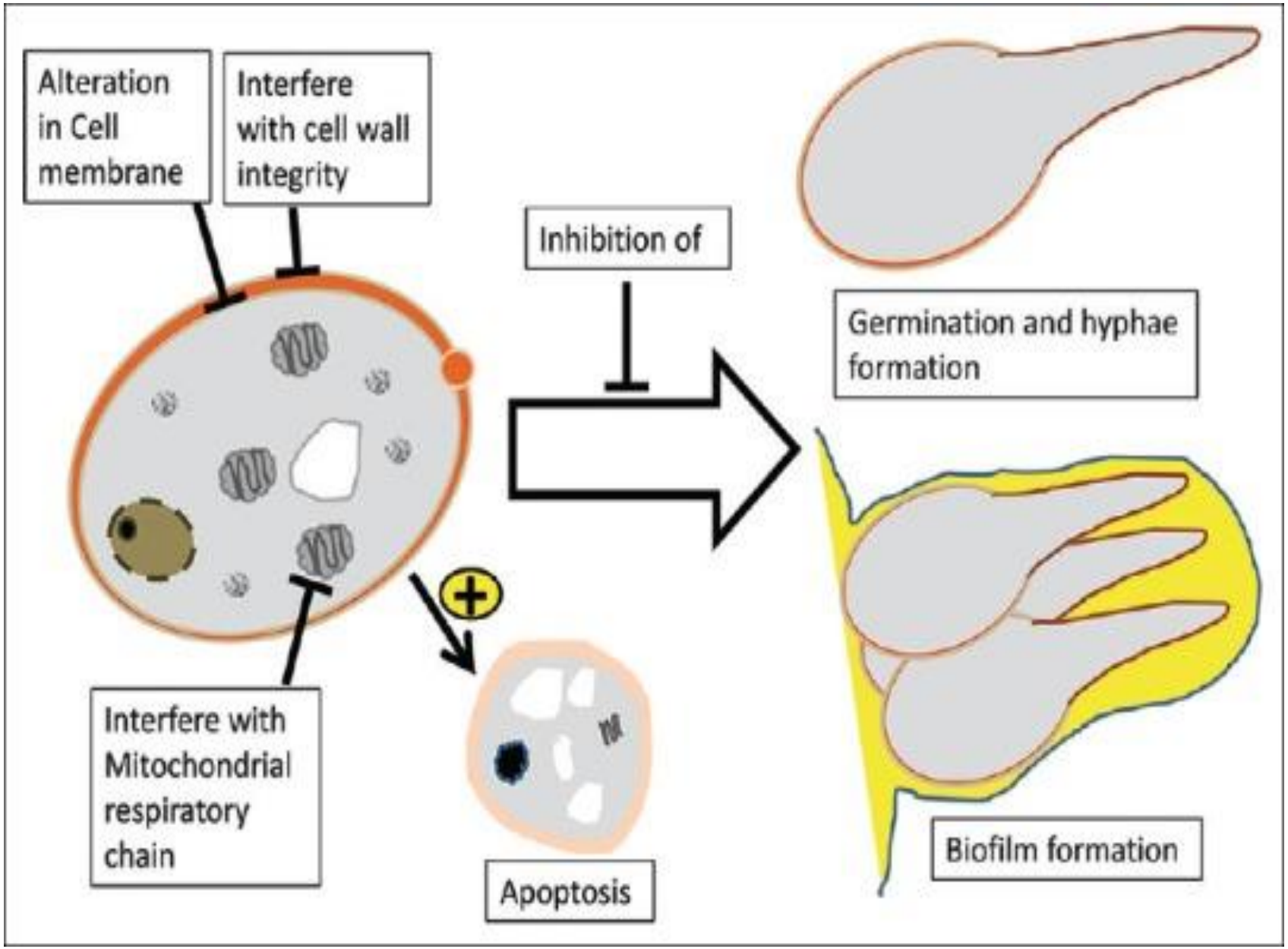
- The formation of biofilms in *Candida* and the transition from planktonic to sessile form are mainly associated with highly resistant phenotype. Other mechanisms of resistance include the expression of resistance genes, particularly those encoding efflux pumps, and the presence of persister cells. Major synthetic drugs that develop candidal resistance include 5-flucytosin, amphotericin B, azoles, and echinocandin.

MECHANISM OF ACTION OF ANTI-CANDIDA NATURAL PRODUCTS

- The anti-*Candida* mechanisms of action initiated by plant natural products can involve inhibition of germination and biofilm formation, cell metabolism, cell wall integrity, cell membrane plasticity, or can involve induction of apoptosis.

- Numerous essential oils have been tested for *in vivo* and *in vitro* a timycotic activity and some demonstrated to be potential antifungal agents. Their mechanism of action appears to be predominantly on the fungal cell membrane, disrupting its structure causing leakage and cell death; blocking the membrane synthesis; inhibition of the spore germination, fungal proliferation and cellular respiration

- In summary, the results presented in this paper clearly demonstrate the antifungal potential effect of selected plant oils. Black seed oil, lemon oil, ginger oils. These results not only encourage further examination of the efficacy of plant oils against other forms of systemic and superficial fungal infections


Alteration in Cell membrane
Interfere with cell wall integrity
Inhibition of
Germination and hyphae formation
Interfere with Mitochondrial respiratory chain
Apoptosis
Biofilm formation

CONCLUSION

- As concluding remarks, several plant natural products have been tested for anti-*Candida* activities. Several of these plant products can target critical processes in *Candida* biological activities including cell wall integrity, cell membrane plasticity, cell metabolism, respiratory chain, adherence to host cell, germination and biofilm formation, or induction of apoptosis.
- Despite these great anti-*Candida* activities of plant products compared to controls, only few have been tested *in vivo* and few of them have been used as anti-*Candida*. On the other hand, although some of these products including, cinnamon, ginger, are present in the pharmaceutical market for other medical purposes, they have never been used as anti-*Candida*.
- The need for new anti-*Candida* is urgent since *Candida* is known as a serious resistant microbe, and hence promotion of some of the selected plant products for clinical testing will be beneficial

- More Fluconazole/azole-resistant strains need to be included in future studies. The plant oils could find use as anti-*Candida* agents against azole-resistant strains. Most of the oils used in this study have a long history of use in food, confectionery and as components of perfume. However, if they are to be considered in topical preparations a careful exploration of their probable irritating and other undesirable effects in humans need to be undertaken.

References:

- Potential of plant oils as inhibitors of Candida albicans growth Anupama N. Devkatte, Gajanan B. Zore, S. Mohan Karuppayil * School of Life Sciences, SRTM University, Nanded – 431606 (MS), India
-
- Willinger B, Apfalter P, Hirschl AM, Makristathis A, Rotter M, Seibold M. Susceptibility testing of Candida species: comparison of NCCLS microdilution method with Fungitest. Diagn Microbiol Infect Dis. 2000
- Akbari S. Antifungal activity of Thymus vulgaris L. and Origanum vulgare L. against fluconazol-resistant and susceptible Candida albicans isolates. J Med Plants. 2007;6(1):53-62.
-
- Bita A , Rosu AF, Calina D, Rosu L, Zlatian O, Dindere C, et al. An alternative treatment for Candida infections with Nigella sativa extracts Eur J Hosp Pharm 2012; 19:162.
-
- Barry A L, Brown S D. Fluconazole disk diffusion procedure for determining susceptibility of *Candida*species. J Clin Microbiol. 1996;34:2154–2157
-
- Dermoumi H. In vitro susceptibility of yeast isolates from the blood to fluconazole and amphotericin B. Chemotherapy. 1992;38:112–117