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Eco friendly control of candida

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Abstract: Several revisions have revealed that 75% of the female will have at slightest one and 40-50% will have repeated affairs through their life time. Vulvovagnitis is considered by sever itching of vulva, anomalous vaginal exoneration, erythema, edema of vulva, and satellite lesions. In this study, 43 clinical candida samples of patients at some age cluster range from 1to 50 year old and for both masculinity were composed from patients misery from vaginal candidiasis, oral thrush and urinary tract infection who admitted to different Mansoura University Hospitals. All samples were cultured on Sabourad's Dextrose Agar (SDA). The isolation and identification method of yeast isolated were followed by upon the morphological, cultural and biochemical characteristics, such as Germ tube and PCR technique.

Some candida samples showed complete resistance to antibiotics such as (flucanozole 25 and voriconazole). So we investigated the anti microbial activity of the following medical plants; Thyme, Cinnamon ,Garlic, Peppermint and fenugreek. Results showed that Cinnamon (87.5mg/ml) and Peppermint (87.5mg/ml) had good antifungal activity and the inhibition zone diameter was ranged between (25mm-35mm) for Cinnamon and between (12mm- 20mm) for Peppermint.

keywords: Candida. Plant extract, Morphology and Biochemistry, Garlic

1.Introduction

Candida species is a part of normal human microflora and it develops pathogenic after confident situations are present and cause opportunistic infections [1].

Candida causes a variety of clinical syndromes in humans ranging from

superficial infections to invasive diseases in immunocompromised patients [2].

The major etiological agent is *Candida albicans*, while diverse *Candida* species can reason a variability of poisons including *C. tropicalis*, *C. dubliniensis*, *C. parapsilosis*, *C. krusei*, *C. guillermondii*, *C. glabrata*, and *C. kefyer* which represent several medical methods of candidiasis. Certain of these species are faced as inferior contaminations to additional species, for example; *C. parapsilosis* is inferior infection only when *C. albicans* as a origin *Candida endocarditis* [3].

Due to its virulence factors such as adhesion, dimorphism, enzymes, and biofilm formation, *candida* performs to be a pathogen instigating extensive gamut infections in diverse sections. The two enzymes that mainly show an chief role in pathogenicity are phospholipase and secretory aspartic proteinases [4].

Fluconazole is a associate of the triazole family, one of the most widely used antifungal agents. It is an FDA-approved medication to luxury vaginal candidiasis, oropharyngeal and esophageal candidiasis, *Candida* urinary tract infections, peritonitis, and universal *Candida* infections including candidemia, scattered candidiasis, and pneumonia, and cryptococcal meningitis, Prophylaxis is also branded to decline the commonness of candidiasis in patients undertaking bone marrow transfer who receive cytotoxic chemotherapy and/or radiation therapy.

Fluconazole FLC discuses are used for antimicrobial weakness testing of fungal cultures.

Because of the side effects and high cost of the chemicals, expending therapeutic plants is recommended which have fewer side effects,

less poisonous effects on tissues, more economical, and individuals endure them calmer. A lot of therapeutic plants are found in emergent and developed countries [5].

Egypt uses medical plants for treatment of diseases. Medicinal plants are used as herbal medicine for treatment of human infections [6].

For example; the biotic stuff of steroid saponins existing in garlic has antifungal, antitumor avoiding stiffening lump and cytotoxicity possessions [7].The PCR technique is commonly used for identifying *Candida* species from clinical specimens [8].Polymerase chain reaction methods are very reliable because of their simplicity, specificity, and sensitivity [9].

2.Aim of the work

To evaluate the effect of certain medicinal plant extract on *candida* species as a biotherapy.In addition, the studies will be extended to notice ultrastructure changes of *candida* cells treated with plant extract by using electon microscope examination .

3. Materials & Methods

3.1. Sample isolates

Forty-three candida isolates recovered from different samples [vaginal swab (18), blood (5), urine (8), cornea (1), oral swab (5) and Sputum (6)] from patients who were admitted to different Mansoura University Hospitals.

These samples were cultured using **Sabouraud's Dextrose Agar media** and incubated aerobically at 37^{o} C for 48 hours.

3.2. Identification of isolates

3.2.1. Morphology of colony:

Creamy white colonies with creamy texture and yeast odor.

3.2.2. Germ tube test:

0.5 ml of human serum was added into a slight tube. By a Pasteur pipette, trace a gathering of yeast and moderately combine it in the serum.The tube was incubated at 37°C for 2 hours.A drop of the serum was transferred to a slide for inspection.Cover slide and inspect microscopically under low and high influence purposes.

Positive Test: A short hyphal (filamentous) postponement ascending across from a yeast cell, with no constriction at the point of origin. **Examples:** *Candida albicans* and *Candida dubliniensis*

Negative Test: No hyphal (filamentous) allowance ascending from a yeast cell or a short hyphal allowance confined at the point of origin. **Examples:** *C.tropicalis*, *C. glabrata* and additional yeasts.

3.2.3. Molecular identification:

3.3. Polymerase chain reaction (PCR)

The FastDNA™ SPIN Kit, an updated version of the well-known FastDNA™ Kit, is used with any FastPrep® Instrument to lyse and subsequently isolate DNA from up to 200 mg of almost any sample in less than 30 minutes. Features: Separation of genomic DNA from plants, animals, bacteria, yeast, algae, and fungi using a silica spin method: Fast and reproducible sample lysis with the FastPrep ®-24 or FastPrep® FP120 Tool, Lyse and isolate DNA in less than 30 minutes from a variation of classical types, Plastic appearance disdains need for separate DNA isolation kits, No risky organic reagents essential, SPIN Filters are

encompassed to brook Line silica management.

3.4. Antibiotic susceptibility testing

3.4.1. Antibiotic disks

The antibiotic disks tested were (Fluconazole, Voriconazole and Amphotricin B). the name of these antibiotic disks, potency, symbol and the standard evaluation of inhibition zones (CLSI, 2009) were represented in **Table**

Table (1): Inhibition zone diameter(mm) in antibiotic susceptibility test

Antimicrobial category	Antimicrobial agents	Disk symbol	Potency (mcg/disk)	Resistant (R)	Intrmediate (I)	Susceptible (S)
Triazole	**Fluconazole**	FLC	25	≥14	15-18	≤19
Triazole	**Voriconazole**	VRC	1	≥13	14-16	≤17
Polyene	**AmphotricinB**	AP	20	≥15	13-14	≤12

.4.1.1. Disk diffusion method

Antibiotic susceptibility testing of *candida* was carried out by

Kirby-bauer diskette diffusion technique using Mueller-Hinton agar according to (9) method. This method was done by inoculation standard method; inoculum was equipped by picking five disconnected colonies of approximately 1mm from 24 hours old culture grown on Sabouraud Dextrose Agar and gestated at 35 ± 2^{o} C. Colonies are suspended in 5ml of sterile 0.85% saline. Then vortex the resultant holdup and adjust the turbidity to yield 1x10^{6} - 5x10^{6} cells/ml (i.e.0.5.McFarland standard).

The Candia suspension was inoculated by sterile cotton swab on Muellure-Hinton agar plates in 3 directions on the agar plates surface to get a uniform inoculum. Then, the cups were permitted to dry for 3-5 minutes before adding antibiotic disks. Then the plates were incubated into an incubator at 37^{O}C for 24 hours.

3.4.1.2: Detection of candida susceptibility to different medicinal plant extracts

The following medicinal plants, which are tabulated in **Table (2)** were used to prepare ethanolic plant extracts. The plant samples were collected from herbalists in Mansoura, Egypt.

Table (2) Scientific and Arabic names and parts used from each medicinal plants used in preparing extracts

English name	Scientific name	Arabic name	Family	Used part
Garlic	*Allium* spp.	**الثوم**	*Liliaceae*	Bulbs
Cinnamon	*Cinnamomum* spp.	**القرفه**	*Lauraceae*	Flower buds
Thyme	*Thymus vulgaris*	**الزعتر**	*Lamiaceae*	Leaves
Peppermint	*Mentha* x *Piperita*	**النعناع**	*Lamiaceae*	Leaves
Fenugreek	*Trigonella foenum-graecum*	**الحلبه**	Fabaceae	seeds

3.4.1.3. Preparation of Inoculum

Inocula were prepared by direct colony suspension. A sterile broth was transferred into a sterile test tube. Then some gatherings of the test organism were engaged from the dish then immersed into the test tube. The check was accustomed to match the half McFarland typical which had alike entrance of an instant broth culture by count distilled water.

3.4.1.4. Preparation of plant extract

Twenty-five grams of dried plant material (garlic, thyme, cinnamon, peppermint and fenugreek) were soaked in 100ml of ethanol (ethyl alcohol 95%) for 3 days at room temperature. After that, the ensuing extract was filtered over Whitman filter paper (NO.1). The purification filtrate was reextracted twice using the identical system. Then the filtrates obtained were disappeared using a revolving evaporator to dryness.

4. Results

In this practice, 43 clinical *candida sample* isolated were obtained from (Male & female). Patients admitted to different Mansoura University Hospitals. All samples were cultured on sabourad's Dextrose Agar (SDA).

Table (3): Antimicrobial susceptibility of 43 candida isolates

Antimicrobial Category	Antimicrobial Agents (Antibiotics)	Symbol	Resistance (R)		Intermediate (I)		Susceptible (S)	
			NO	%	NO	%	NO	%
Triazole	**Fluconazole**	FLC25	36	83.7	0	0	7	16.2
Triazole	**Voriconazole**	VRC1	36	83.7	0	0	7	16.2
Polyene	**AmphotricinB**	AP20	0	0	16		27	62.7
							95.3%	

The results in **Table (4) and Figure (1)** show the antimicrobial susceptibility of 43 candida to 3 antibiotics belonging to 2 antimicrobial categories.

The highest resistance was shown to fluconazole and voriconazole (83.7%) and the highest susceptibility was shown to amphotricin B (62.7)

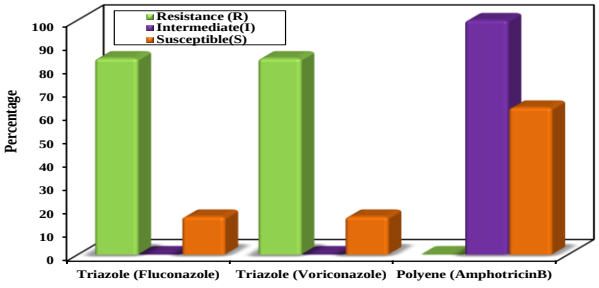


Table (4): Antifungal activity of ethanolic plant extracts against the most resistant candida isolates

Resistant candida isolate NO.	Diameter of inhibition zone (mm) of different ethanolic plant extracts				
	Garlic	Cinnamon	thyme	Peppermint	Fenugrek
5	0	30	0	20	0
12	0	35	0	15	0
19	0	30	0	13	0
21	0	30	0	0	0
39	0	30	0	0	0
40	0	25	20	0	15

The results in Table (4) showed that the cinnamon (87.5mg/ml) and peppermint (87.5mg/ml) had good antifungal activity. The inhibition zones diameter (mm) ranged between 25 and35 for cinnamon and between12and20 for peppermint. while antibiotics (Flucanazole and Voriconazole) were completely resistant.

Table (5): Comparison between activity of some antibiotics and the most effective ethanolic plant extract aginst the most resistant candida isolates

Candida isolateNO.	Diameter of inhibition zone (mm)				
	Cinnamon	Pepprmint	Ap20	FlG25	VRC1
5	30	20	15	0	0
12	35	15	15	0	0
19	30	13	15	0	0
21	30	12	15	0	0
39	25	0	15	0	0
40	30	0	15	0	0

Ap20 =AmphotricinB, FLC 25 = Fluconazole, VRC1= Voriconazole

Table (5) showed that Cinnamon and peppermint were completely resistant to FLC=25 and VRC1 and the inhibition zone of plant extract were more than antibiotic (Ap20

Table (6): Diameter of inhibition zone of different concentrations of cinnamon against the most resistant candida isolates

Resistant candida isolateNo		Diameter of inhibition zone (mm) of different concentrations (mg/ml) of cinnamon extract				
	50 % mg /ml	25%mg/ml	12.5%mg/ml	6.25% mg/ml	3.125% mg/ml	Blank (DEMSO)
5	15	15	15	15	0	0
12	17	17	17	16	0	0
19	27	25	22	25	17	0
21	22	20	18	12	0	0
39	21	20	18	16	0	0
40	18	18	14	0	0	0

The results in Table (6) showed that the MICs (mg/ml) were at 50, 25, 12.5, 6.25 and 3.125 for isolate number 5, 12, 19, 21, 39, 40 respectively.

Table (7) diameter of inhibition zone of different concentrations of peppermint against the most resistant candida isolates

Resistant candida isolate No.	Diameter of inhibition zone(mm) of different concentrations (mg/ml) of peppermint extract					
	50% mg/ml	25% mg/ml	12.5 %mg/ml	6.25% mg/ml	3.125 %mg/ml	Blank (DEMSO)
5	20	15	15	0	0	0
12	15	15	13	0	0	0
19	13	13	0	0	0	0
21	12	11	11	0	0	0
39	0	0	0	0	0	0
40	0	0	0	0	0	0

DEMSO= Dimethyl sulfoxide

The results in **Table (7)** showed that the MICs (mg/ml) were at 50, 25, and 12.5 for isolates number 5, 12, 19 and 21, respectively.

Table (8): Minimum inhibitory concentration values of cinnamon and peppermint against the most resistant candida isolates

Resistant candida isolate NO.	MIC in mg/ml	
	cinnamon	**peppermint**
5	5.4	10.8
12	5.4	10.8
19	2.7	21.7
21	5.4	10.8
39	5.4	0
40	10.8	0
MIC value(mg/ml)	5.85	13.52

The result in **Table (8)** showed that MIC value of Cinnamon =5.85 mg/ml and MIC value of Peppermint =13.52 mg/ml.

The results in **Table (8)** demonstrated that the MIC values (mg/ml) were 5.85 for cinnamon and 13.52 for peppermint.

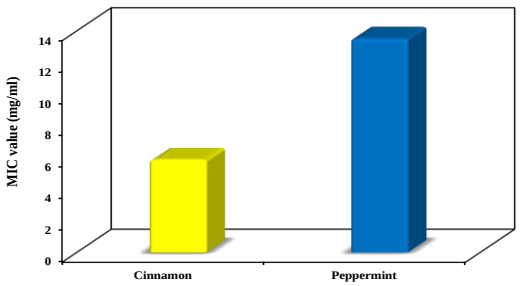
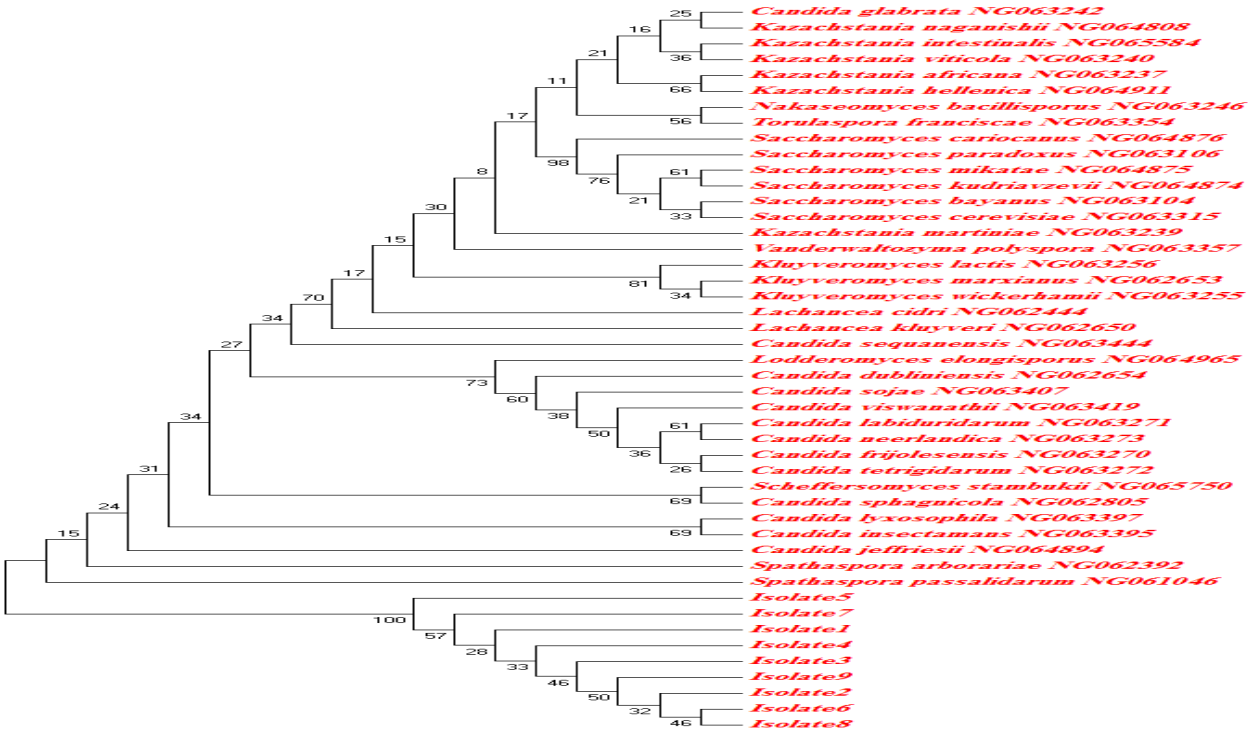


Figure (2): Minimum inhibitory concentration values of cinnamon and peppermint against the most resistant candida isolates

Table (9) show identification of *candida* isolates by using PCR

Isolates	Size (bp)	Closely related fungi	Accession number	Homology (%)
1	455	Scheffersomyces stambukii	NG065750	92
2	1242	Candida dubliniensis	NG062654	98
3	843	Candida neerlandica	NG063273	98
4	1668	Candida glabrata	NG063242	91
5	1181	Candida glabrata	NG063242	99
6	1115	Kluyveromyces marxianus	NG062653	99
7	1078	Candida dubliniensis	NG062654	97
8	917	Kluyveromyces marxianus	NG062653	99
9	1074	Kluyveromyces marxianus	NG062653	100



5. Discussion

Candidiasis is solitary of the record various fungal infections that can central to apparent, such as vaginitis, to universal and hypothetically life frightening diseases.

Genital contribution of women is one of the most public appearances due to candida. Vulvovaginal candidiasis results from unusual advance of candida in the genital tract mucosa and has augmented intensely in the recent years. This infection is a international health difficult [10]**.**

While in dentistry, universal and resident use of antimicrobial factual is more common and entrance worn is usually used to decrease microorganisms of oral hollow. These biological doorway washes such as chlorhexidine have some side effects [11]**.** Therefore result medical plants that have antimicrobial effects and using them as opening wash have bootees as lessening supposed side effects, diminishing deadly effects on tissues, and also they are cheaper.

In former educations antimicrobial effects of some plant quotes have been studied with different methods. One of them, in vitro antimicrobial movement of different normal agents beside a few mouth bacterial has been appraised. Amongst them cinnamon, clove oil and spices excerpt have inhibitory properties on louth bacteria [12, 13].

A training done about effect of 45 Indian plants on human tough pathogens to different drugs, 40 plants showed antimicrobial activity against one or more bacteria and 24 plants showed antifungal activity against candida [14].

This study presented antifungal effect of five plant remove against candida species using standard disc dispersal method. In comparison with effect of fluconazole, voriconazole and Amphotricin B, ethanol extract of cinnamon, peppermint and thyme showed good antifungal

activity. This is similar to grades of the research done [15].

In a research done in 2001, the biologic stuff of steroid saponins present in garlic was traveled. That study unfurnished that this material has antifungal, antitumor averting condensing lump and cytotoxicity effects [7].

Ghannoum clear that water abstract of garlic shows antifungal effect by oxidation of thiol groups of proteins. Thiol corrosion causes unplugging of enzymes and blushing of microbial growth [16]. In other study, the effect of the garlic on *Candida* Albicans was evaluated and observed that allinmin of this plant has inhibitory effect on mycosphaerella arachidicola [17].

trainings, inveterate the inhibitory effect of cinnamon on changed microorganisms, such as *E.coli*, *Sacharomyces cerevisiae*, *Bacillus subtilis*, and oral bacteria, such as *Streptococcus* sp, *Actinomyces* sp, *Actinobacillus* sp, *Bacteroides* sp, *Capnocytophaga* sp, *Eikenella* sp, *Fusobacterium* sp, *Propionibacterium* sp and a few species of *Candida* [12, 18-20]..

Dalirsani, et al (2011) trained notorious cinnamon aldehyde as the active fungitoxic fundamental of cinnamon [21].

In my recent study, ethanol quotation of garlic and thyme shows little or no antimicrobial effect on *Canidia* species.

The modification between our accomplished results in the present study and some of the previous research can be due to different *Candida* strains. These plants have inhibitory effect on one strain of *Candida* and these plants do not have any inhibitory effect on other strains such as *Candida albicans*.

6. Conclusion

Among plants being studied, methanol extract of cinnamon and peppermint had greater inhibitory activity against Candida isolates.

Cinnamon and peppermint showed better inhibitory consequence than antibiotics used.

Since of the antimicrobial properties of some medical plants which have minimal cross effects in assessment with chemical treatments, more in vivo and in vitro investigations about antimicrobial and antifungal effects of different plants on *Candida* infections are mentioned

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