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STUDY THE EFFECT OF SOME PLANT EXTRACTS EFFICIENCY TO REDUCE THE PATHOGENESIS *CANDIDA ALBICANS* ISOLATES FROM DIFFERENT BODY AREAS IN THE PATIENTS WITH DIABETES II

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Abstract

This study was conducted to investigate the inhibitory role of alcoholic extract of *Salvadora persica* and aqueous extract of *Mentha longifolia*, *Thymus vulgaris* and *Cinnamon* extracts, *Nigeria sativa* oil on the inhibition of *Candida albicans*. The results revealed that the inhibitory effect of alcoholic extract was more effect (52mm) in concentration 10%, on *C. albicans*, while, *N. sativa* oils in (46mm) in some concentration 10%, on the other hand the aqueous extraction include *Thymus vulgaris*, *Cinnamon*, *Mentha longifolia* have less inhibitory effect (38, 31, 23) in respectly. whereas all plant extract and Nigeria *Sativa* oils were inhibition 100mm in concentration 100%. Also susceptibility test of these yeast for use some antibiotics include fluconazole, voriconazole, itraconazole, nystatin was more effective against clinical isolates of *C. albicans* while Amphotreicin B with less effect on these yeast.

Key words :

Introduction

Candida species are harmless saprophyte yeasts, belong to the normal microbiota of the human in the gastrointestinal tract, oral and vaginal mucosae. These yeasts can cause superficial infections such as thrush and vaginitis; however, if the immunocompromised host, they can cause severe systemic infections. Risk factors for patients include infection by the HIV, anticancer therapy, organ transplantation, abdominal surgery, catheters, diabetes and the use of broad-spectrum antibiotics (Maria *et al*., 2010). *C. albicans* and other *Candida* species have been highly associated with several opportunistic fungal pathogen and the major cause of orophoryngeal candidiasis, gastrointestinal and female genital flora. Opportunistic pathogens are accounted for a substantial morbidity rat and can result in hospitalization and expensive therapies and they also reduce the survival rate of people with HLV infection (Gholampour *et al*., 2015).

Candida spp have many virulence factors, have ability to adherence, protolytic enzyme (protease), biofilm formation on tissue and other enzymes (Silva *et al*., 2011). *C. albicans* is one of important yeast that cause many series public of health challenge and that high frequency when isolated in hospitalization (Almirante *et al*., 2005; Lai *et al*., 2012).

C.albicans is wide spread infection that cause of nosocomial candidiasis and cutaneous or subcutaneous infections in vaginal, mouth, nail and skin especially diabetes, renal failure and AIDS (Beck–Sague and Jarvis, 1993).

The antibiotics are importance using from plant because the safety and ensure high active materials and low side effect comparison with chemical or synthetic antibiotics (Shabana and El-Adly, 2016). Efforts to using the plants extract to alternate to chemical drugs for antifungal agents to less toxic and high activity because the purity of this drug against microorganism, animal and plants (Cleff *et al*., 2010).

The present study aimed to identify inexpensive, simple and effective plants, which can prevent and control the growth of *C. albicans*, to evaluate the antimicrobial effects of plants like tea leaves, onion leaves, onion bulb, aloe vera, mint leaves and curry leaves on *C. albicans*

involved in causing candidiasis and compared it's with activity some antibiotics of inhibition *C. albcans*.

Materials and Methods

Specimen collection and culture

Collected samples (50) from urinary tract, urogenital tract, mouth, burn and sputum from diabetes mellitus were collected from patients suffering from various clinical signs during the period 6month to AL-Sadder Medical City during the studied period. All specimens were treatment directly by (McGinnis, 1980) and then cultured on Sabouraud dextrose agar (SDA), then they were incubated at 37ºC for 48h, the most frequently used media for primary isolation of *Candida* spp (Odds, 1991). It permits the growth of *Candida* and suppresses the growth of many pathogens.

Many methods were used to identification *Candida* including Germ tube production by Eills (1994), Forbes *et al*. (2007). Surface growth by Milne (1996) and Chlamydospores production by Eills (1994), growth at 45C^{o} (Kim *et al*., 2002; Forbes *et al*., 2007). All the *Candida* spp were diagnosis by Koneman *et al.* (1978), Eills (1994), Biochemical tests (Carbohydrate fermentation) according to the methods of Koneman *et al*. (1978).

Preparation of the plant extracts

Adopted methods (Hernandez *et al.*, 1994), fresh leaves and stem of plant were thoroughly cleaned twice using distilled water. They were cut into pieces with the help of scissors/ knife and grinded using a sterile electric grinding jar into fine texture powder form, aqueous plant extract was prepared by take 10gm of powder plant with 200ml in distilled water in electric mixture for 24 hours at 37°C, afterwards filtered using medical gauze to clearance from unresolved plant, then using centrifuge quick 3000cycle/min at 10min, then runny extract using filterer paper to obtainable aqueous solution, to dry extract with using hot air oven to 40°C, then conserves to refrigerator with using.

While prepared alcoholic plant extracts were depending methods of Ladd *et al*. (1978) by dissolving the 20gm powdered form of plant materials in 200ml from Ethanol alcohol by Soxhlet extraction for 24 hours, subsequently extract dry using hot air oven to 40°C. *Nigeria sativa* oils were obtained from markets.

Effect aqueous, alcoholic plant extract and *Nigeria sativa* oils against *C. albicans*

The effect plant extract and oils against *C. albcans* was determining by using the well diffusion method, SDA medium was inoculated with *C. albicans* suspension by swap, then 5 mm wells were caved in it by Pasteur pipette. Then 50 µl of each concentration were added to wells the plates were incubated at 35ºC for 48h. after The inhibition zone was measured determined in millimeters (Gholampour *et al*., 2015).

Activity some antibiotics of inhibtion *C. albcans*

The following antibiotics were used: Itraconazole, Voriconazole, Fluconazole, Nystatin, Amphotericin B. Antifungal activity assessment according (Maroszyñska *et al*., 2013) using Agar disc diffusion method *C.albcans* inoculums (103 cfu/mL) in 0.85% from NaCl solution and spread on the YPG agar. Filter paper discs about 6mm with 50 µg nystatin and Amphotericin B, 25 µg fluconazole, 1 µg of voriconazol and 10 µl of Itraconazole in the concentration of 5 µg/ml were placed on the inoculated plates.

Statistical analysis

All laboratory results were analyzed according to (C.R.D) Design and averages were compared by test teams less moral L.S.D and at the level of probability of 0.05.

Results and Discussion

Result of microscopic and biochemical identification on 50 clinical sample intake from Urinary tract, Urogenital tract, Mouth, Burned and Sputum in diapetes, that showed in Fig. 1 to *C. albicans* the prevailing at all yeasts isolated as it was a (50, 60, 60, 50, 80%) isolated from the places the urinary tract, urogenital tract, mouth, burn and sputum respectively. The high incidence *C. albicans* in sputum that reached to 80% followed by yeasts *C. krusei* and *C. glabrata* by frequency.

C. albicans were prevailing all yeasts that isolated from different place may be beyond to change the balance between the host and the microorganism among which there are individuals infected with the human immunodeficiency virus, with nutritional deficiencies, malignancies, or with metabolic disorders like diabetes mellitus and HIV (Tekeli *et al*., 2004).

C. albicans is the more frequecy in patients that cause of Candidiasis (in 60-80% of the cases) and *C. glabrata* that second pathogen infection causing Candidiasis (Redding *et al*., 2002).

The high incidence by *C. albicans* may come back to the weakness of the immune system with diabetic patients and no balance between the integrity of host defense mechanisms and the intensity of exposure to potentially pathogenic in the host's especially how

suffering from lack of the immune e.g cancer, renal transplant , diabetes. and wide spectrum antibiotics treatment with corticosteroids and cytostatic, that all factor lead to the development of infections in yeast (Durango *et al*., 2002; Vento and Cainelli, 2003).

On the other hand, *C. albicans* have ability to adhesion cells epithelial lining of urinary tract, urogenital tract, mouth surface as the existence of a number of receptors surface especially ic3b, which increases the expression weakness in the case of the concentration of sugar glucose in the media is equal to 20 Mm as when found a high concentrations of sugars, especially glucose leads to an increase this receptors on the surface of the gas invader filament form is working on the inhibition of white blood cells multi-core to do the process of phycocytosis and increase the shortcoming winning in function lymphocytes that result in an important role in defense body against infection tissue mucosa (Willis *et al*., 2000; Hedderwick and Kauffman, 1997).

Identification of *Candida* spp

Morphological features and Virulence factors

Table 1 refer that many characteristics that sure that yeasts cultured *Candida* spp on SDA appear the colonies are cream to yellowish, in color through 3-5 days, the texture of the colony smooth, glistening or dry, wrinkled depending on the species and many type appear Pseudo hypha and consist odder featured yeasts and consist sediment in bottomless tube in drip media after 24-48 h this status share with other type *Candida* except *C. krusei* and *C. tropicalis* (Conant *et al*., 1971; Bhavan *et al*., 2010).

On the other hand, Virulence factors including Germ tube production appear only of *C. albicans* but the other species give negative test and the results appear *C. albicans* production Chlamydospore on media, but the other give negative test.

Results are showed that the ability of *C. albicans* on the growth at 45^0C in 48 h at incubation, but the other haven't ability to growth at 45ºC.

These results are in agreement with many studies Since the germ tube and Chlamydospore is a characteristic morphology observed only in *C. albicans*, confirmation these features is available as a rapid method for identifying *C. albicans* and to recognize *C. albicans* on the other species (Kwon-Chung and Bennett, 1992; Berman and Sudbery, 2002; Ha *et al*., 2011; Kumar and Shakla, 2010).

Table 2 show the biochemical test to identification of *Candida* spp. is based on assimilation and fermentation

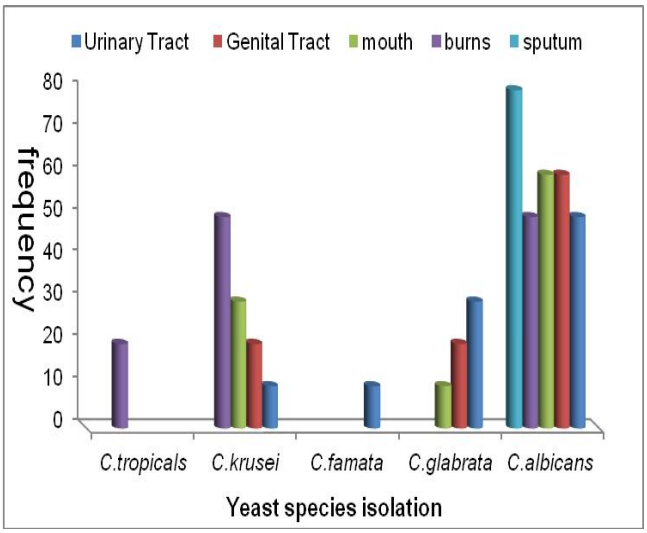

Fig. 1 : Yeast species isolation from patients diabetes mellitus Type II.

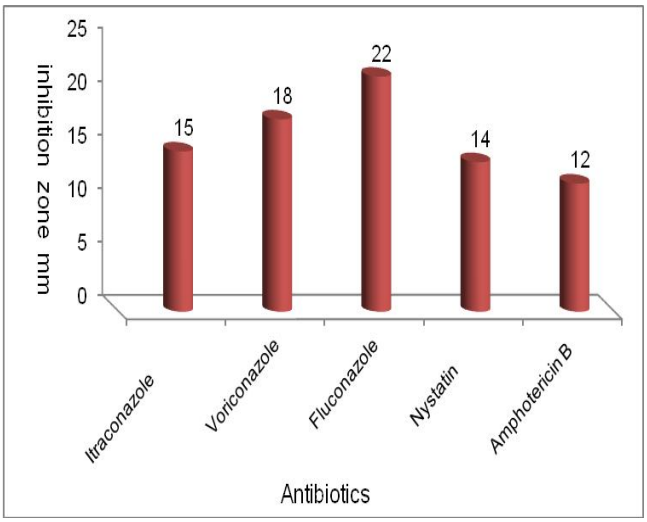

Fig. 2 : Diameter zone of growth inhibition of antibiotics aginst *C. albicans*.

of carbohydrates. The pattern of carbohydrate assimilation is considered a reliable test and is generally used for the correct identification of yeasts of clinical interest. This result show *C. albicans* not ferment Galactose sugar, *C. gabrata* ferment Glucose fermenter, *C. tropicalies* not ferment Sucrose sugar, *C. fumata* ferment Maltose and Sucrose sugar, this result disagreement with Hussain Qadri and Nichols (1978), while *C. krusei* show ferment Glucose and not ferment other sugar this result agreement with Marinho *et al*. (2010).

Effect aqueous, alcoholic plant extract and *Nigeria sativa* oils aganist *C. albicans*

Data presented in table 3 indicated that all essential plant extract and *N. sativa* oils under test process showed antimicrobial activity against yeasts. Most of these plant

Table 1 : Many of test used to identification *Candida* spp.

Germ tube	***C. albicans***	+
	C. glabrate	_
	C. famata	_
	C.krusei	-
	C.tropicalis	_
Surface growth	*C.albicans*	Pseudo hypha
	C.glabrate	_
	C.famata	_
	C.krusei	Pseudo hypha heavy and clear
	C.tropicalis	Pseudo hypha with gas bubbles
Chlamydospores production	*C.albicans*	+
	C.glabrate	_
	C.famata	_
	C.krusei	+
	C.tropicalis	_
Growth at 45C°	*C.albicans*	+
	C.glabrate	_
	C.famata	_
	C.krusei	_
	C.tropicalis	_

extract and oils delayed condition of fungi. The feature of antimicrobial activity varied not only from one plant extract and essential oil to another, but also among microorganisms.

This result show alcoholic extraction of *Salvadora persica* as greater inhibition (52mm) in concentration 10%, this study agreed with other it was seen that even the alcoholic extracts of *S. persica* (obtained from Pakistan) had no inhibitory effect on *Streptococcus mutans*, *Staphylococcus aureus* and *C. albicans* (Almas, 2001). This result agreement with other such studies were made by Brandi *et al*. (2006), Al-Rashedi and Al-Habib (2011), Al-Terehi *et al*. (2015) have confirmed that the ethanol extracts of the tested plants have a higher biological effects than the aqueous extracts on the growth of *Candida* sp.

While, *N. sativa* oils in 46mm in some concentration 10%. It is possible that the plant extract contains active ingredient(s), which may directly stimulate the granulocytes and monocytes to generate no leading to an excellent anti-fungal activity, which in turn kills *C. albicans*.

Table 2 : Carbohydrate fermentation to identification of *Candida* spp.

Type yeasts	**Sugar fermentation**				
	Glucose	**Maltose**	**Galactose**	**Lactose**	**Sucrose**
C.albicans	+	+	-	-	+
C.gabrata	+	-	-	-	-
C.krusei	+	-	-	-	-
C.tropicalies	+	+	+	-	-
C.fumata	-	+	-	-	+

Table 3 : Antimicrobial activity of aqueous and alcoholic plant extracts against *C. albicans*.

C. albicans	**Concentration**			
	10%	**30%**	**70%**	**100%**
Mentha longifolia	23	19	12	0
Salvadora persica	52	43	28	0
Nigeria sativa oil	46	40	32	0
Thymus vulgaris	38	36	20	0
Cinnamon	31	25	18	0

Many study refer to action *Nigella* oil that attributed in find of β-sitosterol and oleic acid as the important components this oil of and presence long-chain fatty acid that may be play role to fungistatic against many strains of *Candida* (*Ouraïni et al.*, 2007; Asdadi *et al.*, 2014).

On the other hands, oils inhibit growth yeast because have strong to shatters the cell wall and weakens of the operation inside cell through overlap function of the cytoplasmic membrane that interfering of protein synthesis and disable process that work to transferring of the ions and salts through such membrane (Al-Qaysi, 2008).

While aqueous extraction include *Thymus vulgaris*, *Cinnamon*, *Mentha longifolia* (38, 31, 23) in respectly, whereas all plant extract and Nigeria *Sativa* oils were inhibition 100mm in concentration 100%.

The ability of plants and their extracts and oils is beyond to production of primary or secondary metabolites such as phenolics, polyphenols, tannins, quercetin, flavones, flavonols, alkloids, terpenoids, lectins, polypeptides and complex mixtures that effect to inhibition growth of microorganisms (Cowan, 1999).

Test ability antibiotics to inhibition *C. albicans*

Five antibiotics were test sensitivity *of C. albicans*. All isolates were found sensitive to Fluconazole expressed by growth inhibition zones ranged 22 mm but Amphotericin B give less inhibition its 12 mm. Fig. 2 other antibiotics give the lowest effectiveness. Azoles previously using instead of Amphotreicin B because little toxicity this group

were chemical antibiotics include the Imidazole e.g Ketoconazol Clotrimazole, Miconazole and copund Itriazol e.g Itraconazol and Fluconazol that using in mouth and treatment topical and systematic infection (Jawetz *et al.*, 1997).

The action Mechanism of these drugs is play role to synthesis of ergosterol in the fungal plasma membrane, with the participation of cytochrome P450 and inhibits the synthesis of chitin. Voriconazole also inhibits the synthesis of chitin (Szymankiewicz and Dancewicz, 2008).

Nystatin back to polyine group have same activity Amphotreicin B that using to treatment candidiasis in mouth, viginia, gastrointestinal and haven't side effect to human (Lortholary *et al*., 1999).

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