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DIAGNOSIS OF SOME TYPES OF YEASTS ASSOCIATED WITH THE HUMAN BODY AND TREATED IN THE GARLIC AND COLOCYNTHIS EXTRACTS

Hadi Alwan Mohammed AlSaidy,
Department of Animal Resources. College of Agriculture. University of Diyala .Baquba, Iraq

ABSTRACT: *97 swabs were collected from the vagina, the mouth, middle ear, skin and urine of clinical cases for patients with candidiasis disease in Diyala province for the period of July 2010 - February 2011 for the diagnosis of some types of yeasts associated with the human body and treated with garlic and colocynthis extracts. The results of diagnosis showed that Candida albicans and C.tropicalis yeasts associated to the human body by 69.35% and 13.3%. colocynthis extract showed cellular toxicity of red blood cells. Hexane extracts of garlic and colocynthis gave highest percentages 52% and 63%, followed Alcoholic extracts 50% and 45%, then the hot and cold water extracts 33%,35% 0.25% and 30%, respectively,alcohol and hexane extracts of garlic and colocynthis showed inhibition effectiveness higher than in cold and hot water extracts.The inhibition effectiveness of garlic extracts better than colocynthis extracts against C. albicans and C.tropicalis yeast, hexane extract of garlic at 100 mg/ ml gave higher inhibition percentages 100% against C.tropicalis, followed by alcoholic extract 98.88% against C. albicans and C.tropicalis, and the lowest inhibition percentages 57% at 20 mg / ml for the cold water extract of the garlic plant too. The results showed that MIC of Fluconazole 12.5 and 6.5 µg / ml against C.albicans and C.tropicalis , and then Ketacanazole and Nystation 25 and 12.5 µg / ml respectively . Whereas MFC of antifungal 50 µg / ml. hexane extraction for garlic and colocynthis at 100 mg / ml have been showed inhibition effectiveness equalized to inhibition effectiveness for antifungal of Nystatin at 2 mg/mI*

KEYWORDS: Garlic , Colocynthis , Antifungal, culture media and patients

INTRODUCTION

candidiasis are organisms eukaryotic , single-cell, unicellular cells and sometimes spherical or oval prolonged, diameter 4-6 µm asexually Reproduction are in budding or binary fission, there are pseudohyphae resulting from the continuation of budding yeast cells without separation from each other, [14,31], known many species of the genus *Candida* spp. however, the species and pathogens that cause disease in humans Candida were few, and the fungus *C. albicans* is the most widespread species, and is the president that causes candidiasis, and then comes the importance pathogenicity of other types, including *C.tropicalis* , *C.parapsiosis* , *C.krusei* , *C.glabrata* , *C.kefyr* , *C.guilliermondii* and *C.pseudotropicalis* [32], And grow speices of *Candida* spp. in temperatures ranging between 20-40 m º and pH between 3-8 [34], and its colonies developing on private agricultural medium such as Sabouraud Dextrose Agar and, Malt Extract Agar characterized being the soft and smooth with a creamy white color (cream colored), and convex and its smell yeast odour, either the old colonies shall be large and irregular edges and rough [12, 14]. Pharmacology interested in studying the antibiotics with natural sources such as Aspirin (The buslic salicyat structure) extracted from the plant willow (Salix), and Opioids extracted from the poppy plant (*Opium poppies*) and the pill (Steroid structure) extracted from the wild yam in Mexico and other [30]. In this study, garlic plant (*Allium sativum*) and colocynth(*Citrullus colocynthus*) were used.This study aims to:

determine the inhibitory efficiency of extracts of garlic and colocynth and some types of antifungal against *C. albicans* and *C.tropicalis* yeast isolated from some patients in Diyala / province of Iraq.

MATERIALS AND METHODS

Collection of sample

97 samples were Collected mediated by cotton swabs from the vagina, mouth, middle ear, skin, fingernails and urine of people infected with Candidiasis disease from General Teaching Hospital and Al Batoul teaching hospital for the birth and Child and Health Center in Canaan in Diyala, swabs were putting in a sterile test tubes containing physiological saline solution, kept in the refrigerator until use.

Examination of samples

Direct microscopic examination Samples were examined directly by putting the swab in physiological saline solution and shake well, took a drop of suspension on a glass slide and examined under an optical microscope (40X, 100X) to observe the yeast cells and pseudohyphae. glass slide stained again after you install it with gram to observation the positive yeast cells to this stain [15, 27]. the results of direct examination were compared with results of laboratory planting to measure the sensitivity of direct examination[28] and according to the following equation:

$$\boldsymbol{sensitivity} = \frac{\boldsymbol{The\ number\ of\ positive\ cases}}{\boldsymbol{the\ number\ of\ false\ negetive\ cases + number\ of\ positive\ cases}} \times \mathbf{100}$$

Samples culturing

Swabs were cultured on Sabouraud Dextrose Ager Chloramphenicol (SDAC) in sterile Petri dishes (9 cm diameter), incubated at 25-30 ºC for a period of 2-4 days, been observed phenotypic characteristics of yeast colonies such as color, texture, colony shape from my part dish during the period of incubation.

Staining of the Samples

Taking part of the colony of yeast-mediated loop and mixing with a drop of Lacto phenol cotton blue (LPCB) stain on a glass slide, and is covered by cover slipe to observe the mycelium and large spores at the examination, and took a second swab on a glass slide and stained with gram and set on flame to observation the budding.

Diagnosis of yeasts

Fungi diagnosed according to the method of Cletus and Jack [14], and the confirmation of the diagnosis was depending on some of the culturing manifestations and biochemical tests according to the method of Cletus and Jack [14] and Milne[31]. Including:

Germ Tube Formation (GT)

2 ml of egg whites was putting in a sterile test tubes, inoculated in a part of developing yeast colony on SDAC and incubated at 30 °C for 2-3 hrs. Took a drop on a glass slide and examined under an optical microscope to observe the formation of germination tube [6].

Chlamydospores formation (Ch)
Three parallel lines of 10 mm a length and of 45-degree angle worked on the medium of Corn Meal Ager (CMA), And inoculated with yeast required to diagnose, put sterile cover slipe on the midium surface, the dish is incubated at 37 ºC for 48 hrs,Then examined with a microscope to observe the Blastoconidia and chlamydospore as well as Pseudohyphae [26].

Surface Growth
inoculated test tubes containing SSB in a part of the colony of yeast, And incubated the dish at 25- 30 °C for 24 hour, to observe the Surface Growth [43].

Sugar Assimilation
Use a glass rod to spread 1 ml of yeast solution aged 24-48 hour in plastic dish containing Sugar Assimilation Medium (SAM), Add Sugar Stock Solution (SSS) in hole diameter of 6 mm in the SAM medium, Dishes were incubated at 30 °C for 2-4 days to observe the presence or absence of yeast growth in the hole [38].

Sugar Fermentation
2 ml was added 0f Sugar Fermentation Medium (SFM) to test tubes containing Durham tube upside down, and was added its as well as 2 ml of SSS medium, then was added its drops of Phenol red color change until the SFM medium of to red, then inoculated tubes in yeast suspension and incubated at 30 ºC. Results were followed up daily for 10 days to observe change in the red color to yellow and the formation of gas in Durham tube [24].

Virulence factors in yeast
Estimated some virulence factors (pathogenicity) of Candida genus, including:

Estimation of efficiency of the phospholipase enzyme
The inoculum were attended by putting a part of the yeast colony of *Candida albicans* and *C. tropicalis* developing for 18 hrs at SDAC in 5 ml of sterile Physiological Saline Solution (PSS),the number of cells was adjusted to 10^6 cells / ml using the Haemocytometric Counter, planting 10 µl of suspension to the Sabouraud Egg yolk Agar (SEAM) Medium, dishes were incubated at 37 °C for 4 days, were measured colony diameter and sedimentation zone diameter to calculate the effectiveness of the enzyme (Pz value) which is the ratio between the colony diameter and sedimentation zone diameter [1].

Estimating the mechanism of adhesion
Attended the epithelial cells of the lining of the mouth of a healthy person, taking swab from the epithelial cells of the mouth by using cotton swabs, swab placed in a glass tube containing 20 ml of Phosphate Buffer Saline solution (PBS), the solution Centrifuging at 250 r / min for 5 minutes, epithelial cells were suspended in 4 ml of PBS after washing them three times by using 20 ml of PBS, haemocytometeric Counter used to calculate the concentration of epithelial cells and adjust its concentration to 2×10^5 cell / ml, taking 0.05 ml of yeast suspension contain 2×10^6 cells were incubated with 0.5 ml of epithelial cells suspension for 90 minutes at a temperature of 37 ºC in a shaker water bath, and used 2 repeater/sample, and the adhesion assay by using a microscope [2].

Plants used
The garlic *(Ailium sativum*) of Alliaceae family and colocynthus *(Citrulius colocynthus*) of Cucurbitaceae family fruits were dried at room temperature under air stream and then grinding samples were used in this study to get the powder required for plant extracts.

Plant extracts
Four types of extracts of garlic and colocynthus, a hot and cold water and alcohol and acetone, were prepared and used in this study.

Cold water extracts
Following the method of Parekh and Chanda [36] 10 g of powder plant were mixed with 100 ml of distilled water in glass beaker, the flask was putting in an shaker incubator for 24 hours and at 37 ºC , then the mixture was nominated by using medical gauze in glass tubes, and centrifuging at 5000 r / min for 10 minutes, output was nominated by using filter paper, the filtrate is evaporated in an oven at 40 ºC for dry powder extract and save that in a dark glass bottle and sealed in frozen (- 20 ºC) until use.

Hot water extract
According to the method of El-fallal and El-kattan [17] 10 g of the powder plant were mixed with 100 ml of boiled distilled water in glass beaker, the glass beaker was putting in the shaker incubator at 28 ºC ˚ for 30 minutes, then the mixture was nominated by using medical gauze, filtrate was distributed in glass tubes and have centrifuging at 3000 r /min for 10 minutes. The filtrate collection in glass dishes (diameter 20 cm) and dry it in the oven at 40 ˚C until the water evaporates completely, to get the powder of hot water extract, which saved the same way above.

Alcoholic extracts
Following the method of Shtayeh and Abo-Ghdeib [41] 10 g of the powder plant were mixed with 100 ml of 70% ethanol, the mixture were putting in the shaker incubator at 35 °C for 24 hour, then the mixture was nominated by using medical gauze, filtrate was distributed in glass tubes and centrifuging at 3000 r /min for 10 minutes. The filtrate collection in glass dishes (diameter 20 cm), and dry it in the oven at a temperature 40°C , to get the powder of alcoholic extract which saved the same way.

Acetone extracts
Following the method above replacement with ethanol, acetone.

Preparation concentrations of garlic and colocynthis plant extracts
The microbial concentrations 20, 40 60, 80 and 100 mg / ml were prepared in dissolving 10 g of powder plant extract in 10 ml of distilled water, and using the law of general dilution C1V1 = C2V2 and was sterilized by using millipore filter (0.22µm).

The sensitivity of *C. albicans* and *C.tropicalis* to antifungal tests
The effects of three antifungal Nystatin, Ketocanazole and Flucanazole were tested against *C. albicans* and *C.tropicalis* to find the Minimal Inhibitory Concentration (MIC) and the Minimal Fungicidal Concentration (MFC) by using the SSB in the dilution method, according to the method of Shadomy et al [40] as follows: -

Preparation of inoculums

The inoculums were prepared at a rate of 1×10^5 cell/ml from the implanted two species of *C. albicans* and *C.tropicalis* developing on SDA, at the age of 24 hours.

Preparation of antifungal solution

Antifungal were prepared in the dissolving 0.01 g of the antifungal in100 ml of organic solvent Dimethyl Sulphoxide. Then the dilutions required were prepared.

Preparation of the Minimum Inhibitory Concentration (MIC) and Minimum Lethal Concentration (MFC)

The original concentration of the anti-fungal 100 µg / ml were prepared , And after that prepared a series of concentrations of multiplexed antifungal 0.05-50 µg / ml, 12 tube were prepared containing 2 ml of SSB, 2 ml of antioriginal concentration was added to the first tube to get concentration of 50 µg /ml, and the other concentration were prepared by multiplexed dilution method, with a tube of positive control (without antifungal) and a tube of negative control (without inoculums). Tubes were inoculated with 0.05 ml of suspended isolation , shake the tubes and incubated at 30 °C for 48 hrs, the growth was observed and compared with the control tube, MIC values are represent the lowest concentration, who does not appear growth . MFC value were determined by implanting 0.01 ml of each tubes that did not appear growth and control tubes on SDA free antifungal, then incubated at 30 °C for 48 hrs, and examined after the emergence of growth in the control tube. The MFC values represent the lowest concentration anti-fungal, which gives a negative result after secondary implantation.

Estimating of Cellular toxicity of garlic and colocynthis extracts

The cellular toxicity of garlic and colocynthis extracts were estimated by the method of Xin-Guo and Ursella (1994), 0.8 ml of extract was putting in a sterilized test tube with 0.2 ml of human red blood cells, to become the final volume 1 ml, the tube was shaking for 30 minutes and incubated at 37°C , and centrifuging at 1000 r / min for 5 minutes , hemolysis was observed comparison with control treatment (test tube containing blood only) to observe the differences in the hemolysis.

Estimating the percentage and acid function of garlic and colocynthis extracts

The plant extracts percentage were estimating by the method of Al-Balany [5].

$$\% \text{ Extract} = \frac{Extract\ weight}{Plant\ powder\ weight} \times 100$$

pH was measured to the plant extracts after drying and solvent.

Inhibitory effect of garlic and colocynthis extracts in growth of *C. albicans* and *C.tropicalis*

Followed a method of El-Kady et al [18] to make sure no contamination of extract, by planting 0.01 ml of plant extracts in SDA and incubated for 3-7 days, the dried plant extracts were mixed with SDA dissolved and coolant to 40 ºC, in concentration of 0.0, 20, 40, 60, 80 and 100 mg / ml with two replicates each concentration, SDA was inoculated with a disk (diameter 6 mm) at the dish center from yeast colony developing in SDA for 7 days, and two types of comparison were using, positive control with antifungal Nystatine at concentration 2 mg / ml, and negative control without antifungal, all the dishes were inoculated in the same fungus, and incubated at 28-30 °C for a week, diameter of growing colonized was measured (orthogonal diameter rate), and the percentage of inhibition was calculated by using the following equation:

$$\text{Inhibition percentage} = \frac{\text{Diameter of fungi in comparing plates} - \text{diameter of fungi in treatment plates}}{\text{Diameter of fungi in comparing plates}} \times 100$$

Statistical Analysis

The data were analyzed by practical experiment using CRD and the significant differences LSD with levels 0.01 and the SPSS program was use to analyze data .

RESULTS AND DISCUSSION

Examination of samples

A direct microscopic examination and cultured on SDAC results Table 1 Showed that the 40 swabs positive(+) in direct microscopic examination and the implant and increased by 41.24%, 35 swabs positive in planting and negative in direct microscopic examination and increased by 36.08%, 10 swabs positive in direct microscopic examination and negative(–) in planting and increased by 10.30%, And 12 swab negative for both of tests and increased by 12.38%, the reason for the emergence of the negative results of direct microscopic examination and implant attributable to the inadequacy sample collected, or not to be causative fungus [31], Or that the reason is due to the use of topical treatments randomly without consulting the specialist doctor because of the discomfort caused by this infection [15].

Table 1. Direct microscopic examination and implant on SDAC medium on *C. albicans* and *C.tropicalis*

Type of examination		**No. of samples**	**Percentage %**
direct microscopic examination	Culture		
+	+	40	41.24
–	+	35	36.08
+	–	10	10.30
–	–	12	12.38
		97	100

And positive results in implant (75 swab), and by 77.32% are consistent with that of Gravine et al [22] ,the percentage of infection in Candida isolates from oral infections to children with cancer 69.35%, and with Al-Sadik [9] that the infection rate of Candida was 66.6% and with that of Al-Albiad [4] that the infection rate of people who were cancer 76.6% and with Mohammed [33] that the infection rate of Candida was 63.6%, the reason for the different results is attributed to geographical locations and sampling methods [13] the results also showed that the direct examination method is reflective of the actual presence of the yeast Candida in people who suffering from the symptoms of this disease, the percentage of yeast presence in the direct microscopic examination was 51.54% and 77.32% at cultured on DAC, and the sensitivity percentage of direct microscopic examination amounted to 58.8% compared to culturing results , these results are consistent with that of Al-Sadik [9] there was a difference between direct microscopic examination and cultured in the slavs oral infections, and with Majeed [28], which referred to the direct microscopic examination deficit in the discovery of cases of candidiasis, some research has indicated that the cause of infection may be a result of continuing use of the antibiotics in some pathological conditions which lead to the killing of bacteria, anti-candida [35]

Diagnosis of yeasts

Followed a method of Cletus and Jack [14] for the diagnosis of *C. albicans* and *C.tropicalis*, where the genus Candida has been identified depending on phenotypic characteristics and culturing and biochemical tests, Candida individuals appeared a white colony to milky color, smooth, shiny and convex when you development at SDAC for 3-7 days at 37 º C, colony examined under a microscope after staining in gram stain and lactophynol cotton blue , spherical cells were observed to form oval or longitudinal, single cells and the presence of pseudohyphae sometimes.

Germ Tube Formation(GT)

The results in table 2 showed the susceptibility of of *C. albicans* and *C. tropicalis* to form a germination tube, is conceders a diagnosis characteristics of this species [14] and these finding are consist with Mohammed [33] who indicate that the germ tube were formed within two hours of incubation and this is unique diagnosis characteristic of *c.albicans* differentiates it from other fungi .

Table 2. Biochemical and planting tests for the diagnosis of yeast

Yeast	GT	Ch	SG	Sugar fermentation				Sugar Assimilation					
				Glu	Gal	Suc	Mal	Glu	Gal	Suc	Mal	SS	Lac
C.albicans	+	+	–	+	–	–	+	+	+	v	+	+	–
C.tropicalis	+	–	+	+	+	V	+	+	+	–	V	+	–

+ = Positive examination – = Negative examination V = Variable between positive and negative examination, Sucrose=Suc, Maltose=Mal, Starch Solution=SS, Lactose=Lac,

Formation of Chlamydospoer (Ch)
The results Table 2 showed that the yeast colonies *C.albicans* creamy white color, and the appearance clingy, take the form of dendritic branching on agar these finding are consisting with Mohammed [33] who indicates that the *C.albicans* are producing chlamydospores on CMA at 22-25 ˚ C for 48-72 hrs. they were spherical, thick wall and usually produced on suppurating cells that occur along psedohyphae or at the tip of hyphae. *C.tropicalis* doesn't produce chlamydospores.

Surface Growth(SG)
The results in table 2 showed the ability of *C. tropicalis* on the composition of creepy growth towards the top on the wall of test tube containing on SSB medium.

Sugar fermentation and Assimilation
The results in Table 4 have shown the ability of *C.albicans* and *C.tropicalis* on the ferment of sugar glucose (Glu), and portability of *C.tropicalis* species as well as Galactose (Gal) fermentation to indistinguishable from other species, because of the oxidation of carbon-source and production of gas in the tube dirhams and change the medium color from red to yellow.

Table 3 showed that the *C.albicans* is the predominant when diagnosing pathological isolates depending on culturing and microscopic characteristics as well as the biochemical tests, and

increased by 69.3%, followed by 13.3% *C.tropicales*, these results approaching with that of Satana et al. [39] that the percentage of yeast *C. albicans* isolated from the oral cavity of people suffering from oral infections estimated at 73.1% and C. *tropicles* by 1.5%, as well as the Gravina et al. [22] the percent of *C. albicans* 42.55% *C. tropicles* by 12.76% and isolated from the oral cavity of patients with cancer, Al-Sadik [9] indicate to sovereignty the yeast *C. albicans* by 78.7%, followed by *C. tropicalis* 13.7%, the superiority of *C. albicans* possesses were attributable to many virulence factors, such as the bilateral format that enables it to switch from yeast to filamentary form, where the strands begin to grow and colonize the surface of the mucous membranes [19], as well as the ability to adhesion in the epithelial membranes cells with a high degree compared to other species, this is attributed to the presence of a number of surface receptors that lead to increase the capacity of the yeast *C. albicans* adhesion to epithelial tissue cells to the host's body, as well as on their ability to secretion of digestive enzymes of the protein, the most important Aspartic Proteinase responsible for the protein analysis and accelerate the entry into force of yeast cells into the host tissue and cause infection process as well as the excretion of phospholipase enzyme responsible for phospholipids analysis, which are the main component of cell membranes [20].

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Table 3. Yeasts ratios in the samples

Yeast	**N0. of samples**	**Ratios**
C.albicans	52	69.3
C.tropicales	10	13.3
Total number of samples examined	75	

Virulence factors in yeast

Estimation of efficiency of Phospholipase enzyme

The results in Table 4 have shown the ability of Candida species to produce phospholipase enzymes, the yeast *C.albicans* showed higher effective for the production of the phospholipase enzyme 0.33 by measuring the diameter of sedimentation zone to diameter colony Candida followed by *C.tropicalis* 0.29 with no significant differences at the level of probability of 0.01, the reason is attributable to the susceptibility of Candida isolates to produce the phospholipase enzyme and its effectiveness which depends on several factors, including the physics related in degree temperature to the production of the enzyme and save heat, and including gene related to the existence of the necessary genes for the production of this enzyme, it was found that the production of the phospholipase enzyme and effectiveness may vary among strains within species based on the installation of these genetic strains. These findings are consistent with that of the Al-Abied et al. [4] that the effectiveness of the production of the phospholipase enzyme from the *C. albicans* was 0.13 -0.21%, and also with that of Al-hujami [7] that the effectiveness

Table 4. Effectiveness of Phospholipase enzyme

Yeast	**Pz Value**
C.albicans	17.5±4.12
C.tropicalis	10±3.00

phospholipase enzyme production by *C. albicans* 0.7-0.8, and the reason is due to the yeast Candida isolates keeping in - 20 ºC and -80 ºC resulted to the loss of its ability to produce the enzyme, and with what indicated by Price et al. [37] that 30-70% of the isolates of *C. albicans*-producing enzyme despite the differences in effectiveness between these strains.

Estimating of the mechanism of adhesion

Results in Table 5 have shown that the proportion of the adhesion of yeast cells in the epithelial cells of the mouth of *C. albicans* and *C. tropicalis* 17.5% and 10% with the existence of significant differences between *C. albicans* and *C. tropicalis*, these results are consistent with Klotz [25] that the adhesion occurs because of *C. albicans* formation the fibrous layer composed of multiple sugars on the surfaces of cells stationary phase of growth, and with Al-Abeid et al. [4] that the adhesion percentages of yeast *C. albicans* and *C. glabrata* 21.0% and 18.4%, and the results vary with Al-hujami [7], which mentioned that the highest percentage of adhesion of yeasts 52% and the lowest 32% of *C.albicans*. And the emergence of differences in the proportion of adhesion is attributable to the affinity of yeast cells to water (hydrophobic)

Table 5. The adhesion percentage of *C.albicans* and *C.tropicalis* yeast in oral epithelial cells

Yeast	Percentage of adhesion
C.albicans	17.5±4.12
C.tropicalis	10±3.00

*** Average number of adhesion in epithelial cells lining the mouth ± standard error when LSD = 8.3**

, if the cells have a few familiarity of water is the biggest adhesion twice as high for water familiarity cells, as well as effect of charge, the adherent cells positive charge ten times bigger than non-adherent cells [25], it was also observed that the adhesion out of the body (in vitro) increases when the *Candida albicans* growth on the media containing high concentrations of sugars, such as sucrose and galactose (Carbone as a source of growth) [29].

The sensitivity of *C. albicans* and *C.tropicalis* yeast for antifungal

The results in Table 6 showed that the antifungal Flucanazole group have inhibitory effectiveness against *C. albicans* and *C.tropicalis* growth, Where the MIC 12.5 and 6.25 µg/ml, Followed by the two groups of Ketacanazole and Nystatin 25 and 12.5 µg/ml respectively, These findings are consistent with Van-den Bossch [42] that a group Fluconazole two effects, the first effect on Cytochrome P450 (CYP) that leads to the inhibition of Ergosterol produces from the removal of 14. α methylsterol, And second effect which resulting from direct overlapping anti-fungal with innate fat cell membrane, which leads to destroying it, and with Ingroff et al [23] that a polyenes group including anti-fungal Nystatin wide effect at union with sterols in the cell membrane, and thus lead to leakage of important components of cell and death, the results are different with Al-Sadik [9] results that MIC from Nystatin 32 µg/ml against *C. albicans* ,the results are consistent with the results of Al-hujami [7] that the value of MIC from Nystatin ranges between 12.5-25 µg/ml against Candida yeast, and vary with the results of Arikan et al [11] that the value of MIC for Nystatin antifungal ranges between 1-2 µg/ml, and the difficulty of determining the value of the concentration of MIC is attributable to the wide and indiscriminate use of anti-fungal, and to the difference of sensitivity between the types of genes towards antifungal, and to the emergence of strains differ in the genotype from wild strains [16, 25].The results in table 6 showed that the concentration of MFC 50 µg/ml of the *C. albicans* and *C.tropicalis* fungus.These findings are consistent with Al-Janabi [8] that the effectiveness of anti-fungal and duration required to kill the yeast depends on the concentration. Colocynthis extracts of

Table 6. The MIC and MFC values for antifungal

Yeast	Antifungal	MIC (µg/ml)	MFC (µg/ml)
C. albicans	Flucanazole	12.5	50
	Ketacanazole	25	50
	Nystatin	25	50
C.tropicalis	Flucanazole	6.25	50
	Ketacanazole	12.5	50
	Nystatin	25	50

Estimating of cellular toxicity of watery, alcoholic and hexane of garlic and colocynthis extracts

watery , alcohol and hexane showed cellular toxicity of human red blood cells by hemolysis test in vitro Table 7, and garlic plant extracts did not show toxicity cellular, the reason for this is attributed to the low concentrations of saponin compounds in garlic plant, and cellular toxicity is attributed to the emergence of colocynthis extract to the affinity for the sterols in the plasma membrane of the cell, Where the removing the plasma membrane and liberation of hemoglobin [30].

Table 7. Toxicity function to the garlic and colocynthis extract

Extraction method	Toxicity function of garlic	Toxicity function of colocynthis
Cold water extract	–	+
Hot water extract	–	+
Alcoholic extract	–	+
Hexane extract	–	+

The percentage and acidic function of garlic and colocynthis extracts

Alcoholic extracts gave the best percentages of 50% and 45% Table 8, followed by hexane extracts 52% and 63% and hot water extracts 33% and 35%, then the cold water extracts of 25% and 30% for the garlic and colocynthis respectively, the acidic function for alcoholic extracts for garlic and colocynthis 5.33 and 5.63, followed by hexane extracts 4.66 and 5.47, then hot and cold water extracts 5.45, 5.54 and 5.66, 4.56, respectively.

Table 8. The percentage and acidic function for garlic and colocynthis extract

Extraction method	percentage %		Acidic function	
	colocynthis	Garlic	colocynthis	Garlic
Alcoholic extract	50.00	45.00	5.33	5.63
Hexanes extract	52.00	63.00	4.66	5.47
Hot water extract	33.00	35.00	5.45	5.54
Cold water extract	25.00	30.00	5.66	4.56

Effect of garlic and colocynthis extract at inhibition of *C. albicans* and *C.tropicalis* yeast growth

Results in table 9, have shown non-significant differences ($P \leq 0.01$) between the cold and hot water extracts of garlic and colocynthis plants, and the existence of significant differences

between alcoholic and hexanes extracts in inhibiting the growth of *C. albicans* and *C.tropicalis*, the reason is attributed to soluble active substances for the garlic and colocynthis well in organic solvents such as ethanol, methanol and not soluble in water well [3], and the results for garlic extracts showed the highest effective inhibition than the effective inhibition for the colocynthis extracts against the *C. albicans* and *C.tropicalis*, these findings are consistent with that of Al-Thuwaini et al [10] that garlic extract has a high effective inhibition against Candida species, Cold water extracts is ranked first among the extracts, where the results in table 9 showed that the cold water extract of garlic has effective inhibition against *C. albicans* and *C.tropicalis* higher than the effective inhibition of cold water extracts for the colocynthis, and *C.tropicalis* has a higher sensitivity for cold water extract of garlic of *C. albicans*, cold water extract of garlic has given highest inhibition percentages 96.73% for *C.tropicalis* at 100 mg / ml, and cold water extract of colocynthis has given the lowest percentage of inhibition 57.60% for *C. albicans* at 20 mg / ml.

Table 9. Effect of cold water extracts of garlic and colocynthis in the inhibition of *C. albicans* and *C.tropicalis* yeast growth

Plants	Yeasts	Growth diameter (mm)/Extract concentration (mg/ml)							L.S.D
Garlic		Con+	0.0	20	40	60	80	100	C=0.79
	C. albicans	0.0	46.0	18.0	12.5	8.5	6.5	2.5	
	C.tropicalis	0.0	46.0	17.0	11.0	8.0	4.0	1.5	
Colocynthis	*C. albicans*	0.0	46.0	19.5	12.5	8.5	7.0	3.0	
	C.tropicalis	0.0	46.0	16.0	14.5	10.0	4.5	1.5	
A=0.56	B= 0.56	D = 1.05							P≤0.01

A = plant B = species C = method D = concentratim
Con + = control treatment with antifungal Nystatin at concentration of 2 mg / ml

Hot water extracts for garlic and colocynthis came in second place table 10, hot water extract of garlic gave the highest percentage inhibition 95.65% to *C. albicans* at 100 mg / ml, and hot water extract of colocynthis gave the lowest percentage inhibition 59.34% to *C. albicans* at 20 mg / ml.

Table 10. Effect of hot water extracts of garlic and colocynthis at inhibition of *C. albicans* and *C.tropicalis* yeast growth

Plants	Yeasts	Growth diameter (mm)/Extract concentration (mg/ml)							L.S.D
Garlic		Con+	0.0	20	40	60	80	100	C=0.79
	C. albicans	0.0	46.0	17.0	11.0	8.5	6.0	2.0	
	C.tropicalis	0.0	44.5	15.0	10.0	7.5	3.0	2.0	
Colocynthis	*C. albicans*	0.0	45.5	18.5	16.0	10.0	6.5	2.5	
	C.tropicalis	0.0	46.0	16.5	13.5	10.0	6.0	2.0	
A=0.56	B= 0.56	D = 1.05							P≤0.01

And alcoholic extracts were came in third Table 11, alcoholic extract of garlic gave the highest inhibition percentage 98.88% at 100 mg / ml for *C. albicans* and *C.tropicalis*, and the lowest inhibition percentage 67.02% at 20 mg / ml for *C. albicans*. The alcoholic extract of garlic showed inhibition effectiveness at 100 mg / ml equal to the inhibition effectiveness of antifungal Nystatine at 2 mg / ml against *C. albicans* and *C.tropicalis*, and inhibition

effectiveness of alcoholic extract for colocynthis at 100 mg / ml equally with inhibition effectiveness for antifungal Nystatine at 2 mg / ml against *C.tropicalis* as well.

Table 11. Effect of alcoholic extracts of garlic and colocynthis at inhibition of *C. albicans* and *C.tropicalis* yeast growth

Plants	Yeasts	Growth diameter (mm)/Extract concentration (mg/ml)							L.S.D
Garlic		Con+	0.0	20	40	60	80	100	
	C. albicans	0.0	45.0	14.5	9.5	4.50	3.0	0.5	
	C.tropicalis	0.0	45.0	12.5	9.5	4.5	3.5	0.5	C= 0.79
Colocynthis	*C. albicans*	0.0	47.5	15.5	11.0	5.5	2.5	2.0	
	C.tropicalis	0.0	51.5	15.0	11.0	5.5	2.5	1.0	
A= 0.56	B = 0.56	D = 1.05							P≤0.01

Then hexane extracts came in fourth Table 12, hexane extract of garlic gave the highest inhibition percentage 100% at 100 mg / ml against *C.tropicalis*, it is equal to the inhibition percentage of antifungal Nystatine at of 2 mg / ml, and the lowest inhibition percentage of hexane extract of garlic 72.72% at 20 mg / ml against the *C. albicans*.

Table 12. Effect of hexane extracts of garlic and colocynthis at inhibition of *C. albicans* and *C.tropicalis* yeast growth

Plants	Yeasts	Growth diameter (mm)/Extract concentration (mg/ml)							L.S.D
Garlic		Con+	0.0	20	40	60	80	100	
	C. albicans	0.0	44.0	12.5	8.0	4.0	2.5	1.0	
	C.tropicalis	0.0	49.0	12.5	7.5	3.0	2.0	0.0	C= 0.79
Colocynthis	*C. albicans*	0.0	46.5	11.5	7.5	2.5	2.0	1.0	
	C.tropicalis	0.0	47.0	8.00	5.0	3.0	2.5	1.0	
A= 0.56	B = 0.56	D = 1.05							P≤0.01

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