بسم الله الرحمن الرحيم

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Title

Detection of Antifungal Activity of Cinnamon Extract on Candida Species Isolated from Diabetic Patients with Urinary Tract Infection.

A thesis submitted in partial fulfillment for the requirement of MSc degree in (Microbiology)

by

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الايـــــــــة

قال تعالى:

بسم الله الرحمن الرحيم

﴿اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ﴿1﴾خَلَقَ الْإِنْسَانَ مِنْ عَلَقٍ﴿2﴾اقْرَأْ وَرَبُّكَ الْأَكْرَمُ﴿3﴾الَّذِي عَلَّمَ بِالْقَلَمِ﴿4﴾عَلَّمَ الْإِنْسَانَ مَا لَمْ يَعْلَمْ ﴿5﴾﴾

صدق الله العظيم

سورة العلق : الايات 1- 5

Dedication

To my wonderful parents who strongly supported me all throughout.

To my beloved sisters and adorable brothers.

To all those whom I always love, care and respect.

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List of Abbreviations

ALS	[agglutinin–like sequence (Als1–7 and Als9)]
(BTB)	Bromothymol blue
(BCP),	Bromcresol purple
GTT	Germ Tube Test
HWP1	(Hypha associated GPI–linked protein)
MBC	Minimum bactericidal concentration.
MIC	Minimum inhibitory concentration.
MSU	Mid-stream urine.
NCCLS	National Committee for clinical laboratory standards.
pH	Potential of hydrogen.
STI	Sexual Transmitted Infection
UTI	Urinary tract infection.

Abstract

Background: Diabetic patients are more susceptible to develop fungal Urinary tract infections (UTI). UTI is a major health problem. Finding alternative antimicrobial agents from plant extracts has received growing interest. *Cinnamon* a safe, nontoxic, cheap beverage that has been reported to have antimicrobial effects against various Candida species.

Objectives: This study aimed to evaluate the antifungal activity of Cinnamon extract on Candida species isolated from diabetic patients suffering from urinary tract infection.

Methodology: A descriptive cross sectional study was conducted in Shendi locality in a period from May to August 2018, to determine the activity of cinnamon extract against Candida species isolated from urine of diabetic patients.

Four Candida species were isolated, in vitro sensitivity testing using well diffusion technique against aqueous cinnamon extract.

Results: Crude extract of Cinnamon was tested for antifungal activity using Cup plate method.

Cinnamon extract showed good antifungal activity against Candida species.

Largest zone of inhibition against *Candida tropicalis* was (25 mm) and least zone of inhibition against *Candida kefyr* was (10 mm). The results of antimicrobial activity of crude extract was compared with the positive control (Clotrimazole) for evaluating their relative percentage inhibition and statistical significance the aqueous extract exhibited maximum relative percentage inhibition against *Candida kefyr* (89%) and minimum relative percentage inhibition against *Candida glabrata* was (12.3%). The p.value is (0.018) its statistically significant.

Minimum inhibitory concentration*:*

Antibacterial activities of extracts were checked by well diffusion method. The concentrations of green leaves aqueous, extract used was 1, 0.5, 0.25 and 0.125g/100ml**.** MIC values of aqueous extracts of cinnamon on test organisms which the lowest concentration of Cinnamon aqueous extract able to inhibit the growth of Candida was (0.187g/100ml).

Conclusion: The antifungal activity of crude extract was compared with that of standard antimicrobial based on the mean diameter of inhibition zone. The aqueous extract exhibited maximum relative percentage against *Candida kefyr* (89%) and minimum relative percentage inhibition against *Candida glabrata* was (12.3%).

Key word: Candida spp, Cinnamon, Shendi, Antifungal activity, Diabetic, UTI.

المستخلص

المقدمة : نقص المناعة عند مرضي السكري يجعلهم أكثر الفئات عرضة للإصابة بإخماجات المجاري البولية الذي يعتبر من أكبر المشاكل الصحية.تواجد بدائل من عوامل مضادة للميكروبات مستخلصة من النبات وجد طريقا للاهتمام. نبتة القرفة هي نبتة آمنة ، غير سامة و رخيصة الثمن للتحصل عليها و قد أثبتت التقارير أن لها فعالية مضادة للميكروبات الممرضة و خاصة الخميرة .

الهدف :هدفت هذه الدراسة إلى معرفة مدى تأثير المستخلص المائي لنبتة القرفة على الأنواع المختلفة للخميرة المعزولة من مرضى السكري الزين يعانون من إخماج المجاري البولية.

أجريت هذه الدراسة التقاطعية في جامعة شندي– كلية علوم المختبرات الطبية – قسم الأحياء المجهرية في الفترة مايو 2018 الى أغسطس 2018م . وقد تضمنت عدد مائة من مرضى السكرى يعانين من إخماجات المجاري البولية من أعمار مختلفة.

المنهجية :أربعة انواع خميرة معزولة مختلفة مسببة لإخماج المجاري البولية أختبر تأثيرمستخلص القرفة عليها داخل المعمل بإستخدام تقنية الثقوب النافذة أو المنتشرة.

النتائج :متوسط أعمار مرضى السكري المصابون بإخماج المجاري البولية 56.34أربعة أنواع مختلفة من الخميرة وكانالعامل الرئيسي المسبب لإخماج المجاري البولية في مجتمع الدراسة هي خميرة

اكبر نطاق للنثبيط ظهر ضد خميرة المبيضة المدارية (25.)،.تراكيز المستخلص المائي للقرفة اللتي أستخدم هو0.125,0.25,0.5،1. 1 ملجم / 100مل.أقل تركيز من المستخلص المائي للقرفة قادر على تثبيط نمو الخميرة هو0.187جم/100مل

الاستنتاجات : نتائج فعالية مستخلص القرفة ضد الخميرة قورن مع نوع فعال من الأدوية لإيجاد نسبة معدل التثبيط المقرب والدلالة الاحصائية.معدل التثبيط المقرب أظهر أعلى نسبة ضد مبيضة كيفير (89%) وأقل نسبة ضد الميضة الجرداء(12.3%).القيمة المعنوية (0.018) اي انها دالة احصائيا .

الكلمات المفتاحية : شندي، خميرة مبيضة، القرفة،السكري،التهاب المجاري البولية،نشاط ضد الخميرة.

Chapter one

Introduction

Rationale

Objectives

1.1 Introduction

Since time immemorial, mankind has searched for medications to remove pain and cure various diseases. Evidence exists for the use of medicinal plants up to 60,000 years ago but more recently, a 5000 year-old Sumerian clay slab was discovered verifying the utilization of medicinal plants for the preparation of drugs.

Nowadays it has been estimated that more than 50% of available drugs have originated in some way from plants. [(1)]

While medicinal plants were used primarily in simple pharmaceutical formulations such as macerations, infusions and decoctions, between the 16th and 18th centuries, the demand for compounded drugs was very much on the increase. These compounded drugs comprised medicinal plants along with drugs of animal and plant origin. If the drug was prepared from a number of medicinal plants, minerals and rare animals, it was highly valued and sold at a premium. [(1)]

Many medicinal plants are employed as self-medications or are used upon the recommendation of a physician or pharmacist. They are employed both independently and/or in combination as complementary medicines to synthetic drugs. It is imperative for an adequate and successfully applied therapy that an accurate early diagnosis is made of the illness along with selection of the appropriate pharmacological effects associated with the specific herbal components. [(1)]

Community-acquired Urinary Tract Infections (UTIs) are a frequent problem worldwide which are caused by microbial invasion to different tissues of the urinary tract. Fungi are a portion of microbial population that may contribute as fungal uropathogens in UTIs. In the last 2.5 decades the fungal UTIs due to Candida genus yeast has increased significantly [(2).]

The predisposing factors of UTIs including gender, genetic predisposition, behavioral factors, urologic structural abnormalities, diabetes, immune-suppression, pregnancy, hypertension, stone formation, nosocomial acquired infections and instrumentation like catheterization [(3).]

The healthy urinary tract is sterile so, the presence of Candida yeasts in the urine implicates a variety of clinical situations. Candiduria can be demonstrated as symptomatic or asymptomatic UTIs; the incidence of lower urinary tract infections caused by yeasts is fourfold more common in women than in men. Furthermore, the results of different studies have indicated that UTIs in women are very common [(3).]

Lower UTI with Candida usually occurs in patients with urinary catheters, typically after antibiotic therapy, although candidal and bacterial infections frequently occur simultaneously. *C. albicans* prostatitis occurs infrequently in patients with diabetes, usually after instrumentation.

Renal candidiasis is usually spread hematogenously and commonly originates from the GI tract. Ascending infection is possible and occurs mainly in patients with nephrostomy tubes, other permanent indwelling devices, and stents. At high risk are patients with diabetes and those who are immunocompromised because of tumor, AIDS, chemotherapy, or immunosuppressant's. A major source of candidemia in such high-risk hospitalized patients is an indwelling intravascular catheter. Renal transplantation increases the risk because of the combination of indwelling catheters, stents, antibiotics, anastomotic leaks, obstruction, and immunosuppressive therapy. [(4)]

The consumption of cinnamon and its use for a variety of other purposes has a long history. Even Moses used cinnamon in his holy oil in the Bible’s Old Testament. The spice trade of historic Europe thrived on cinnamon as one of

its primary luxury goods. Cinnamon is now grown in many parts of the world and has been used medicinally for some time. With the advent of modern technology; scientists have also verified that cinnamon is a viable treatment for some bacterial and fungal conditions. Getting rid of yeast infections is one medicinal use cinnamon shows excellent efficacy in. You likely have some cinnamon bark powder already in your house; you can add it to your yeast infection treatment to bolster the power of your current therapy. [5]

1.2Rational

This research was done to evaluate the effectiveness of Cinnamon to eliminate the yeast cell which can be used as preventive agent for recurrent urinary tract infection in diabetic patients. And it was done first time in this area. Also used in traditional medicine ,no side effect and have good antimicrobial activity.

1.3 Objectives

1.3.1General objective:

To detect the antifungal activity of Cinnamon extract on Candida species isolated from diabetic patients suffering from urinary tract infection.

1.3.2 Specific objectives:

1- To evaluate the effectiveness of aqueous extraction of Cinnamon *on Candida albicans* and the other species of Candida.

2- To determine the (MIC) of Cinnamon extract for descript the antifungal activity against Candida species.

3- To determine the frequency of the Candida in urine of diabetic patients.

Chapter 2

Literature Review

2. Literature review

2.1 Cinnamon:

2.1.1 Scientific Classification:

Kingdom: plantae

Clade: angiosperms

Clade: magnoliids

Order: laurales

Family: lauraceae

Genus: Cinamomum

Species:c. verum

Few human trials have been published that investigated theefficacy of cinnamon on physiological parameters and health related conditions. However, recent in vitro and invivo researchhas discovered new properties of C zeylanicum and C. cassia, which may be of interest for clinical use. The treatment ofdiabetes (type 2) has been investigated in several clinical trials. And is probably the most well-documented health benefit of this spice for humans. Nevertheless, additional research is needed to determine whether cinnamon can help control this disease in free-living patients. Furthermore, some evidence suggests that cinnamon may be effective in the supportive treatment of cancer, infectious diseases, and complaints associated with modern life style due to its anti-inflammatory, antimicrobial, antioxidant, and blood pressure-lowering effects. Unfortunately, human data in this area is limited. One trial on Helicobacter pylori infection yielded negative results for cinnamon ingested at daily doses of 80 mg. pilot trial on candidiasis in HIV-patients (daily dosage of cinnamon not reported). [6]

Dose-response trials are of paramount importance, as the clinical studies on

the hypoglycemic properties of cinnamon have shown. The studies which did not yield statistically significant results were carried out with a daily dose of ≤1.5 g of cinnamon. Significant positive effects were only found in studies utilizing 3 to 6 g of cinnamon daily. One teaspoonful of cinnamon powder weighs approximately 1.5 g. It seems reasonable that up to 2 teaspoons of this spice could easily be integrated into a normal diet. [15]

Preclinical and Clinical Evidence Anti-inflammatory properties. The studies cited in this sectionrefer to the ability of cinnamon to affect inflammation, e.g. By counteracting the cyclooxygenase (COX) enzyme. Studiesdealing with related mechanisms of action are cited in the appropriatesections—for example, the antioxidant activity mayinfluence the immunomodulary properties of a drug, which in turn may cause an anti-inflammatory effect. The inhibitors of prostaglandin biosynthesis and nitric oxide production are potential anti-inflammatory and cancer chemo preventiveagents. Cinnamomum cassia extracts showed potent inhibition of cyclooxygenase-2 (COX-2) activity in lipopolysaccharide(LPS)-induced mouse macrophage RAW264.7 cells.The main constituents of cinnamon, eugenol, and Cinnamaldehyde, were found to inhibit COX-2 in vitro in a rapid semi-homogeneous COX-2 enzymatic assay. [6]

The redox sensitive, pro-inflammatory nuclear transcription factor NF-kappaB plays a key role in inflammation. Cinnamaldehyde derivatives based on 2_-hydroxycinnamaldehyde isolated from the bark of C. cassia significantly inhibited lipopolysaccharide (LPS)-induced nitric oxide (NO) production and NF-kappaB transcriptional activity in a dose-dependent manner. [6]

2_-Hydroxycinnamaldehyde had the strongest inhibitory effect on NO production among the cinnamaledhyde derivatives through inhibition of NF-kappaB activation, and thus could be used as an anti-inflammatory agent due

to its antioxidant properties. Recently examined cinnamaldehyde further for its molecular modulation of inflammatory NFkappaB activation via the redox-relatedNF-kappaB/IχBkinases (NIK/IKK) and mitogen-activated protein kinase (MAPK) pathways through the reduction of oxidative stress. Results show that age-related NF-kappaB activation upregulated NF-kappaB targeting genes, inflammatory iNOS, and COX-2, all of which were effectively inhibited by cinnamaldehyde. Cinnamaldehyde furthermore inhibited the activation of NF-kappaB via three signal transduction pathways—NIK/IKK, extracellular signal regulated kinases, and p38 MAPK. It is likely that the antioxidative effect of cinnamaldehyde and the restoration of redox balance are responsible for its anti-inflammatory action. (6)

As this section has suggested, the bark of C. cassia, probably due to its cinnamaldehyde content, demonstrates clear anti-inflammatory properties in vitro. Additional information is provided below in the sections on "antioxidant properties" and "immunomodulatory properties.

2.1.2 Antibacterial properties:

Spices have been traditionally used since ancient times for their antiseptic and disinfectant properties. Preliminary screening for antimicrobial activities of 35 different Indian spices. Cinnamon, among others, has potent antimicrobial activity against the test organisms *Bacillus subtilis* and *Escherichia coli.*

Cinnamon bark oil as well as cinnamaldehyde and eugenol showed potent antibacterial effects against Bacillus cereus, *Campylobacter jejuni, Enterococcus faecalis, Escherichia coli, Listeria monocytogenes, Haemophilus influenzae, Salmonella choleraesuis, S. enterica, Pseudomonas aeruginosa, Staphylococcusaureus, Streptococcus pneumoniae, and S. pyogenes,* as well as *Yersinia enterocolitica*. In general, Gram-positive

bacteria were more sensitive to inhibition by the plant essential oil than Gram-negative bacteria. *Campylobacter jejuni* was the most resistant of the bacteria investigated. [6]

2.1.3 Antifungal properties:

The essential oils of several Cinnamon species have been shown to have anticandidal(Candida albicans, C. glabrata) and antidermatophytic (Microsporum canis, Trichophyton mentagrophytes, T. rubrum)activity in vitro. The essential oil of the leaves of C. zeylanicum demonstrated only modest antifungal properties. However, the essential oil of C. zeylanicum (plant part notspecified) showed the strongest antifungal activity comparedto Aniba roaeodora, Laurus nobilis and Sassafras albidum against 17 micromycetes (Aspergillus niger, A. ochraceus, A.versicolor, A. flavus, A. terreus, Alternaria alternata, Aureobasidium pullulans, Penicillium ochrochloron, P. funiculosum, Cladosporium cladosporioides, C. Fulvium, Trichoderma viride, Fusarium tricinctum, F. sprortrichoides, Phoma macdonaldii, Phomopsis helianthi, Mucor mucedo) in vitro.Transcinnamaldehydewas the most active component in the oil of C.zeylanicum. Cinnamic aldehyde as the active fungitoxic constituent of C. zeylanicum bark oil. The fungitoxic properties of the vapors of the oil/active constituent were establishedagainst fungi involved in respiratory tract infections (mycoses), i.e., Aspergillus niger, A. fumigatus, A. nidulans A.flavus, Candida albicans, C. tropicalis, C. pseudotropicalis, and Histoplasma capsulatum. The authors concluded that these inhalable vapors appear to approach the ideal chemotherapy forrespiratory tract mycoses. Cinnamon oil demonstrated inhibitory activity against Aspergillus flavus, A. parasiticus, A. ochraceus, Fusarium moniliforme, F. graminearum and F. proliferatum as well as Saccharomyces cerevisiae in several further studies.

Oral candidiasis is a frequent occurrence in patientswith HIVinfection.

Treatment of this conditionwith an oral azole is generally effective. However, fluconazole-resistant Candida species are an emerging problem. C. zeylanicum shows in vitro activity against fluconazole-resistant and -susceptible Candida isolates. [6]

2.1.4 Antiviral Properties:

A Cinnamomum cassia bark extract was highly effective against HIV-1 and HIV-2 replication in terms of inhibition of virus-induced cytopathogenicity in MT-4 cells infected with HIV. Cinnamaldehyde derived from cinnamon bark has an inhibitory effect on the growth of influenza A/PR/8 virus in vitro (Madin-Darby canine kidney cells) and in vivo (mice infected with the lung-adapted PR-8 virus.

The available in vitro data demonstrate that C. cassia bark oil as well as aqueous and ethanoic extracts have potent antibacterial and highly effective antiviral properties against Gram positive and Gram-negative bacteria as well as HI- and influenza virus, respectively. [6]

2.1.5 Clinical trials:

Pilot study in five patientswith HIV infection and oral candidiasis to investigate the activityofcinnamon (*Cinnamomum zeylanicum*) against fluconazole resistant and –susceptible Candida isolates. All patients studied had pseudo membranous candida infection confirmed by culture. Patients were given eight lozenges of a cinnamon candy daily (no further information given). The commercially available extract was administered for one week. Three of the five patients had improvement of their oral candidiasis (no further details given). The pilot study was neither randomized nor blinded, and the sample size was very small. Further clinical trials will be necessary to determine the usefulness of cinnamon for the treatment of mucosal candidiasis. [6]

2.2. **A urinary tract infection (UTI):**

Is an infection that affects part of the urinary tract. When it affects the lower urinary tract it is known as a bladder infection (cystitis) and when it affects the upper urinary tract it is known as kidney infection (pyelonephritis). Symptoms from a lower urinary tract include pain with urination, frequent urination, and feeling the need to urinate despite having an empty bladder. [7]

Symptoms of a kidney infection include fever and flank pain usually in addition to the symptoms of a lower UTI. Rarely the urine may appear bloody.

In the very old and the very young, symptoms may be vague or non-specific.

The most common cause of infection is Escherichia coli, though other bacteria or fungi may rarely be the cause. Risk factors include female anatomy, sexual intercourse, diabetes, obesity, and family history. Although sexual intercourse is a risk factor, UTIs are not classified as sexually transmitted infections (STIs). [8]

Kidney infection, if it occurs, usually follows a bladder infection but may also result from a blood-borne infection. Diagnosis in young healthy women can be based on symptoms alone. In those with vague symptoms, diagnosis can be difficult because bacteria may be present without there being an infection. In complicated cases or if treatment fails, a urine culture may be useful.[9]

2.3 Fungal UTI:

Anatomically, urinary tract infections (UTIs) –whet-her caused by fungi or bacteria– are categorized into two sections (lower and upper tract infections) which may occur in asymptomatic or symptomatic forms. Our knowledge

about the incidence of candidal UTIs is obtained from diverse published reports in different countries worldwide, just like a puzzle which is completed by putting together puzzle pieces. It is important to control the pattern of etiologic microbial agents regularly in order to find new options to manage and prevent the related infections.

According to numerous investigations, Candida species and in particular, *Candida albicans* (*C. albicans*) are the most remarkable opportunistic pathogenic fungi causing nosocomial UTIs.

Candida albicans and non–C. albicans Candida (NACA) species are considered important parts of microbial normal flora in the oral cavity, alimentary canal and vagina in a vast range of the healthy people. Furthermore, they colonize on the external side of the urethral opening in premenopausal and healthy females. Immune deficiencies may lead to an imbalance between *C. albicans*, NACA yeasts and the other host normal flora. In this condition, the commensal yeasts of Candida may convert into opportunistic. The presence of *C. albicans* and NACA species in urine is known as candiduria, which may occur in both asymptomatic and symptomatic UTIs. [9]

2.3.1 Candida:

Scientific classification

Kingdom: Fungi

Division: Ascomycota

Class:Saccharomyces's

Order: Saccharomycetales

Family: Saccharomycetaceae

Genus: Candida

Candida is a genus of yeasts and is the most common cause of fungal infections worldwide. Many species are harmless commensals or

endosymbionts of hosts including humans; however, when mucosal barriers are disrupted or the immune system is compromised they can invade and cause disease. Candida albicans is the most commonly isolated species, and can cause infections (candidiasis or thrush) in humans and other animals. In winemaking, some species of Candida can potentially spoil wines. [10]

2.3.2. Candida species:

Many species are found in gut flora, including *C. albicans* in mammalian hosts, whereas others live as endosymbionts in insect hosts. Systemic infections of the bloodstream and major organs (candidemia or invasive candidiasis), particularly in immunocompromised patients, affect over 90,000 people a year in the U.S. [11]

Antibiotics promote yeast infections, including gastrointestinal Candida overgrowth, and penetration of the GI mucosa. While women are more susceptible to genital yeast infections, men can also be infected. Certain factors, such as prolonged antibiotic use, increase the risk for both men and women. People with diabetes or impaired immune systems, such as those with HIV, are more susceptible to yeast infections. [12]

2.3.3 Candida albicans and virulence factors:

C.albicans as a diploid dimorphic fungus ranks first for causing systemic candidiasis and fungal nosocomial UTIs worldwide. The shape flexibility, as in switching between yeast and filamentous forms, is one of the most well-known pathogenic factors in the dimorphic fungus C. albicans. Additionally, there are several attributes such as adhesion, invasion, discharging hydrolytic enzymes, stereotropism (thigmotropism) and biofilm formation which are absolutely considered as pathogenic mechanisms pertaining to C. albican. [13]

2.3.4 Candida albicans and polymorphism:

Morphology of *C. albicans* determines the strategy of fungal colonization and infection. The three forms of *C. albicans* include spheroid–ovoid shape of single–celled budding yeast, loose septate pseudo hyphae with an elongated ellipsoid appearance of the hyphae divisions as well as septate true hyphae. In accordance with recorded reports, yeast cells and true hyphae both directly contributed to UTI candidiasis and the pseudohyphal form of *C. albicans is* known as a switch construction of the fungus in vivo conditions. Therefore, an obvious morphological evolutionary pathway is seen in the life cycle of *C. albicans*. [13]

The filamentous form of *C. albicans is* an invasive morphology of the fungus which is observed in solid tissues, such as the kidneys, and is able to produce a huge amount of proteases. These enzymes are able to hydrolyze, disrupt and progress within the host tissues at an accelerated rate. In contrast, the yeast form of C. albicanswith slight invasion ability is an effective pathogenic morphology for disseminating in different parts of mucosal membranes and liquid–form structures of the host. [13]

2.3.5 Adhesion and invasion:

In both life styles, including commensalism and pathogenesis, *C. albicans* utilizes a special set of proteins called adhesions to have successful adherence to the other cells of *C. albicans*, host cells or inanimate surfaces. Therefore, the first and essential factor for colonization of commensal or pathogenic strains of *C. albicans* is a strong attachment to prevent being washed away. Two sets of protein families belonging to *C. albicans*, including Als [agglutinin–like sequence (Als1–7 and Als9)] and Hwp1 (Hypha associated GPI–linked protein) adhesions, mediate the activity of adhesion in the filamentous form of *C. albicans*. Among Als proteins, the Als3 has the key role in adhesion. The aforementioned proteins are the products

of als and hwp1 genes, respectively. [13]

On the other hand, invasion is a natural mechanism in the hyphal structure of pathogenic strains of *C. albicans*. Generally, there are two complementary invasion processes in which invasions are mediated for invading host cells. These processes are consisted to trigger endocytosis and the Trojan Horse mechanism (hyphal active penetration). [13]

The triggered endocytosis mechanism is mediated by determined proteins on hyphal cells' surfaces called invasins. Invasins in both dead and living fungal cells are able to bind to host cells ligands, including E–cadherin on epithelial cells and N–cadherin on endothelial cells. The most important invasins involve Als3 and Ssa1 proteins. Als3 is an adhesion–invasion protein which is applied for adhesion and invasion in fungal hyphae of *C. albicans*. Furthermore, Ssa1 protein is a member of heat shock protein 70 (HSP70) which acts as an invasion in parallel with Als3 in *C. albicans* hyphal structures. It seems that the triggered endocytosis mechanism is necessary for the early steps of invasion. [13]

The second mechanism, known as Trojan Horse (hyphal active penetration), occurs only in living cells to penetrate deeper into tissues. Pathogenic C. albicanspossesses 10 secreted aspartic protease (Sap) isoen. [13]

2.4 Diabetes mellitus:

Diabetes mellitus is recognized as being a syndrome, a collection of disorders that have hyper glycaemia and glucose intolerance as their hallmark, due either toinsulin deficiency or to the impaired effectiveness of insulin's action, or to a combination of these. In order to understand diabetes, it is necessary to understand the normal physiological process occurring during and after a meal. Food passes through the digestive system, where nutrients, including proteins, fat and carbohydrates are absorbed into the bloodstream. The presence of sugar, a carbohydrate, signals to the

endocrine pancreas to secrete the hormone insulin. Insulin causes the uptake and storage of sugar by almost all tissue types in the body, especially the liver, musculature and fat tissues. [14]

Unfortunately, there is no cure for diabetes yet but by controlling blood sugar levels through a healthy diet, exercise and medication the risk of long-term diabetes complications can be decreased. Long-term complications that can be experienced are:

- Eyes – cataracts and retinopathy (gradual damaging of the eye) that may lead to blindness.
- Kidneys – kidney disease and kidney failure
- Nerves – neuropathy (gradual damaging of nerves)
- Feet – ulcers, infections, gangrene, etc.
- Cardiovascular system – hardening of arteries, heart disease and stroke. [14]

The progressive nature of the disease necessitates constant reassessment of glycemic control in people with diabetes and appropriate adjustment of therapeutic regimens. When glycemic control is no longer maintained with a single agent, the addition of a second or third drug is usually more effective than switching to another single agent. Insulin dependent diabetes mellitus (IDDM, type I diabetes) and non-insulin dependent diabetes (NIDDM, type II diabetes). The evidence of this heterogeneity is over whelming and includes the following:

a) There are many distinct disorders, most of which are individually rare, in which glucose intolerance is a feature.

b) There are large differences in the prevalence of the major forms of diabetes among various racial or ethnic groups world-wide.

c) Glucose tolerance presents variable clinical features, for example, the differences between thin ketosis-prone, insulin dependent diabetes and obese, non-ketotic insulin resistant diabetes.

d) Genetic, immunologic and clinical studies show that in Western countries, the forms of diabetes with their onset primarily in youth or in adulthood are distinct entities. [14]

Chapter 3

Materials and Methods

3. Materials and Methods

3.1 Methods:

3.1.1 Study design:

A cross-sectional, Hospital based study.

3.1.2 Study area:

Shendi locality, River Nile State, Sudan. Shendi is a town in northern of Sudan on the east bank of the River Nile 150 km northeast of Khartoum (16°41'N 33°25'E). The area is inhabited by the Ga'aleen Tribe.

3.1.3 Study duration:

From May 2018 to August 2018.

3.1.4 Study population:

Diabetic patients in Shendi hospitals.

3.1.4.1 Inclusion criteria:

Diabetic patients with different ages and with urinary tract infection.

3.1.4.2 Exclusion criteria:

diabetic patients without urinary tract infection.

3.1.5 Sample size:

Hundred urine sample from diabetic patients.

3.1.6 Scientific & Ethical considerations:

The study proposal was reviewed and ethically approved by the scientific and the ethical committee of medical laboratory sciences in Shendi University.

3.1.7 Data collection:

Data was collected by using questionnaire.

3.1.8 Samples collection:

After take consent, patients at risk to become infected with fungal infection

sample well be collected by standard procedure (Midstream urine MSU) by giving the patient a sterile, dry, wide-naked, leak-proof container and explain the importance of collecting a specimen with as little contamination as possible and cultured in S.D A media (Sabrouds Dextrose Agar), then incubated for 24h. If the culture shows significant growth, doing gram stain, biochemical tests to identify the causative agent then susceptibility testing to cinnamon extract will be done.

3.1.9 Data analysis:

Data was analyzed using SPSS program (Statistical Package for Social Science).

3.1.10 Data presentation:

Data will be presented inform of tables & figure

3.1.11 Specimen collection:

Midstream urine (MSU) was collected as follows:

1-The patient was given a sterile, dry, wide-necked, leak proof container and requested to collect 10–20 ml of urine specimen.

2-The container was labeled with the date, the name and number of the patient, and the time of collection. When immediate delivery to the laboratory was not possible, the patient was requested to refrigerate the urine at 4–6 °C until delivery not more than 24 hours. [16]

3.1.12 Culture of urine specimen:

1- Urine sample were mixed well by rotating urine container several times.

2-Beside opened Bunsen burner urine container was opened and Nichrome loop was inserted after sterilization by flaming and cooling.

3-Small amount of urine sample was taken by loop and inoculated by making firstly well in Sabrauds dextrose agar media then making primary lines from the well then secondary lines from primary lines then tertiary

lines from secondary lines finally zigzag from last line of tertiary lines.

4-The inoculated plates were incubated in incubator at 37ºC for 24h under aerobic condition.

3.1.12.1Interpretation of culture growth:

The plates were examined for any significant yeast growth. The isolated yeast was then identified by colonial morphology, Gram stain and biochemical tests.

3.1.13 Microscopic examination:

Preparation of smear:

1-On clean dry slide one drop of normal saline was putted and by loop after sterilization small amount of well grown single bacterial colony was taken from the agar plate and mixed with normal saline.

2- Bacteria and normal saline were well mixed and spread on slide in area about 1 cm.

3-Slide was left to air dry then fixed by heating by flame by passing the slide in flame 3 times.

Gram stain:

Principle:

Differences in Gram reaction between bacteria is thought to be due to differences in the permeability of the cell wall of Gram positive and Gram negative organisms during the staining process. Following staining with a triphenyl methane basic dye such as crystal violet and treatment with iodine, the dye–iodine complex is easily removed from the more permeable cell wall of Gram negative bacteria but not from the less permeable cell wall of Gram positive bacteria. Retention of crystal violet by Gram positive organisms may also be due in part to the more acidic protoplasm of these organisms binding to the basic dye (helped by the iodine).

Procedure:

1-After making heat fixed smear, the slide was putted in staining rack.

2-The smear was covered with the basic stain crystal violet then left for 1 minute.

3-Washed by tape water then covered the smear with the mordant lugol´s iodine for 1 minute then washed by tape water.

4-The smear was covered with the decolorizer 95% acetone alcohol for 5 seconds then washed by tape water.

5-Finally, the smear was covered with the counter stain Saffranin and left it for 2 minutes then washed by tape water.

6-The smear was dried by air and examined under microscope using 100X lance.

Results:

Gram positive microorganism . . . . Dark purple.

Gram negative microorganism . . . . Pale to dark red.

Result candida species is gram positive yeast cells.

3.1.15 Biochemical test:

Germ tube test:

Germ Tube Test is a screening test which is used to differentiate Candida albicansfrom other yeast. Germ tube (GT) formation was first reported by Reynolds and Braude in 1956. When Candida is grown in human or sheep serum at 37°C for 3 hours, they form germ tubes, which can be detected with a wet KOH films as filamentous outgrowth extending from yeast cells. It is positive for *Candida albicans* and *Candida dubliniensis*. Approximately 95 – 97% of *Candida albicans* isolated develop germ tubes when incubated.

Principle:

This is a rapid test for the presumptive identification of C.

albicans.Procedure of Germ Formation of germ tube is associated with increased synthesis of protein and ribonucleic acid. Germ Tube solutions contains tryptic soy broth and fetal bovine serum, essential nutrients for protein synthesis. It is lyophilized for stability. Germ tube is one of the virulence factors of Candida albicansTube Test

1. Put 0.5 ml of sheep or human serum into a small tube. Note: Fetal bovine serum can also be used instead of human serum.
2. Using a Pasteur pipette, touch a colony of yeast and gently emulsify it in the serum.
3. Note: Too large of an inoculums will inhibit germ tube formation.
4. Incubated the tube at 37°C for 2 to 4 hours.
5. Transfer a drop of the serum to a slide for examination.
6. Cover slip and examine microscopically under low and high power objectives.

Results and Interpretation of Germ Tube Test:

Positive Test: A short hyphal (filamentous) extension arising laterally from a yeast cell, with no constriction at the point of origin. Germ tube is half the width and 3 to 4 times the length of the yeast cell and there is no presence of nucleus. **Examples:** *Candida albicans* and *Candida dubliniensis*

Negative Test: No hyphal (filamentous) extension arising from a yeast cell or a short hyphal extension constricted at the point of origin. **Examples:** *C. tropicalis*, *C. glabrata* and other yeasts.

Carbohydrate fermentation test:

The carbohydrate fermentation test is used to determine whether or not bacteria can ferment a specific carbohydrate. Carbohydrate fermentation patterns are useful in differentiating among bacterial groups or species.

it tests for the presence of acid and/or gas produced from carbohydrate fermentation. Basal medium containing a single carbohydrate source such as

Glucose, Lactose, Sucrose or any other carbohydrate is used for this purpose. A pH indicator (such as Andrade's solution, Bromcresol purple (BCP), Bromothymol blue (BTB) or Phenol red) is also present in the medium; which will detect the lowering of the pH of the medium due to acid production. Small inverted tubes called Durham tube is also immersed in the medium to test for the production of the gas (hydrogen or carbon dioxide).

Principle:

When microorganisms ferment carbohydrate an acid or acid with gas are produced. Depending up on the organisms involved and the substrate being fermented, the end products may varies. Common end-products of bacterial fermentation include lactic acid, formic acid, acetic acid, butyric acid, butyl alcohol, acetone, ethyl alcohol, carbon dioxide and hydrogen.
The production of the acid lowers the pH of the test medium, which is detected by the color change of the pH indicator. Color change only occurs when sufficient amount of acid is produced, as bacteria may utilize the peptone producing alkaline by products.

Inoculation and Incubation:

- Aseptically inoculate each test tube with the test microorganism using an inoculating needle or loop. Alternatively, inoculate each test tube with 1-2 drops of an 18- to 24-hour brain-heart infusion broth culture of the desired organism.
- Incubate tubes at 35-37°C for 18-24 hours. Longer incubation periods may be required to confirm a negative result.

Interpretation of the results

Positive: After incubation the liquid in the tube turns yellow (indicated by the change in the color of the phenol red indicator). It indicates that there is

drop in the pH because of the production of the acid by the fermentation of the carbohydrate (sugar) present in the media.

Negative**:** The tube containing medium will remain red, indicating the bacteria cannot ferment that particular carbohydrate source present in the media.

Gas Production:

Positive**:** A bubble (small or big depending up the amount of gas produced) will be seen in the inverted Durham tube.

Negative**:** There won't be any bubble in the inverted Durham tube i.e. bacteria does not produce gas from the fermentation of that particular carbohydrate present in the media i.e. anaerogenic organism.

Assimilation Test:

The assimilation test, used for the taxonomy in yeast, is an agar that is made up and inoculated with a yeast and a carbohydrate. It is then incubating anywhere from 1-3 days, or ever up to 24 days to allow the identification of yeast that are slow metabolizers. It can be run in carbon and nitrogen based media made with distilled water. [17]

The assimilation test checks the ability of yeast to use a carbohydrate as its sole carbon source and can be run with nitrogen or carbon. If nitrate is used instead of carbon, the test tests the ability of yeast to use nitrate as sole nitrogen source. One observes the growth for 1 to 3 days. If there is an increase in growth then the yeast has a certain enzyme to assimilate that certain carbohydrate and the opposite is true too, if there is no growth then the yeast lacks a certain enzyme. In short, the pattern of how the yeast takes up the carbohydrate is observed and this characteristic pattern is called the yeast's auxogram. There are many ways to apply the carbohydrates and inoculums onto the agar plate. They can be seeded into the agar, streaked onto the plate, applied via a filter, or applied drop-wise. When using the

assimilation test, there are many options for techniques to use. There is the Wickerham Broth version in which a chemical broth is made in a tube and inoculated with a yeast colony that had been starved of nutrients. The yeast is allowed to grow for 48 hours and the growth is measured based on turbidity of the solution. Another technique would be to take the broth, add agar and allow a slant to form. These can then be stored for months before being used. These slants are usually used with Bromocresol blue indicator. When the slants pH changes (from the neutral 7) it changes color indicating a change in the pH and thus a change in the yeast Ahearn, D, Roth, F, Fell, J, Meyers, S. Use of Shaken Cultures in the Assimilation Test for Yeast Identification. Finally, there is the dye pour plate auxanographic technique (DPPA). This method has the ability to test yeasts' assimilation of 14 carbohydrates at a time. [18]

Application in Wine Microbiology:

This test is one of many to aid in the identification of yeast. It identifies the yeast based on whether or not it uses specific carbohydrates as carbon sources. It can also be used with nitrate to determine whether or not yeast can nitrate successfully as a nitrogen source. If the yeast can or cannot use the certain carbohydrate or nitrate, then it can be compared to the traits of certain yeasts. The agar can be infused with different carbohydrates or nitrogen sources to test for traits specific to the yeast testes for. This can be seen in the test by where potassium nitrate is used. This is an example of where a specific nitrogen source is used to test whether or not the yeast can use it and then can help to classify the organism. [19]

Urease test:

(Prprinciple:

The test organism is cultured in a medium which contains urea and the indicator phenol red. When the strain is urease producing, the enzyme will break down the urea (by hydrolysis) to give ammonia and carbon dioxide. With the release of ammonia, the medium becomes alkaline as shown by a change in color of the indicator to pink-red.

3.1.16 Preparation of Cinnamon extract:

The plant sample was coarsely powdered using mortar and pestle. Coarsely sample was successively extracted with petroleum ether using soxhelt extractor apparatus. Extraction carried out for about five hours till the color of solvents at the last siphoning time returned colorless.

Solvents were evaporated under reduced pressure using rotary evaporator apparatus. Finally, extracts allowed to air in Glass container till complete dryness and the yield percentage were calculated as followed:

Weight of extract obtained / weight of plant sample X100[(20)]

Sample	Weight of sample in gm	Weight of extract in gm or volume of the oil in ml	Yield %

3.1.17Procedure of inoculation in Mueller Hinton agar plates and applying cinnamon extract:

1- By the loop the tops of each of 3–5 colonies were touched, of similar appearance, of the organism to be tested.

2- The growth was transferred to a tube of sterile saline and mixed then compared the tube with the turbidity standard and adjusted the density of the test suspension to that of the standard by adding more bacteria or more sterile saline.

3- The plates were inoculated by dipping a sterile swab into the 27inoculums. The excess 27inoculums was removed by pressing and rotating the swab firmly against the side of the tube above the level of the liquid.

4- The swab was streaked all over the surface of the medium three times, rotating the plate through an angle of 60º after each application. Finally, the swab was passed round the edge of the agar surface. The inoculums were left to dry for a few minutes at room temperature with the lid closed.

5- By using glass poorer of size 6 mm in diameter, 5 pores were made in agar plate then the pores were filled by cinnamon extract by using automatic pipette in volume 50 µ.l of concentrations 1, 0.5, 0.25, 0.1.5g/100ml.

6- The plates were incubated for 24h in incubator under aerobic condition in 37ºC.

3.1.18 interpreting the sensitivity of cinnamon extract:

1- The diameter of each zone (including the diameter of the disc) had been measured and recorded in mm.

2- The measurements was made with a ruler on the under-surface of the plate without opening the lid.

3.1.19 Calculation of relative percentage of inhibition:

Relative percentage inhibition = 100 x (x – y) / (z – y)

x: total area of inhibition of the test extract.

y: total area of inhibition of the solvent.

z: total area of inhibition of the standard drug.

The total area of the inhibition was calculated by using area = πr^2; where, r = radius of zone of inhibition.

- π value = 3.14

X: Total area of inhibition of the test extract = 3.14 x (radius of zone inhibition of green tea extract in mm)^2.

Y: Total area of inhibition of the solvent = 3.14 x (radius of zone inhibition of water in mm)^2.

Z: Total area of inhibition of the standard drug = 3.14 x (radius of zone inhibition of Clotrimazol in mm)^2.

3.1.20 Statistical analysis:

Data was analyzed by using Statistical Package for Social Science (SPSS) V.16.

3.2 Materials:

3.2.1 Mueller–Hinton agar:

Mueller–Hinton agar was prepared from a dehydrated base according to the manufacturer's instructions.

3.2.2 Cinnamon powder:

Dried cinnamon powder.

3.2.3 Turbidity standard (0.5 McFarland standard):

The turbidity standard was prepared by pouring 0.6 ml of a 1% (10 g/l) solution of barium chloride dehydrate into a 100 ml graduated cylinder, and filling to100ml with 1% (10 ml/l) sulfuric acid. The turbidity standard solution was placed in a tube identical to the one used for the broth sample. It cans be stored in the dark at room temperature for 6 months, provided it is sealed to prevent evaporation (Vandepitte, et al. 2003).

3.2.4 Swabs:

Sterile wooden swab with applicator from Ningbo MFLAB medical instruments Co.Ltd.

3.2.5 Sterile Normal saline concentration 0.85%:

Prepared by dissolve 8.5g of Nacl in 1000 ml of Distill water.

3.2.6 Disposable plastic Petri dish:

90 mm size disposable plastic Petri dish (Marina Co. Ltd).

3.2.7 Glass poorer:

6 mm diameter glass poorer.

3.2.8 Automatic pipette:

- Automatic pipette variable (5 – 50 µ.l).
- Automatic pipette variable (100 – 1000 µ.l).

3.2.9 Disposable plastic automatic pipette tips:

- Blue tips (size: 1000 µ.l)
- Yellow tips (size: 200 µ.l).

3.2.10 Serology small glass tubes:

12 x 75mm glass test tube.

3.2.11 large size glass tubes:

15 x100mm glass test tubes.

3.2.12 glass Erlenmeyer flask:

500ml size flask and 250ml size flask.

3.2.13 glass Beaker:

300 ml size.

3.2.14 bacteriological loops:

- Nichrome ring loop and needle loop(HI-MEDIA).

3.2.15 test tubes racks:

3.2.16: Autoclave (Dixon), Incubator (Thermo Scientific), Oven and Bunsen burner.

Chapter 4

Results

4. Results

A total of one hundred patients with UTI patients were enrolled in this study. The mean age was (56.34± 6.7) years (Table 1).Male frequency is (24%) and female frequency is (76%).

4.1 Isolated and identified yeast pathogens:

The main causative agent of UTI caused by Candida species in the study population was *Candida glabrata*n=7(33.4%), Candida *tropicalisn*=4 (19%), *Candida albican* n=4(19%) and *Candida kefyr* n=3 (14.3%) *Candida krussein*=3(14.3%).(table 2).

4.2 Susceptibility testing of cinnamon:

Antimicrobial susceptibility of cinnamon extract has largest zone of inhibition against *Candida tropicalis* was (25 mm) and least zone of inhibition against *Candida kefyr* was(10 mm) (Table 3).The results of antimicrobial activity of crude extract was compared with the positive control (Clotrimazole) for evaluating their relative percentage inhibition, the aqueous extract exhibited maximum relative percentage inhibition against *Candida kefyr* (89%) and minimum relative percentage inhibition against *Candida glabrata* was (12.3%) (Table 4).

4.3 Minimum inhibitory concentration:

Antibacterial activities of extracts were checked by well diffusion method. The concentrations of green leaves aqueous, extract used was 1, 0.5, 0.25 and 0.125g/100ml.MIC values of aqueous extracts of cinnamon on test organisms which the lowest concentration of cinnamon aqueous extract able to inhibit the growth of Candida was (0.187g/100ml) appear against *Candida krussei* followed by *Candida kefyr* (0.208g/100ml), *Candida tropicalis*(0.343g/100ml), *Candida albican*(0.375g/100ml)and *Candida glabrata*(0.458g/100ml).

Table (4.1) Distribution of the population according to age and gender:

Age group	Number	Male	Female	percentage
Les than40	14	3	11	14%
41 – 50	16	2	14	16%
51 – 60	31	12	19	31%
More than 60	39	7	32	39%
Total	**100**	**24**	**76**	**100%**

Age (mean ± SD) = (56.34 ± 6.7)

Table (4.2): percentage of Candida isolated from urine of study population:

Candida species name	Number	Percentage
Candida glabrata	7	33.4%
Candida albican	4	19%
Candida tropicalis	4	19%
Candida kefyr	3	14.3%
Candida krussei	3	14.3%

Table (4.3): Mean inhibition zone of Cinnamon and Clotrimazole.

Test	Mean ± SD	P-value
Clotrimazole	21.16 ± 6.7	0.018
Cinnamon	11.23 ± 2.66	

Table (4.4): antimicrobial susceptibility of cinnamon extract compared to Clotrimazole:

Candida species	Inhibition zone diameter (mm)		
	Cinnamon extract (1 g/100ml)	Positive control Clotrimazole (1 g/100ml)	MIC g/100ml
Candida glabrata	7.4	21.4	0.458
Candidaalbican	12.25	25	0.375
Candida tropicalis	14.5	28.5	0.343
Candida kefyr	10	10.6	0.208
Candida krussei	12	20.3	0.187

Table (4.5): **relative percentage inhibitions of Cinnamon extract compared to Clotrimazole:**

Test organisms	Relative percentage inhibition (%)
Candida glabrata	12.3%
Candida albican	24%
Candida tropicalis	25.9%
Candida kefyr	89%
Candida krussei	35%

Chapter 5

Discussion

Conclusion

Recommendations

5.1 Discussion

Twenty-one out of hundred patients (21%) were positive for Candiduria,the recovery rate of Candida species from urine samples varies in different studies. In the presentstudy, in a study conducted by Nademi *et al.,* nosocomial candiduria was reported in 4.3% (5 out of 115) of hospitalized patients. Furthermore, Yismaw et al. reported significant candiduria in 7.5% and 17.1% of asymptomatic and symptomatic patients with diabetes, respectively. The overall candiduria rate was 8.3% (35 out of 422). Similarly, Goyal *et al*., *Zarei et al.,* and Padawer *et al*. reported candiduria in 2.36%, 16.5%, and 19.49% of their study populations, respectively. In a comparative study by da Silva Krenke et al., candiduria was reported in 30% and 16% of diabetic and non-diabetic patients, respectively. The disagreements between the results of various studies may be due to differences in study populations, underlying factors, preventive measures of patients and hospitals, and geographical location. Although *C. albicans* is reported as the major Candida species causing candiduria in some studies, an increasing trend in the prevalence of candiduria due to non-albicans Candida species was shown by some researchers.

The study found that*C. glabrata* was the most isolated species (33.4%) followed by *C. albicans* (19%), *C. tropicalis*(19%), *C. krussei*(14.3%), and *C. kefyr* (14.3%), the higher incidence of non-albicans Candida species, including *C. glabrata,* may be due to their ability to adapt to the urinary tract condition, as well as their intrinsic and/or acquired resistance to the conventional antifungals. Considering the high drug resistance of non-albicans Candida species, especially C. glabrata, their dominance should be considered as a health concern and a probable cause of treatment failure. The

association between candiduria and different demographic and laboratory data was assessed in the present study. Female gender, decreased (acidic) urine pH, high FBS followed by glycosuria, and uncontrolled diabetes (HbA1c ≥8) were associated with candiduria.

Antimicrobial susceptibility of Cinnamon extract shows highest zone of inhibition against *Candida tropicalis* (14.5 mm) and lowest zone of inhibition against *Candida kefyr* (10 mm).

MIC values of Cinnamon extract on test organisms which the lowest concentration of Cinnamon extract able to inhibit the growth of Candida was (0.187g/100ml) appear against *Candida krussei* followed by *Candida kefyr* (0.208g/100ml), *Candida tropicalis* (0.343g/100ml), *Candida albican*(0.375g/100ml) and *Candida glabrata* (0.458g/100ml; susceptibility present is *C. krussei*(66.6%), C. *kefyr* and *C. tropicalis* were (100%). *C. albican*(75%) and *C. glabrata*(42.8%).According to Nidhai Goel *et .al* the percent of anti-candida susceptibility of Cinnamon oil was 58.83% for *C. albicans*, 46.51% for *C. tropicalis*, 44.45% for *C. krussei* and 57.12% for *C. parapsilosis.*[(21)]

The results of antimicrobial activity of crude extract was compared with the positive control (Standard drugs) for evaluating their relative percentage inhibition while the aqueous extract exhibits maximum relative percentage inhibition against *Candida kefyr* (89%) and minimum relative percentage inhibition against *Candida glabrata* (12.3%) and it is statistically significant (P= 0.018).

5.2 Conclusion

- Considering the high incidence rate of candiduria in diabetic patients, diabetes, predisposing factors, and causal relationships between diabetes and candiduria. In addition, as the non-albicans Candida species were isolated more than *C. albicans*.
- Cinnamon extract has ability to inhibit Candida species specially *C. tropicalis.*

5.3 Recommendations

- Antifungal susceptibility testing for determination of susceptibility/resistance profile of isolates could be helpful for appropriate treatment.
- Use of alternative medicine for complicated cases with combination with anti-fungal.

Chapter 6

References

Appendix

6.1 Reference

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6.2 Appendices

Appendix (1): Questionnaire

University of Shendi

Faculty of medical laboratory sciences

College of Graduate Studies

Detection of antifungal activity of Cinnamon Extract On Candida species Isolated from Diabetic Patients with Urinary Tract Infection

Name……

City…… Village ……..

1- Age:

a- ≤40 (……) c- 41 – 50 (……). d- 51 – 60 (……...). e- > 60 (……).

2- Gender

a- male (……) b- Female (……)

3- Diabetes Treatment:

a- Tabs (……). b- Insulin (……).

4- History of urinary tract infection in previous……...

5- UTI treatment……

6- Response to treatment:

a- Good (…...) b- Few (……) c- No response

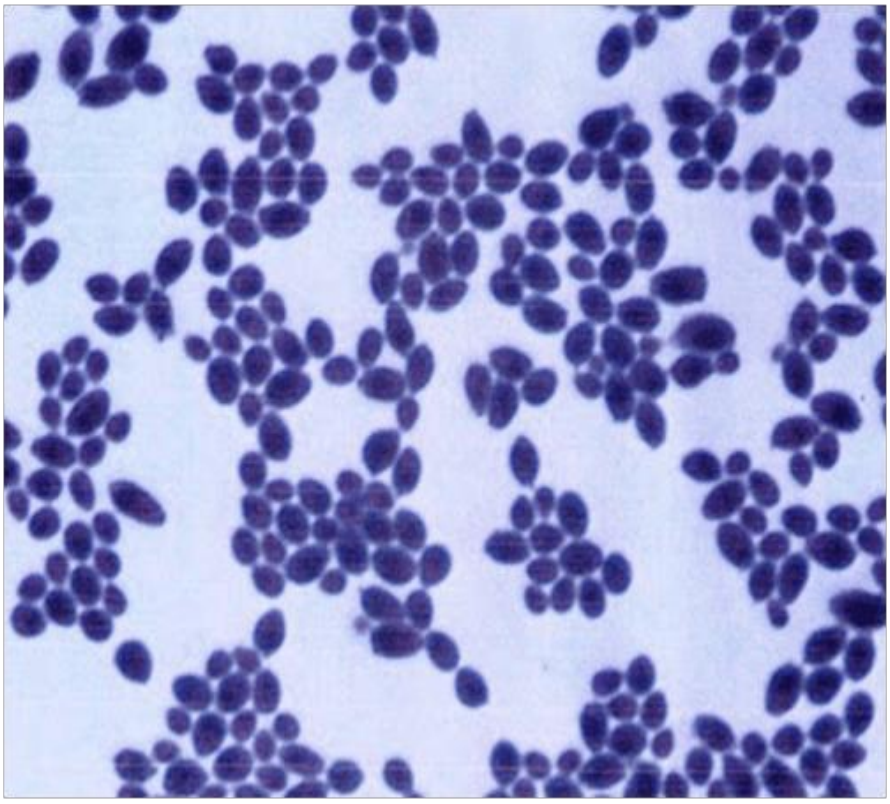
7- Use of herbal

a- yes (……). b- no (…….).

8- Other disease:

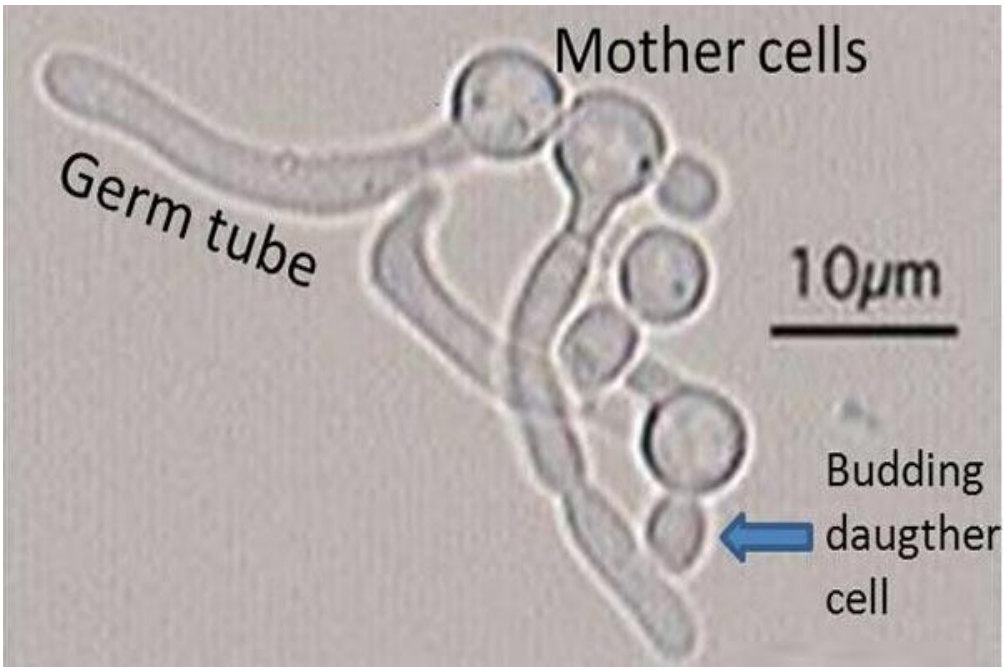
a- yes (……) b- No (……)

Appendix (2)



Gram stain of Candida species

Appendix (3)

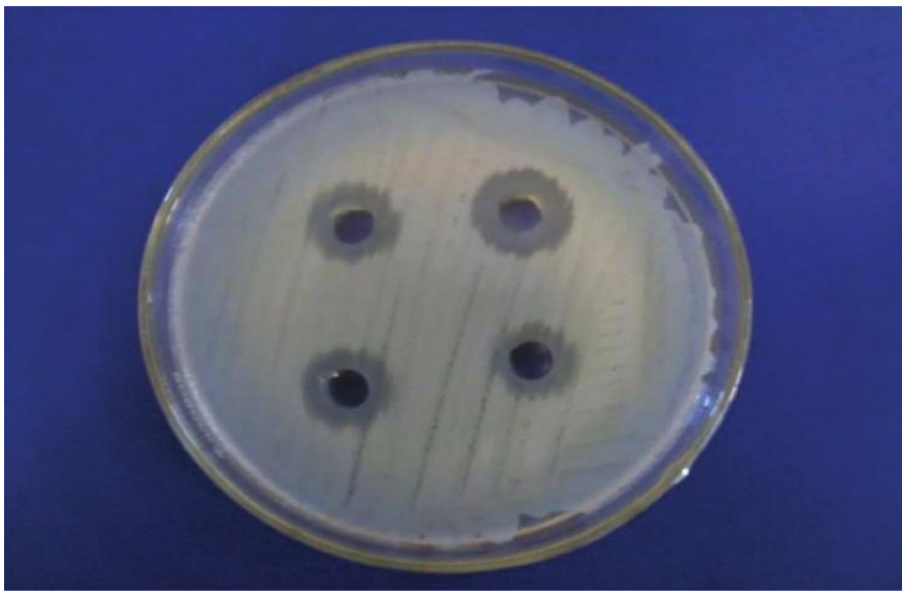


Germ Tube Test (GTT)

Appendix (4)

Phenol red carbohydrate broth

Appendix (5)



Inhibition zones of Cinnamon extract

Appendix (6) :Standard formula and uses for some materials

Materials	Standard formula	Gram/liter	Preparation
Muller Hinton agar (HI-MEDIA)	-Meat, infusion solids from 300g -Casein acid hydrolysate. -Starch. -Agar.	2.0 17.5 1.5 17.0	Suspend 38 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15.
Sabrouraud Dextrose Agar (SDA) (HI-MEDIA)	-Dextrose -Pepton -Agar	40 10 15	Suspend 36 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15.
Urea agar base (Christensen). (HI-MEDIA)	.Peptic digest of animal tissue. . Dextrose. . Sodium chloride. . Disodium phosphate. . Monopotassium phosphate. . Phenol red. .Agar.	1.0 1.0 5.0 1.2 0.8 0.012 15.0	Suspend 24.01 grams in 950 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 10 lbs pressure (115°C) for 20 minutes. Cool to 50°C and aseptically add 50 ml of sterile 40% Urea Solution (FD048) and mix well.
Phenol red carbohydrate broth	▪ Sodium Chloride (NaCl): 5 g ▪ Beef extract (optional): 1 g ▪ Phenol red (7.2 ml of 0.25% phenol red solution) ▪ Carbohydrate source	5 1 0.018 50	Fill 13 x 100 mm test tubes with 4-5 ml of phenol red carbohydrate broth, insert Draham tube and sterilizationby autoclaving at 10 lbs pressure (115°C) for 15 minutes

Appendix 6: biochemical reactions

Candida	Glucose	Lactose	Maltose	Sucrose	Galactose	Trehalose	GTT	Urase
C.albican	F	-	F	-	F	F	+	-
C.glabrata	F	-	-	-	-	F	-	-
C.tropicalis	F	-	F	F	F	F	-	-
C.krussei	F	-	-	-	-	-	-	+
C.kefyr	F	F	-	F	F	-	-	-

Sugar fermentation reactions

Candida name	Glucose	Lactose	Maltose	Sucrose	Trehalose
C.albican	+	+	+	+	+
C.glabrata	+	-	-	-	+
C.tropicalis	+	-	+	+	+
C.krussei	+	-	-	-	-
C.kefyr	+	+	-	+	-

Sugar assimilation reactions