

Original Research Article

Detection of Antifungal Activity of *Cinnamon* Extract on *Candida* Species Isolated from Diabetic Patients with Urinary Tract Infection Pre-COVID-19 Era

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Abstract: This study aimed to evaluate the antifungal activity of Cinnamon extract on *Candida* species isolated from 100 diabetic patients suffering from urinary tract infection between May to August 2018. Twenty-five *Candida* species were isolated, different *Candida* species were isolated then identified biochemically, *in vitro* sensitivity testing using well diffusion technique against Cinnamon extract was done. The cinnamon extract showed good antifungal activity against *Candida* species. The largest zone of inhibition against *Candida tropicalis* was (25 mm) and the least zone of inhibition was against *Candida kefyr* was (10 mm). The results of antimicrobial activity of crude extract were 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. values* (0.018) are statistically significant.

Keywords: *Candida spp*, *Cinnamon*, Shendi, Antifungal activity, Diabetic, UTI.

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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 additionally, 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]. These compounded drugs comprised medicinal plants along with drugs of animal and plant origin. If the drug was prepared from several medicinal plants, minerals, and rare animals, it was highly valued and sold at a premium [1]. Many medicinal plants are employed as self-medication or are used upon the suggestion of a physician or pharmacist. They are employed both independently and/or in combination as

complementary therapies 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 a selection of the appropriate pharmacological effects associated with the specific herbal components [1].

Community-acquired Urinary Tract Infections (UTIs) are a frequent problem worldwide that is caused by microbial invasion to different tissues of the urinary tract. Fungi are a part of the microbial inhabitants that may contribute as fungal uropathogens in UTIs. In the last 2.5 decades, the fungal UTIs due to *Candida* genus yeast have increased significantly [2]. The predisposing factors of UTIs include gender, genetic predisposition, behavioral factors, urologic structural abnormalities, diabetes, immunosuppression, pregnancy, hypertension,

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stone formation, nosocomially 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 more subordinate urinary tract infections induced by yeasts is fourfold more expected in females than in males. Furthermore, the results of different studies have indicated that UTIs in women are very common [3].

Lower UTI with *Candida* usually happens in patients with urinary catheters, generally after antibiotic treatment, although candidal and bacterial infections regularly happen simultaneously. *C. albicans* prostatitis happen infrequently in patients with diabetes, usually after instrumentation. Renal candidiasis is usually spread hematogenously and commonly originates from the GI tract. Ascending infection is likely and occurs mainly in patients with nephrostomy tubes, another permanent indwelling instrument, and stents. At high risk are patients with diabetes and those who are immunocompromised because of a tumor, AIDS, chemotherapy, or immunosuppressants. The main original of candidemia in such high-risk hospitalized patients is an indwelling intravascular catheter. kidney transplantation raise the risk to of the combination of indwelling catheters, stents, antibiotics, anastomotic leaks, obstruction, and immunosuppressive therapy [4]. The consumption of cinnamon and its usage 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 business of historic Europe thrived on cinnamon as one of its primary luxury interests. Cinnamon is now grown in many regions of the world and has been used medicinally for some time. With the advent of modern technology; scientists have even proved that cinnamon is a viable treatment for some bacterial and fungal infections. Getting rid of yeast diseases is one medicinal use cinnamon offers remarkable 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]

MATERIAL AND METHODS

Study design

A cross-sectional, Hospital based study.

Study period

From May 2018 to August 2018

Study population

Diabetic patients in Shendi hospitals- Sudan

Sample size

Hundred urine sample from diabetic patients

Ethical considerations

The study was reviewed and approved by the s

cientific and the ethical committee of Shendi University

Data collection

Data was collected by using questionnaire.

Study setting

Shendi locality, River Nile State, Sudan. Shendi is a town in northern of Sudan beside the River Nile and Almatamah town also far 150 km northeast from Khartoum the capital of Sudan (16°41'N 33°25'E). The area is inhabited by the Ga'aleen Tribe.

Method

Specimen collection

After take consent, patients at risk to become infected with fungal infection sample will be collected by standard procedure (Midstream urine MSU) by giving the patient a sterile, dry, wide-necked, leak-proof container and explain the importance of collecting a specimen with as little contamination as possible and cultured in S.D A media (Sabourds 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.

Culture of urine specimen

Urine sample were mixed well by rotating urine container several times. Beside opened Bunsen burner urine container was opened and Nichrome loop was inserted after sterilization by flaming and cooling. 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 37°C for 24h under aerobic condition.

Interpretation 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.

Preparation of plant 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

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

By the loop the tops of each of 3–5 colonies were touched, of similar appearance, of the organism to

be tested. 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. 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. The swab was streaked all over the surface of the medium three times, rotating the plate through an angle of 60° 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. By using glass pooper 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, 0.15g/100ml. The plates were incubated for 24h in incubator under aerobic condition in 37°C.

Interpreting the sensitivity of cinnamon extract

The diameter of each zone (including the diameter of the disc) had been measured and recorded in mm. The measurements were made with a ruler on the under-surface of the plate without opening the lid.

DATA ANALYSIS

Data was analyzed by using online web site <https://www.graphpad.com> to determine the mean and standard deviation of the data. Proportional data were presented as frequencies and percentages.

RESULT

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%). The main causative agent of UTI caused by *Candida* species in the study population was *Candida glabrata*=7(33.4%), *Candida tropicalis*=4 (19%), *Candida Albicans* n=4(19%) and *Candida kefir* n=3 (14.3%) *Candida krussei*=3(14.3%) (Table2). Antimicrobial susceptibility of cinnamon extract has the largest zone of inhibition against *Candida tropicalis* was (25 mm) and the least zone of inhibition against *Candida kefir* was (10 mm) (Table 4). The results of antimicrobial activity of the crude extract were compared with the positive control (Clotrimazole) for evaluating their relative percentage inhibition, the aqueous extract exhibited maximum relative percentage inhibition against *Candida kefir* (89%) and minimum relative percentage inhibition against *Candida glabrata* was (12.3%) (Table 3& 4). Antibacterial activities of extracts were checked by well diffusion method. The concentrations of 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 kefir* (0.208g/100ml), *Candida tropicalis* (0.343g/100ml), *Candida albican* (0.375g/100ml) and *Candida glabrata* (0.458g/100ml) (Table 5).

Table-1: Shows population distribution according to ages and gender

| Age group | Number | Male | Female | % |
|--------------|------------|-----------|-----------|-------------|
| Lessthan40 | 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-2: Shows percentage of Candida isolated from the urine.

| Candia species | Number | % |
|---------------------------|--------|-------|
| <i>Candida glabrata</i> | 7 | 33.4% |
| <i>Candida albican</i> | 4 | 19% |
| <i>Candida tropicalis</i> | 4 | 19% |
| <i>Candida kefir</i> | 3 | 14.3% |
| <i>Candida krussei</i> | 3 | 14.3% |
| Total | 21 | 100 |

Table-3: Shows Mean inhibition zone of Cinnamon and Clotrimazole

| Type of tea used | Number | P-value |
|------------------|--------------|---------|
| Clotrimazole | 21.16 ± 6.7 | 0.018 |
| Cinnamon | 11.23 ± 2.66 | |

Table-4: Shows Relative percentage inhibitions of Cinnamon extract compared to Clotrimazole

| Test organisms | Relative percentage inhibition (%) |
|---------------------------|------------------------------------|
| <i>Candida glabrata</i> | 12.3% |
| <i>Candida albican</i> | 24% |
| <i>Candida tropicalis</i> | 25.9% |
| <i>Candida kefyfyr</i> | 89% |
| <i>Candida krussei</i> | 35% |

Table-5: Shows Antimicrobial susceptibility of cinnamon extract compared to Clotrimazole

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

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 present study, 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). Likewise, 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 outcomes of different studies may be due to differences in analysis populations, underlying factors, preventative measures of patients and hospitals, and geographical area. 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. kefyfyr* (14.3%), the higher incidence of non-albicans *Candida* species, including *C. glabrata*, maybe 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 assembly between candiduria and several demographic and laboratory data was assessed in the present investigation. Female gender, decreased (acidic) urine pH, high FBS followed by glycosuria and uncontrolled diabetes (HbA1c ≥ 8) were associated with

candiduria. The antimicrobial susceptibility of Cinnamon extract shows the highest zone of inhibition against *Candida tropicalis* (14.5 mm) and the lowest zone of inhibition against *Candida kefyfyr* (10 mm). MIC values of Cinnamon extract on test organisms which the lowest concentration of Cinnamon extractable to inhibit the growth of *Candida* was (0.187g/100ml) appear against *Candida krussei* followed by *Candida kefyfyr* (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. kefyfyr* and *C. tropicalis* were (100%). *C. albican* (75%) and *C. glabrata* (42.8%). According to NidhaiGoel *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*. The results of antimicrobial activity of the crude extract were compared with the positive control (Standard drugs) for evaluating their relative percentage inhibition while the aqueous extract exhibits maximum relative percentage inhibition against *Candida kefyfyr* (89%) and minimum relative percentage inhibition against *Candida glabrata* (12.3%) and it is statistically significant (P= 0.018).

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 can inhibit *Candida* species especially *C. tropicalis*.

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