The Effect of Some Chemical Materials against Virulence Factors of Candida Spp Isolated from UTI Patients

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Abstract

During the period from September 2016 to December 2017, a total of 135 urine samples were collected from urinary tract infection patients attending to AL-Zahraa Hospital in AL-Najaf Governorate. The present study was conducted to isolate and identify Candida spp. isolated from urinary tract infection patients by different methods including direct examination, laboratory culture, biochemical test and by modern techniques (Api Candida kit) and determine the virulence factors phenotypic to Candida spp. which involved (bio film formation, phospholipase and germ tube). The percentage of females to males was as following, female (84) 62.2 % (21) infected and male (51) 37.8% (1) infected with all age categories. The results in this study explain that some Candida spp. such as C. albicans, have high susceptible to eugenole follow by phenol and umbellulone. The efficiency of some chemical substances such as (eugenole, umbellulone, and phenol) was evaluated to inhibit the growth of Candida spp as well as some virulence factors such as bio film formation, germ tube and phospholipase which were studied in this research. Statistically analysis results have been significance difference between the results of the substance concentrations and the concentrations of the different other substances.

Keywords: Eugenole, Umbellulone, Candiduria.

Introduction

Urinary tract infection (UTI) is a term applied to a variety of clinical conditions ranging in severity from asymptomatic which is carrier status in the urine to symptomatic acute infection of the kidney with resultant sepsis [1]. It is usually classified by the infection site: an infection of the lower urinary tract, urethra (urethritis) and urinary bladder (cystitis); an infection of the upper urinary tract, ureter and kidneys (pyelonephritis); and an infection of the renal pelvis (pyelitis) [2]. In community-acquired UTIs, women are significantly more likely to experience these infections during their life time than men where such cases are rare except in association with anatomic or functional abnormalities in the first year of life [3]. The presence of Candida spp. in urine is a condition known as candiduria.

A common clinical problem is deciding whether candiduria represents urinary tract infections or merely bladder colonization or contamination [4]. Cells of this genus are Globose, ovoid, cylindrical or elongate and sometimes irregularly shaped. Most spp. in the genus Candida multiplies by forming blast spores and pseudohyphae. The virulence factors are proteins encoded by genes on the pathogen city islands found in many Candida spp. The virulence of Candida sp. is attributed to certain factors like adherence, bio film formation, and the production of tissue damaging extracellular hydrolytic enzymes [5]. Extracellular hydrolytic enzymes like phospholipase and proteinase are important for colonization and invasion of host tissue [6]. The antifungal are a type of antimicrobial drug used in the treatment and prevention of Candida infections. They may either kill or inhibit the growth of Candida spp. (utilizing antibiotics agents effectively will preserve present day medication [7].

Aim of the study

This study aimed to evaluate the efficacy of alternatives chemical materials against C. albicans and some virulence factors formed by of C. albicans for application.

Material and Methods

Specimen's Collection

The patients who complain of urinary tract infection in Medical AL-Zahraa Hospital were
given clean dry sterile plastic tubes; return the specimen as soon as possible to the laboratory. The samples were processed immediately by loop was inserted vertically into the urine sediment and streaking in isolation media.

**Identification of Candida Ssp**

*Candida ssp* was identified depending on the morphological features on culture medium and other characteristic formation as shape, size, color and texture after culturing on Sabouraud dextrose agar and CHROM Agar.

**Preparation of Candida Suspension**

Preparation of this suspension was by taking up 10 ml of distil water in test tube and inoculating wire loop colony which activate in Saubroud dextrose broth or culturing on Sabauroud dextrose Agar .The in the test tube then calibrated to McFarland tube NO.3 where it was available to use [8].

**Phospholipase Production Test**

According to [9]

**Bio film Formation Test**: According to [10].

**Api 10 Candida Examination**

Identification candida ssp by using api 10 candida kit according to [11].

**Antifungal test**

The method in this study to the effect of examination of Antifungal (fluconazole) used ready discs placed in Petri dish contain culture media and inoculate colony of *Candida species* then incubation at 37°C for 48 hours to reading and measured zones size. Antifungal inhibition considerable standard to it compared with inhibition of chemical substances [12].

**Results and Discussion**

**Isolation**

One hundred thirty five specimens were obtained from attending patients to AL- Zahra Hospital were susceptible to incidence urinary candidiosis, Where were the percentage 84(62.2%) samples are female and 51(37.8%) samples are male infected with urinary tract infection, The results in Figure (1) showed that infected female more than male. This is due to several reasons; the urethra (the tube that carries urine away from the bladder) is shorter in women than in men.

![Figure 1: Showing percentage female and male with UTI](image)

Frequent sexual intercourse also increases a woman’s risk of developing UTIs, Contraceptive spermicidal and diaphragms are additional risk factors. When women reach menopause, the decrease in estrogen thins the lining of the urinary tract, which increases susceptibility to Candida infections this conforms to [13] explained that infection occurs in about 30% of women who are taking a course of antibiotics by mouth [14]. According to [15] He appears that is broad-spectrum antibiotics kill healthy bacteria in the vagina, such as *Lactobacillus*. These bacteria normally help to limit yeast colonization.
Identification of *Candida* Spp

**Morphological and Microscopic Features**

*Candida* spp. showed colonies on SDA after incubation at 37°C to 48 hours white to creamy, the texture of the colony appeared to be pasty, smooth, glistening or dry, wrinkled and dull, as showing in Figure 2.

![Figure 2: Growth of Candida spp. on SDA at 37°C for 48 hours](image)

On SDA, The colonies of *C. albicans* appeared white to creamy in color with round edges, soft and smooth associated with curved top, with yeast odor. The yeast growth reached to the typical form within three days this agree with [16]. While the colony of CHROM agar media revealed different species of *Candida* isolate independed of colony color where *C. albicans* was green, *C. krusie* was purple fuzzy, *C. parapsilosis* was white or pale and *C. galabrata* was light pink (Figure 3). CHROM agar is a selective medium for the isolation of yeast that simultaneously provides direct differentiation and identification of several *Candida* spp. [17]. The yeasts produce enzymes that react with chromogenic substrates in the CHROM agar medium, producing colonies of different colors. While the macroscopically feature result revealed that when staining with gram stain, *C. albicans* isolates appear gram positive, purple or blue in color and shown as a clusters form oval or round shape (Figure 4).

![Figure 3: Morphological features of Candida spp. isolated from patients with UTI cultured of CHROM agar media at 37°C for 48 hours Green colonies of C. albicans.B-Purple fuzzy colonies of C. krusie. C-White or pale colonies of C. parapsilosis.D-Light pink colonies of C. galaprata](image)

**Microscopic Examination**

When staining with gram stain, *C. albicans* isolates appear gram positive, purple or blue in color and shown as a clusters form oval or round shape Figure 4.
Figure 4: Microscopic feature of *Candida* spp. isolated from UTI patients with gram stain under microscope

**Germ Tube Production Test**

The isolates of *C. albicans* formed germ tube after incubation in serum at 37 °C for 3 hours. This germ tube was seen as an extension arising laterally from a yeast cell, with no constriction at the point of origin, the germ tube Figure 5.

Figure 5: Germ tube of *C. albicans* in human serum at 37°C for 3hr (40X)

**Biochemical Test**

The result of Api10 *Candida* kit revealed that only four species of Candidiosis were identified depending on specific code to each Species present in table, according to the Table 1.

<table>
<thead>
<tr>
<th>Candida Species</th>
<th>GLU</th>
<th>GAL</th>
<th>SAC</th>
<th>TRE</th>
<th>RAF</th>
<th>βMAL</th>
<th>αAMY</th>
<th>βXYL</th>
<th>βGUR</th>
<th>URE</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Candida albicans</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>Candida galabrata</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>Candida parapsilosis</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>Candida Krusie</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Table 1

Figure 6: Results of API 10 Candida spp. revealed card box specific
A- C. galaprata Biochemical result  B- C. albicans Biochemical result
C- C. parapsilosis Biochemical result  D- C. kruzie Biochemical result

Frequency of Candida spp
Results in (Figure 7) indicated that the majority of the isolates were C.albicans59% compared with the other species which were C. galabrata 22%,C. krusie14% andC.parapsilosis4% , the predominance of Candida albicans compared with other Candida spp. C. albicans and 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 communal yeasts of Candida may convert into opportunistic or pathogenic microorganisms causing Candida UTIs in the host [18].

Figure 7: Frequency of difference Candida spp. from isolated from samples of UTI patients

Antifungal Activity of Some Chemical Substance on Candida albicans Eugenole
(Figure 8) The result of well diffusion method revealed antifungal activity of eugenol, eugenole within concentration 25mg/ml gave 38mm inhibition zone against C. albicans and 35mm inhibition zone against C. kruzei , C. galaprata and C. parapsilosis was 35mm. With concentration 33mg/ml of eugenole inhibition zone against C. albicans was 29mm, while in C. krusei was 17mm and C. galabrate was 26mm, whereas in C. parapsilosis was 35mm. and with concentration 50mg/ml of eugenol inhibition zone against C. albicans was 37mm, while in C. krusie was 10mm and C. galabrate was 26mm ,whereas in Candida parapsilosis was 36mm.The main content of clove oils (Eugenia caryophyllus), an alone eugenole have been capability to interact with an architecture of an envelope of C. albicans and altered morphogenesis of an envelope and
inhibition of *C. albicans* colonization and infectiousness. An effect of eugenole against *Candida* spp. when exposure *Candida* to eugenole stimulated the dramatic changes in the appearance of an envelope and the progressive increased in a number of breakdown cells with wrinkled and rough surface, flatten cells with the surface folds, the cells with holes, the ghosts and collapsed cells, *Candida* spp. an affects the function and regulation of the necessary membrane-bound enzymes which are catalyses the synthesis of the number of a major cell wall polysaccharide contents [19,20] explain this polysaccharide contents such as β-glucans, chitin and mannan, thus this chemical substance disturbing cell growing and an envelope morphogenesis. From the results in this study are concluding that an effect of eugenole is approximately similar to an effect of fluconazole. On *Candida* spp at concentration 25 mg/ml

![Figure 8](image8.png)

**Figure 8:** Effect of eugenole activity against *C. albicans* isolated from UTI patients on SDA media under 37°C for 48 hours which

1- Concentration 25 mg/ml ➔ 38mm
2- Concentration 33mg/ml ➔ 29mm

**Umbellulone**

Table (2), (Figure 9) the result of well diffusion method revealed antifungal activity of umbellulone at all didn't give any antifungal effect against *C. albicans* and *C. galapratra*, while inhibition zone of *C. krusei* in concentration 25mg/ml was 11mm and *C. parapsilosis* was 12mm, and with concentration 33mg/ml of umbellulone an inhibition zone against *C. krusei* was 10mm, while in *C. parapsilosis* was 16mm, and with concentration 50 mg/ml of umbellulone an inhibition zone against *C. krusei* was 12mm, while in *C. parapsilosis* didn't inhibition[21]. Explain an effect of umbellulone that are known to cause cell membranes damage, causing leakage of cellular materials and ultimately the microorganism death. Mode of action is considered that these components have several sites of action at the cellular level.

![Figure 9](image9.png)

**Figure 9:** Effect of umbellulone activity against *C. krusei* isolated from UTI patients on SDA media under 37°C for 48 hours which

1- Concentration 25 mg/ml ➔ 11mm
2- Concentration 33mg/ml ➔ 10mm
3-Concentration 50mg/ml ➔ 12mm
Gold Nanoparticles

The Table (2) the result of agar well method revealed antifungal activity or antifungal activity of gold nanoparticles are usually in one concentration that is 100µg/ml, The inhibition zone of C. albicans was 11mm. Gold nanoparticles are inhibit H+-ATPase enzyme activities at their respective MIC values (100µg/ml) this result in this study agree with [22].

Table 2: Effects of some chemical substances against Candida spp. isolated from UTI patients using well diffusion method

<table>
<thead>
<tr>
<th>Chemical substances</th>
<th>Concentration</th>
<th>C.albicans</th>
<th>C.krusei</th>
<th>C.glaprata</th>
<th>C.parapsilosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eugenole</td>
<td>25mg/ml</td>
<td>38</td>
<td>35</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>33mg/ml</td>
<td>29</td>
<td>17</td>
<td>26</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>50mg/ml</td>
<td>37</td>
<td>10</td>
<td>26</td>
<td>36</td>
</tr>
<tr>
<td>Umbellulone</td>
<td>25mg/ml</td>
<td>-</td>
<td>11</td>
<td>-</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>33mg/ml</td>
<td>-</td>
<td>10</td>
<td>-</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>50mg/ml</td>
<td>-</td>
<td>12</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Au nanoparticles</td>
<td>One concentration</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Where they were measured by millimeter, (-) means no inhibition.

Table 3: Represents statistical analysis

<table>
<thead>
<tr>
<th>Chemical substances</th>
<th>Concentration</th>
<th>C.albicans</th>
<th>C.krusei</th>
<th>C.glaprata</th>
<th>C.parapsilosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eugenole</td>
<td>25%</td>
<td>38 ± 1.0×b^c</td>
<td>35 ± 1.0×b^r</td>
<td>35 ± 1.0×b^r</td>
<td>35 ± 1.0×b^r</td>
</tr>
<tr>
<td></td>
<td>33%</td>
<td>29 ± 1.0×b^r</td>
<td>17 ± 0.0×b^r</td>
<td>26 ± 0.0×b^r</td>
<td>35 ± 0.0×b^r</td>
</tr>
<tr>
<td></td>
<td>50%</td>
<td>37 ± 1.0×b^r</td>
<td>10 ± 0.0×b^r</td>
<td>26 ± 1.0×b^r</td>
<td>36 ± 1.0×b^r</td>
</tr>
<tr>
<td>Umbellulone</td>
<td>25%</td>
<td>no inhibition b</td>
<td>11 ± 1.0×b^c</td>
<td>no inhibition b</td>
<td>12 ± 0.0×b^r</td>
</tr>
<tr>
<td></td>
<td>33%</td>
<td>no inhibition b</td>
<td>10 ± 1.0×b^r</td>
<td>no inhibition b</td>
<td>16 ± 1.0×b^c</td>
</tr>
<tr>
<td></td>
<td>50%</td>
<td>no inhibition b</td>
<td>12 ± 1.0×b^r</td>
<td>no inhibition b</td>
<td>no inhibition b</td>
</tr>
<tr>
<td>Au nanoparticles</td>
<td>100</td>
<td>11 ± 1.0×c</td>
<td>12 ± 1.0×c</td>
<td>14 ± 1.0×c</td>
<td>11 ± 1.0×c</td>
</tr>
<tr>
<td>LSD</td>
<td></td>
<td>1.86</td>
<td>2.64</td>
<td>2.08</td>
<td>2.28</td>
</tr>
</tbody>
</table>

(a) Significance difference between the different concentrations of the same chemical substance. (b) Significance difference between the chemical substances of the same concentration. (c) Significance difference between the different concentrations of different chemical substances (overlap).

Antifungal Activity of Some Chemical Substances on Virulence Factors of C. albicans

Bio Film Formation

The results in Table (4), (Figure 11) described that eugenol in concentration 25mg/ml effect on growth of C. albicans and inhibition value of bio film formation is trace, while in concentration 33mg/ml and 50mg/ml were inhibition one plus. Umbellulone in concentration 25mg/ml and 33mg/ml produce inhibition value were one plus, while in concentration 50mg/ml no inhibition.

Table 4: Antifungal activity of eugenol,umbellulone and phenole biofilm formation of C. albicans

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Con. 25mg/ml</th>
<th>Con. 33mg/ml</th>
<th>Con. 50mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical substances</td>
<td>Eugenol</td>
<td>trace</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Umbellulone</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

(-)means negative = no inhibition, where inhibition value was measured in pluses.

From the results above is evidence that an effect this chemical substance (eugenol and umbellulone) at concentration 33mg/ml was the best against bio film formation of C. albicans.
Figure 10: (A): Biofilm Formation of C. albicans (positive), (B): inhibition of biofilm formation (negative) with concentration 33mg of eugenol and umbellulone

Explain that is chemical substances are interfere with the biofilm formation represent the novel method to control C. albicans infection. Due to affecting C. albicans virulence characterizes, this may be minimize an appearance of resistance strains. Recently researches have been emphasizing the biofilm growth in majority of infections caused by the Candida spp. Finally gold nanoparticles (Au nanoparticles) at concentration 100µg/ml produce inhibition of biofilm formation are measured two pluses at this concentration Table (5).

Table 5: Effects of Au nanoparticles on Biofilm formation of C. albicans

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Chemical substances</th>
<th>100µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Au nanoparticles</td>
<td>++</td>
</tr>
</tbody>
</table>

Inhibition is measured by pluses. From the results above is evidence that an effect this chemical substance (gold nanoparticles) at this concentration 100µg/ml against biofilm formation of C. albicans. The substances used in this line are able to reduce the viability of biofilm formation was inhibited by this plant extract in different concentration this agree with [8].

B. Germ tube Production

Table 6: Antifungal activity of eugenol, umbellulone and phenol on Germ tube production of C. albicans

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Chemical substances</th>
<th>Con. 25mg/ml</th>
<th>Con. 33mg/ml</th>
<th>Con. 50mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Eugenol</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Umbellulone</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

(-) means no inhibition, inhibition is measured by pluses.

From the results above is evidence that an effects this chemical substances eugenol at all concentrations and umbellulone at concentrations 25mg/ml, 33mg/ml were moderate against germ tube of C. albicans, while phenol no inhibition, but Gold (Au) nanoparticles in at concentration 100µg/ml
produce inhibition was one plus against germ tube of C. albicans Table (7).

Table 7: Antifungal activity of Au nanoparticles on germ tube production of C. albicans

<table>
<thead>
<tr>
<th>Concentration</th>
<th>100µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical substances</td>
<td>Au nanoparticles</td>
</tr>
<tr>
<td>C. albicans</td>
<td>+</td>
</tr>
</tbody>
</table>

Inhibition zone is measured by pluses.

From these results is evidence that an effects this plant extract (gold nanoparticles) with alone concentration 100µg/ml was approximately to precedent clove at all concentrations against germ tube produce by C. albicans All these chemical substances (eugenol, umbellulone, and phenol) tested in this study were able to inhibit the formation of the germ tube of C. albicans with highest activity observed with the chemical substances. C. albicans, requiring different concentrations of essential oil to inhibit germ tube this agree with [24].

C-phospholipids Production

In Table (8), (12) shown results include concentrations 25mg/ml, 33mg/ml and 50mg/ml of eugenol, which didn't have inhibition on phospholipase enzyme production while with same concentrations 25mg/ml, 33mg/ml and 50mg/ml of umbellulone are an effect on phospholipase production with inhibition was one plus.

Table 8: Antifungal activity of eugenol and umbellulone on phospholipase production of C. albicans

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Con. 25mg/ml</th>
<th>Con. 33mg/ml</th>
<th>Con. 50mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical substances</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eugenol</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Umbellulone</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

(+) means no inhibition, inhibition is measured by pluses.

From the results above is evidence that an effects chemical substance umbellulone at all concentrations were moderate against phospholipase production. While gold nanoparticles didn't have inhibition of phospholipids production of C. albicans. Phospholipids’ production has proven the ability of some species of Candida on the production of enzyme phospholipase represented by a change chromatic colonies on isolation after the growth on egg yolk agar and the result was positive in yeast C. albicans and C. krusie, while negative results appear in other species this agree with [26]. The present this study aimed to deter in vitro phospholipase activities in much isolation of C. albicans and C. krusie from the urogenital samples.

Figure 11: Phospholipase production of C.albicans on Egg ylok Agar
References

8. Narkwa PW (2010) Antifungal Susceptibility of Candida Species and Cryptococcus Neformans Isolated from Patients at the Komfo Anokye Teaching Hospital in Kumasi.2010
Patients with Genetic Study of virulence factors.
