EFFECT OF THE ALCOHOLIC EXTRACT OF POMEGRANATE PEELS ON CANDIDA ALBICANS RESISTANT TO ANTIFUNGALS COMPOUNDS

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ABSTRACT

This study was approved due to women high exposed to reproductive infection, so that 75 samples were collected from vagina swab and/or urine (50 vaginal swabs and 25 urine sample) all these samples collected from women suffer from vaginal and/or urinary tract infections (UTI). The results summarized by 41 case infected with fungal microorganism and 34 case infected with another microorganism (did not infected with fungal disease). In the same time the results showed that the highest rate of infection at the age of 25-35 years 51.24%, 26.82% in age 35-45, 12.19 in age 45-55 and the lowest rate of infection was at the age of more than 55 years 9.75%. The results showed the main microorganism was responsible for vaginal infection and UTI was C. albicans, seven from these isolates were resistant to fluconazole and one isolate were resistant to six antifungals (Fluconazole, Voriconazol, Caspofugin, Micafugin, Amphotericin B and Flucytosine).

Key words: pomegranate peels, C. albicans, the alcoholic extract

I. INTRODUCTION

Candida species which consider opportunistic for urogenital system and it is consider the main cause of mortality and morbidity globally, presenting a significant public health threat (Pfaller et al., 2014 and Zghair, 2020). C. albicans is the most commonly isolated pathogenic species and there are several species such as C. glabrata, C. glabrata, C. tropicalis C. parapsilosis C.famata, C.krusei, C.famata, C.famata, C. guilliermondii, and C. guilliermondii lusitaniae have become increasingly isolated in the world (Sultan and Zghair 2020).

C. albicans is the most common causative agent of mucosal and systemic infection among all species, It is responsible for about 70% of fungal infections worldwide (Morad et al., 2018). It is part of the normal flora of mucosal membran in about 50% of humans (Nobile and Johnson, 2015). It is found in the oral cavity, gastrointestinal tract and genital tract of healthy individuals (Mason et al., 2012 ; Liu et al., 2013). C. albicans has been the leading cause of life-threatening invasive infection over the past several decades. Despite the treatment of infected patients the mortality rate is arrived to 40%, especially in the hospital condition (Chen et al., 2020 ; Basmaciyan et al., 2019).

There are many type of antifungal used different means to kill or inhibit the growth of fungal pathogens (Pfaller, 2012 ; Kanafani and Perfect, 2008 ). For the effective treatment of superficial mucosal infection and life-threatening systemic fungal diseases so that a very large number of antifungal drugs have been developed and used for clinical purposes. Antifungals drugs are widely used in the treatment and prevention of most fungal infections, and they can be divided into four main groups: polyenes, azoles, pyrimidine analogues and echinocandins (Carmona and Limper, 2017 ).

When give over dose of antifungal drugs that lead increases resistance to opportunistic pathogens (Revie et al., 2018). The mechanisms of antifungal resistance have been classified as either primary or secondary and associated with intrinsic or acquired characteristics of the fungal pathogen, involving either interference with the antifungal mechanism of its drugs or a decrease in concentration of target drugs. The resistance can also occur when environmental factors lead to the colonization or replacement of susceptible species by resistant species (Pema’n et al., 2009). Some antifungal have effects on cell membrane or cell wall such as polyenes and azoles.
are due to their effect on the fungal cell membrane, while the echinocandins act by disrupting the fungal cell wall (Pfaller, 2012).

Candida have ability to form biofilms drug resistance is an important factor in its contribution to the diseases that affect humans in the same time most fungal have vast majority of microbial biofilms (Rajendran et al., 2010).

Plants are regarded as a natural source of many bioactive compounds, which provide desirable health benefits beyond essential nutrients (Cartea et al., 2011). Medicinal plants are also a good source for the preparation of modern pharmaceuticals and new therapeutic agents as alternative medicines to traditional drug regimens (Paul et al., 2015). In the same time Ali et al., 2014 prove the pharmacological activities of plants are attributed to the presence of polyphenols. Herbal medicine can be used to formulate new antimicrobial drugs to overcome the problem of resistance to synthetic antibiotics available (Yehia et al., 2011). The Phenolic compounds are defined as non-nutritive secondary metabolites that do not participate in plant growth and reproduction (Al-Rawahi et al., 2014). Phenolic compounds from herbal medicine include phenolic acids, flavonoids, and tannins and it is have different bioactivity of phenolic compounds is responsible for their therapeutic properties as antioxidant, anticancer, antimutagenic, antimicrobial, antifungal and anti-inflammatory (Sh et al., 2017).

Punica granatum L. is called the pomegranate and belongs to the family Punicaceae, and it is an important fruit in tropical and subtropical regions. The pomegranate fruit is one of the oldest edible fruits that has received great attention due to its increasing medicinal benefits in the past years (Hmid et al., 2017). Many studies reported that pomegranate peel extracts have antiviral activity against some human pathogens, so they can be used as an alternative to antibiotics in microbial treatments (Ferrazzano et al., 2017). The current study aimed to study the resistance ratio of Candida yeasts isolated from the female urogenital system in Karbala province and to know the effect of plant extracts prepared in college of science laboratory on the Candida isolates, and their ability to inhibit the growth of this Candida.

II. MATERIAL AND METHODS

Samples Collection

The current study included the collection of 75 samples (50 vaginal swabs and 25 urine sample) from women suffer from vaginal infection and urinary tract infections from outpatient clinic in Karbala city. from November 2020 to April 2021. All of the samples were taken to the lab to be identified and studies.

The urine samples were directly examined and implanted on the culture media, while the vaginal samples were implanted directly. Vaginal samples were taken by the doctor using sterile cotton swabs that contain the transport medium. Then the samples were transferred to the laboratory and cultured on Sabouraud's Dextrose agar (SDA) media and incubated at 37 °C for 24-48 hours, then purified to obtain pure colonies, and then the diagnosis by VITEC 2 COMPACT.

Sabouraud's Dextrose agar (SDA) media preparation

This medium was prepared according to the instructions of the Manufacturer by dissolving 62 g of the medium in a liter of distilled water. The medium was sterilized using an autoclave at a temperature of 121 °C and a pressure of 15 pounds/in. After completing the sterilization process, it was cooled to a temperature of (45-50) °C and poured into Sterile Petri dishes. The dishes were used to grow fungal isolates for the purpose of diagnosis.

Diagnosis of specimens

The fungal isolates were distinguished from bacterial isolates initially based on the external form of fungal growth and microscopic examination. After 24-48 hours of incubation, the shape, size, color, edge, and appearance of yeast isolates were examined on SDA media and then the diagnosis was mediated by the VITEC 2 COMPACT system. VITEC 2 COMPACT system is one of the modern and rapid diagnostic systems in bacterial and yeast diagnosis, as it gives accurate results with an accuracy of 99%. The antibiotics used was purchased from the French company BioMerieux including Fluconazole, Voriconazol, Caspofugin, Micafungin, Amphotericin B, and the Flu cytosine.

Plant collection and preservation
Pomegranate peels (Punica granatum L.) was purchased from local markets, stored in dry sacs, pounded to a powder by pistils and stored in a dark glass container at room temperature.

**Preparation of Pomegranate peels extract**

20 g of Pomegranate peels powder was combined with 200 ml of ethanol 96% in a magnetic stirrer for 48 hours. The extract was filtered by using filter paper, and the solvent was then removed by using a rotary evaporator, the residues were kept in freezer till used.

**Sensitivity test**

The antifungals drugs used and prepared from the French company BioMerieux (Fluconazole, Voriconazol, Caspofugin, Micafungin, Amphotericin B and Flucytosine). VITEC 2 COMPACT system was used to determine the sensitivity of isolates Candida species to many antifungals. The sensitivity of the isolates was evaluated by measuring the minimum inhibitory concentration of antifungal (MIC).

### III. RESULT AND DISCUSSION

**Morphological identification (Cultural and Microscopically characteristic)**

When culturing all of the obtained samples (urinary samples and vaginal swabs samples) on SDA medium and incubated at 37 °C for a period of (24-48 hours), the growth of Candida yeast colonies was fast, clear, and yellowish-cream in color and the colonies are smooth, shiny and dry colonies Figure (1). This study agreement with what Bhavan et al., 2010 who showed similar results of Candida morphology after growing on SDA media.

![Candida colonies growth on SDA after 24 hours at 37°C](image)

**Identification by VITEC_2 system**

The VITEC 2 COMPACT system (bioMérieux, Inc., Hazelwood, MO) is a fully automated system that allows species identification and fungal susceptibility testing (Pfaller et al., 2007; Revankar et al., 1998).

All isolated samples were initially diagnosed by microscopic and cultured and morphological diagnosed after that diagnosis by using VITEC 2 system was conducted and the probability of diagnosis with this system ranged between 95% - 99%, as shown in Table (1).

<table>
<thead>
<tr>
<th>No</th>
<th>Types of candida</th>
<th>The probability ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>C. albicans</em></td>
<td>92 - 99%</td>
</tr>
<tr>
<td>2</td>
<td><em>C. galabrata</em></td>
<td>95. % 99</td>
</tr>
<tr>
<td>3</td>
<td><em>C. tropicalis</em></td>
<td>97. % 99</td>
</tr>
<tr>
<td>4</td>
<td><em>C. kefyr</em></td>
<td>99%</td>
</tr>
</tbody>
</table>

**Effect of the alcoholic extract of pomegranate peels on candida spp.**

In this study the results showed that the alcoholic extract of pomegranate peels gave a higher inhibitory activity than oak against the isolated Candida, as the concentration of 25 mg / ml showed an inhibition rate of 2.67 mm
on C. albicans yeast, while the same concentration showed a high inhibition rate of 6.67 mm on C. glabrata in the same time the inhibition rate was 3.67 mm for C. tropicalis, while the lowest inhibition rate was 1.67 mm for C. kefyr. But when used the concentration of 50 mg/ml showed the highest inhibition rate on C. glabrata arrived to 9.00 mm, and the lowest inhibition rate on C. kefyr arrived to 3.33 mm and the inhibition rate for C. albicans was 6.33 mm while the inhibition rate arrived to 5.00 mm for C. tropicalis. The concentration 75 mg/ml of alcoholic extract of pomegranate peels on C. glabrata, which arrived to 10.30 mm but the lowest inhibition rate on C. kefyr was 4.67 mm, and the median between the two values was the inhibition rate of C. albicans and C. tropicalis, which arrived to 7.8 mm. While, when used concentration 100 mg/ml from the extract gave a very high inhibitory activity against Candida, the inhibition rate was 9.67 mm on C. albicans and consider resistant to antifungal drugs, but the same concentration showed a higher inhibition rate arrived to 13.67 mm against C. glabrata and lower than against C. tropicalis. With inhibitor rate arrived to 8.67 mm. While the lowest inhibition rate is 6.33 mm on C. kefyr yeast, as shown in Table (2) and in Figure (2), and our study was agreement with a previous study (Abdealsiede et al., 2020).

Our current results showed that the effectiveness of pomegranate peels is due to the active compounds present in the pomegranate, this results which agreement with previous studies which identified many bioactive compounds with antioxidant activity in the pomegranate peels, such as organic and phenolic acids and flavonoids, sterols, triterpenoids, alkaloids and tannins (Al-Rawahi et al., 2014; Entessar et al., 2012). Also there are many researchers who have demonstrated that pomegranate peels have anti-bacterial, anti-inflammatory, antiviral and anti-fungal activity (Bhandari, 2012; Singh et al., 2020).

Table (2) showed the effect of different concentrations of pomegranate peel extract, fungal type and the inhibition zone.

<table>
<thead>
<tr>
<th>المعالج تأثير الخميرة</th>
<th>التركيز ملغم/مل</th>
<th>C.albicans</th>
<th>C.kefyr</th>
<th>C.galabrata</th>
<th>C.tropicalis</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.75</td>
<td>9.67</td>
<td>6.33</td>
<td>4.33</td>
<td>2.67</td>
<td></td>
</tr>
<tr>
<td>4.00</td>
<td>6.33</td>
<td>4.67</td>
<td>3.33</td>
<td>1.67</td>
<td></td>
</tr>
<tr>
<td>9.92</td>
<td>13.67</td>
<td>10.33</td>
<td>9.00</td>
<td>6.67</td>
<td></td>
</tr>
<tr>
<td>5.92</td>
<td>8.67</td>
<td>6.33</td>
<td>5.00</td>
<td>3.67</td>
<td></td>
</tr>
<tr>
<td>9.59</td>
<td>6.92</td>
<td>5.42</td>
<td>3.67</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[\text{L.S.D. 0.01} = 1.108 \times \text{التركيز} = 1.108 \times \text{الخميره} = 1.108 \times \text{الن.س} = \text{L.S.D. 0.01}\]
REFERENCES


