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Susceptibility of clinical Candida albicans isolates to oak extract that resistance antifungal compounds

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Abstract
The study was conducted with the aim of detecting the causes of vaginitis infection in women and testing the efficacy of some antifungal compounds in treating the disease and the resistance of the isolated types of these antifungals. The study included isolating 125 samples using swabs for women with symptoms of injury in the cities of Al-Hilla and Al-Diwanyia for the period from September 2019 to December 2019. The results showed that the rate of 73% was fungal infection and 25.4% non-fungal infection C.albicans is resistance to against the antifungals, as 6 isolates resistant to fluconazol and nystatin were obtained. While these isolates were sensitive to cherrybark oak plant extract
Keywords: *Candida albicans* , antifungal compound, oak extract

Introduction

Candida species, opportunistic pathogens, are a major cause of morbidity and mortality worldwide and thus represents a serious threat to public health (Pfaller et al ., 2014 ; Matthaiou et al ., 2015 ; Zghair, 2020). Further, Candida species can cause vaginitis, oral candidiasis, cutaneous candidiasis, and systemic infections (Wächtler et al ., 2012). Candidemia is the most frequent hospital infection accounting for up to 15% of blood stream infections, and Candida species are the main causative agents in 50–70% of systemic fungal infections (Cornely et al ., 2012 ; Lionakis and Netea, 2013 ; Barchiesi et al ., 2016).

Candida albicans is the pathogenic species most frequently isolated. However, other species such as C. glabrata, C. tropicalis, C. parapsilosis, C. krusei, C.famata, C. guilliermondii, and C. lusitaniae have been increasingly isolated, (Al-khawaja and Zghair 2020)

mainly in human immunodeficiency virus (HIV)-infected individuals (Brunke and Hube, 2013 ; Ferreira et al ., 2013; Mayer et al ., 2013; Patil et al ., 2015 ; Barchiesi et al ., 2016).

Among the available antifungal agents, azoles are the preferred and most frequently used drugs for treatment of Candida infections. Depending on the type of infection, the anatomical site in which it occurs and the sensitivity profile of species, other antifungals can also be used. Among these, there are polyenes, echinocandins, nucleoside analogs and allylamines. (Pfaller et al ., 2010; Pfaller and Diekema, 2012b; Pfaller et al ., 2013; Pappas et al ., 2016).

The current use of antifungal agents raises concerns about their potential in selecting and spreading resistant fungal strains or species (Klepser M E. , 2006) Studies have reported an increasing incidence of infections caused by yeasts that either have acquired resistance or are intrinsically resistant to the drug in use [Klepser M E , 2006; Kanafani, Z. A., & Perfect, J. R. 2008) One of the acquired

The antifungal spectrum varies according to fungal species, such as: C.albicans, C.dubliniensis and C.tropicalis are normally susceptible to all antifungals used for the treatment of candidemia; C.glabrata is less susceptible and C.krusei is intrinsically resistant to fluconazole. Additionally, C.parapsilosis is less susceptible to the echinocandins (Pappas PG *et al* ., 2009 ; Arendrup MC . , 2013).

Echinocandins and azoles play an important role in the therapeutic management of invasive candidiasis. In recent years, Candida isolates with acquired resistance to azoles and echinocandins have been reported more frequently (Alexander et al ., 2013; Pfaller *et al* ., 2009) .Therefore, antifungal susceptibility testing and the detection of mutations in resistance genes are becoming increasingly important to detect antifungal resistance and determine the

underlying resistance mechanisms.

The overuse of the antifungal leads to the fungal multi-resistance, in addition to the side effects and the toxicity of most antifungal ,those reasons prompt to search for natural alternatives (Alviano, 2009.) Many plants produce natural substances that synthesised to perform versatile biological function to the plant, those substances could be very beneficial in medicine, especially the products of medical plants (Magbool *et al*., 2018) The use of herbal medicines (alternative medicines), date back to (4000-5000) B.C. About 80% of the world population depend on the plant products to maintain their health and approximately 30% of prepared medicines are based on plants (Shinwari, M. I., & Khan, M. A. 1998 ; Gulfraz, 2006)

Among these plants is cherrybark oak , The oak (Quercus pagoda) genus belongs to the family Fagacae, subfamily Quercoideae, and contains about 400 species widespread in Europe, Asia and America. Since medieval times, the bark of these trees has been used in traditional medicine and applied topically to burns and wounds, or applied orally for gastrointestinal diseases (Popović et al,. 2013)..

The well-known rationale for the therapeutic use of Quercus cortex is its direct antibacterial activity against many bacteria pathogenic for humans and animals. Oak bark is usually described as a source of polyphenolic secondary metabolites: hydrolysable tannins, previously known as pyrogallol tannins, and condensed tannins—proanthocyanidins (Haslam, et al.,2007).

Therefore, the study aimed to evaluate the efficiency of the ethyl extract of the Oak bark plant in inhibiting growth of *C.albicans* that resistance antifungal drug.

Materials and methods

Samples Collection

A total of 125 samples were collected from patients with symptoms of vaginal candidiasis, these swab samples were obtained from outpatient clinics in Hilla and Diwania city, from September2019 to December 2019. All the samples were transferred to the laboratory for identification and study.

The samples were taken from region by sterile transport medium swabs; the swabs were transported as soon as possible to the laboratory, and then incubated at 37C° for24-48 hr.

Identification of fungal isolates

Fungal isolates were diagnosed depending on the culture, microscopic characteristics , The shape, size, color, edge and appearance of yeast isolates were studied on SDA media after 24-48 hr of incubation. Chromagar test was used to help in the diagnosis of Candida species depending on color, single cell was picked up from the yeast growth on SDA and culture planning by the loop method incubated for 24-48 hr. at 37C° (Ellis , 1994; Horvath, *et al.*, 2003). CHROMagar Candida is a commonly used phenotypic method for the identification of Candida species (Odds & Bernaerts, 1994.).

Plant collection and preservation

The stem bark of Oak [Quercus sp.] was purchased from local markets, saved in dry sacs,then grounded by pistils to a powder and stored in a dark glass container at roomtemperature**.**

Preparation of Oak extract

100 g of Oak powder was mixed with 500 ml of ethanol [70%] in a magnetic stirrer for48 hours. The extractwas filtered by using filter paper, and the solvent was then removedby using a rotary evaporator, the residues were kept in freezer till used (Harborne.,& Williams, 2000)

Sensitivity test

Inoculation of Test Plates

1. Prepare the inoculum by making a direct broth or saline suspension of isolated colonies selected from an 18- to 24-hour agar plate.
2. Dip a sterile cotton swab into the suspension. Rotate the swab several times and press firmly on the inside wall of the tube above the fluid level. This removes excess fluid from the swab.
3. Inoculate the dried surface of the agar plate by streaking the swab over the entire

sterile agar surface. Repeat this procedure by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum. As a final step, swab the rim of the agar.

4. Leave the lid ajar for three to five minutes, but no more than 15 minutes, to allow for any excess surface moisture to be absorbed before applying the drug-impregnated disks (Tendencia, 2004).

Preparing the disks impregnated with antifungal drugs

Clotrimazole

We had used clotrimazole drug solution manufactured by (Coral Laboratories /India) with a concentration (10mg/30 ml) and by conversion the units from (mg) to (Mg) the concentration is equal to (10000 Mg / 30 ml).

After that we was apply ten–fold dilution by adding (1ml) (1000Ml) from the drug solution to (9 ml) of ethanol, the resulting concentration is (50 Mg/10 ml) in the vial.

The next step was adding (5 Ml) from the vial to the disks and for 5 times (25 Ml for each disk), the resulting concentration in each disk is (250 Mg/25 Ml) because (50 Mg * 5 times = 250 Mg) (Franklin et al., 2012).

Nystatin

- We had used nystatin drug solution manufactured by Egyptian International Pharmaceutical Industries company/Egypt. Each (1ml) of nystatin contains 100000

International Unit (IU), and each (1Ml contains 100 IU). - By using electronic international unit converter we had found that each (1 IU equal to 0.0002 mg). Then each (1 Ml) contains (0.02 mg) of nystatin. - After conversion units from mg to Mg then each (1Ml contain 20 Mg) of nystatin. - By applying ten – fold dilution by adding (1ml) (1000 Ml) of the solution to (9 ml) of methanol, the resulting solution is (2 Mg in each 1 Ml) and (10 Mg in each 5 Ml) of the solution. - The next step is adding (5 Ml) from the vial to the disks and for (5) times (25 Ml for each disk). - The resulting concentration in each disk is (50 Mg / 25 Ml) because (10 Mg * 5 times = 50 Mg) (Franklin et al., 2012).

Placing the disks

1- Using sterile forceps or disk dispenser, place antifungal disk on the surface of the inoculated and dried plate. 2- Immediately press it down lightly with the instrument to ensure complete contact between the disk and the agar surface. Do not move a disk once it has come into contact with the agar surface since some diffusion of the drug occurs instantaneously. 3- Position disks such that the minimum center – center distance is 24 mm and no closer than 10 to 15 mm from the edge of the petri dish. A maximum of six disks may be placed in a 9-cm petri dish and 12 disks on a 150 mm plate. Reduce the number of disks applied per plate if overlapping zones of inhibition are encountered, (Tendencia, 2004).

Reading Plates and Interpreting Results

After 16 to 18 hours of incubation examine each plate. If the plate was satisfactorily streaked, and the inoculum was correct, the resulting zones of inhibition will be uniformly circular and there will be a confluent lawn of growth.

If individual colonies are apparent, the inoculum would be too light and the test must be repeated. Measure the diameters of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk. Measure the zones to the nearest

whole millimeter, using sliding calipers or a ruler, which is held on the back of the inverted Petri plate. Hold the Petri plate a few inches above a black, nonreflecting background illuminated with reflected light.

The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth (Franklin et al., 2012).

Result and Discussion

The result showed that Oak has a broad spectrum antimicrobial effect on *candida* isolates that resistant azole and nystatin as shown in table (1) Which show Susceptibility Test of *Candida* isolates for clotrimazole , nystatin and Cherrybark oak plant .

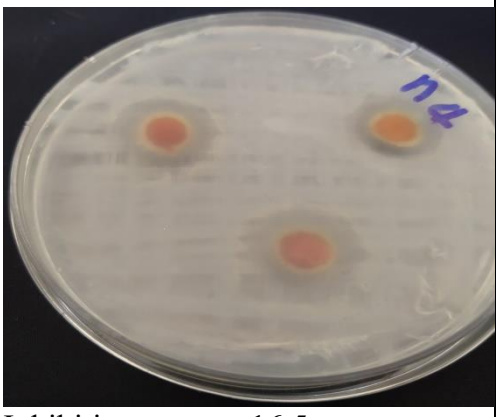
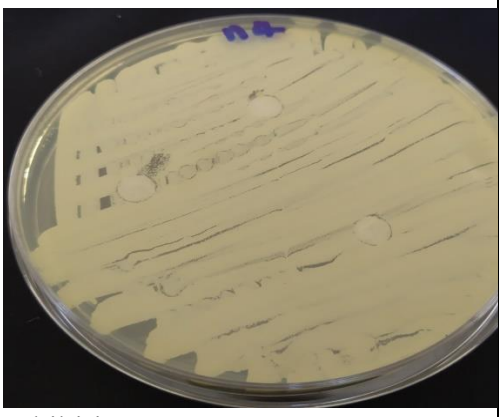
The table (2) show a comparison of the average diameter of the inhibition zone of the oak extract and the azole. Where was the inhibition zone in isolation N4 (16.5)mm , in N22 (24.5)mm , in 47(14.5) mm and in 23(8.5)mm . While these isolates showed resistance to azole derivatives.

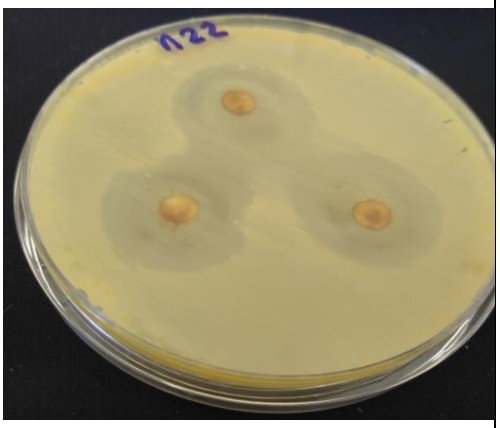
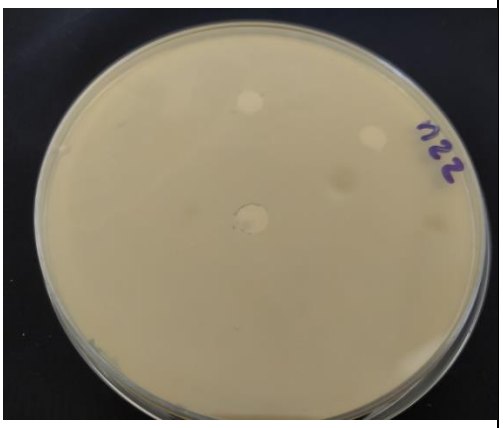
Which confirms the failure of the azole antifungals in the treatment of candidiasis and their resistance to antifungals in recent times and the success of the oak extract in inhibiting the growth of yeast even with resistant isolates. The results of using Cherrybark oak plant extract showed that concentration 250 mg/Ml failed to inhibit the growth of *Candida albicans* isolate, while concentration 350 mg/Ml achieved acceptable results in inhibiting the growth of these antifungal-resistant isolates.

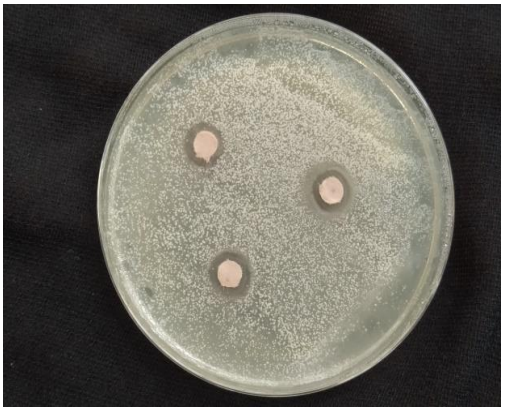
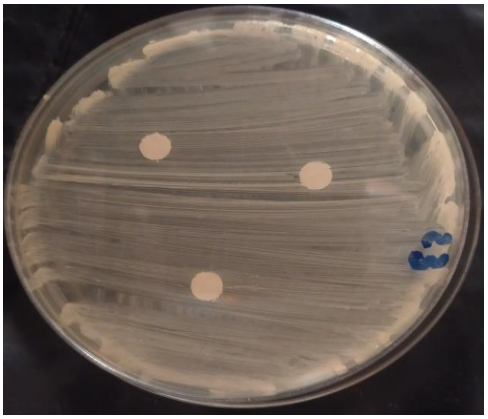
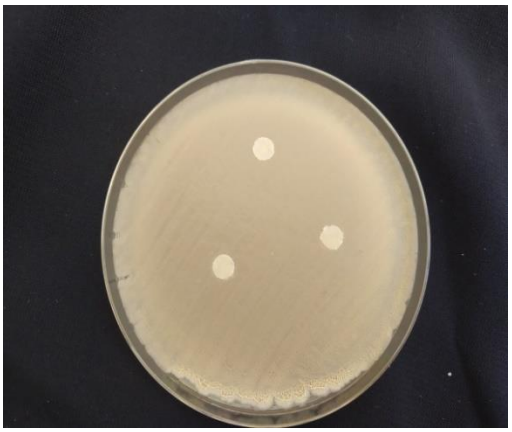
Table 1 Susceptibility Test of *Candida* isolates for some antifungal agents and plant extract.

Isolate No.	Clotrimazole	Nystatin	Cherrybark oak plant
6	Resistant	Resistant	sensitive
23	Resistant	Resistant	sensitive
47	Resistant	Resistant	sensitive
53	Resistant	Resistant	sensitive
N22	Resistant	Resistant	sensitive
N4	Resistant	Resistant	sensitive

Table 2 A comparison of the average diameter of the inhibition zone of the oak extract and the azole.

Isolation number	The average diameter of the inhibition zone of the oak extract	The average diameter of the inhibition zone of the azole
N4	 Inhibition zone = 16.5	 Inhibition zone = 0

N22	 Inhibition zone = 24.5	 Inhibition zone = 0

23	 Inhibition zone = 8.5	 Inhibition zone = 0
47	 Inhibition zone=14.5	 Inhibition zone = 0

The Oak named locally in Iraq as Jaft, and it is prominent used by Iraqi women, after soaking or boiling in water, as antiseptic following birth. Many studies have been revealed the antimicrobial activity of different parts of Quercus species (Cheynier, 2012 ; Welter *et al*., 2012 ; Meziti *et al*., 2019). Phytochemical analysis of Quercus sp. extracts showed the presence of most of the secondary metabolic alkaloids, free amino group, Glycosides,

phenols, saponins and tannins, which agreed with other study(Joshi & Juyal , 2017).

The well-known rationale for the therapeutic use of Quercus is its direct antibacterial activity against many bacteria pathogenic for humans and animals. The antimicrobial effect of Quercus bark extracts was previously revealed against Brucella, Enterobacter, Escherichia, Neisseria, Pseudomonas and Bacillus, while quite potent antibacterial effects have also been shown against Escherichia coli strains (Aldrich & Cavender, 2011). Acorn extract of Quercus showed antifungal activity against vaginal candidiasis [Moshfeghy *et al*.,2018).

In this study, the bark of Oak was extracted in ethanol, and the crude extracts were applied for antifungal screening on *candida* isolates Which proved to be resistant to azole derivatives.

This study is the first of its kind to evaluate the efficacy of the oak plant in inhibiting the growth of *Candida albicans.*

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References

Aldrich, P. R., & Cavender-Bares, J. (2011). Quercus pp. 89–129 in Wild crop relatives: Genomic and breeding resources, ed. C. Kole.

Alexander, B. D., Johnson, M. D., Pfeiffer, C. D., Jiménez-Ortigosa, C., Catania, J., Booker, R., ... & Pfaller, M. A. (2013). Increasing echinocandin resistance in *Candida* glabrata: clinical failure correlates with presence of FKS mutations and elevated minimum inhibitory concentrations. *Clinical infectious diseases*, *56*(12), 1724-1732.

Al-khawaja YA, Zghair FS (2020) First record of Cryptococcus gattii (VGI) that causes Vulvovaginitis in Iraq. Indian Journal of Public Health Research and Development, 11(4).

Alviano, D. S., & Alviano, C. S. (2009). Plant extracts: search for new alternatives to treat microbial diseases. *Current pharmaceutical biotechnology*, *10*(1), 106-121

Arendrup, M. C. (2013). Candida and candidaemia. *Susceptibility and epidemiology. Dan Med J*, *60*(11), B4698.

Barchiesi, F., Orsetti, E., Osimani, P., Catassi, C., Santelli, F., & Manso, E. (2016). Factors related to outcome of bloodstream infections due to *Candida* parapsilosis complex. *BMC infectious diseases*, *16*(1), 387.

Brunke, S., & Hube, B. (2013). Two unlike cousins: C andida *albicans* and C. glabrata infection strategies. *Cellular microbiology*, *15*(5), 701-708.

Cheynier, V. (2012). Phenolic compounds: from plants to foods. *Phytochemistry reviews*, *11*(2-3), 153-177.

Cornely, O. A., Bassetti, M., Calandra, T., Garbino, J., Kullberg, B. J., Lortholary, O., ... & Bille, J. (2012). ESCMID* guideline for the diagnosis and management of *Candida* diseases 2012: non-neutropenic adult patients. *Clinical Microbiology and Infection*, *18*, 19-37.

Ellis,D.H. (1994). Clinical mycology:The human opportunist mycoses. Pfzor, New York :7-14

Ferreira, A. V., Prado, C. G., Carvalho, R. R., Dias, K. S. T., & Dias, A. L. T. (2013). *Candidaalbicans* and Non-C. *albicans Candida* Species: Comparison of Biofilm Production and Metabolic Activity in Biofilms, and Putative Virulence Properties of Isolates from Hospital Environments and Infections. *Mycopathologia*, *175*(3-4), 265-272.

Franklin, R., Patel, J. B., Alder, J., Bradford, P., Dudley, M. N., Eliopoulos, G. M., et al. (2012). Performance standards for antimicrobial disk susceptibility tests, approved standard (11th Ed.). Wilsonville, Oregon: Clinical and Laboratory Standards Institute.

Harborne, J. B., & Williams, C. A. (2000). Advances in flavonoid research since 1992. *Phytochemistry*, *55*(6), 481-504.

Haslam, E. (2007). Vegetable tannins–Lessons of a phytochemical lifetime. *Phytochemistry*, *68*(22-24), 2713-2721.
Horvath, L. L., Hospenthal, D. R., Murray, C. K., & Dooley, D. P. (2003). Direct isolation of *Candida* spp. from blood cultures on the chromogenic medium CHROMagar *Candida*. *Journal of clinical microbiology*, *41*(6), 2629-2632.
Joshi, A. K., & Juyal, D. (2017). Traditional and ethnobotanical uses of Quercus leucotrichophora a. Camus (Quercus oblongata D. Don) in Kumaun and Garhwal regions of Uttarakhand, India: a review. *Int. J Herb Med*, *5*, 06-8.
Kanafani, Z. A., & Perfect, J. R. (2008). Resistance to antifungal agents: mechanisms and clinical impact. *Clinical infectious diseases*, *46*(1), 120-128.
Klepser, M. E. (2006). Candida resistance and its clinical relevance. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy*, *26*(6P2), 68S-75S.
Lionakis, M. S., & Netea, M. G. (2013). *Candida* and host determinants of susceptibility to invasive candidiasis. *PLoS Pathog*, *9*(1), e1003079.
Magbool, F. A., Elnima, E. I., Shayoub, M. E., & Hussein, S. E. O. (2018). Preliminary phytochemical screening of Quercus infectoria galls. *World J Pharm Pharmaceu Sci*, *7*, 77-87
Matthaiou, D. K., Christodoulopoulou, T., & Dimopoulos, G. (2015). How to treat fungal infections in ICU patients. *BMC infectious diseases*, *15*(1), 1-8.
Meziti, H., Bouriche, H., Kada, S., Demirtas, I., Kizil, M., & Senator, A. (2019). Phytochemical analysis, and antioxidant, anti-hemolytic and genoprotective effects of Quercus ilex L. and Pinus halepensis Mill. methanolic extracts. *Journal of Pharmacy & Pharmacognosy Research*, *7*(4), 260-272.
Moshfeghy, Z., Asadi, K., Akbarzadeh, M., Zare, A., Poordast, T., Emamghoreishi, M., ... & Sayadi, M. (2018). Quercus Brantii Lindl. Vaginal Douche Versus Clotrimazole on Vaginal Candidiasis: A Randomized Clinical Trial. *Journal of pharmacopuncture*, *21*(3), 185.
Odds, F. C., & Bernaerts, R. I. A. (1994). CHROMagar *Candida*, a new differential isolation medium for presumptive identification of clinically important *Candida* species. *Journal of clinical microbiology*, *32*(8), 1923-1929.
Pappas, P. G., Kauffman, C. A., Andes, D. R., Clancy, C. J., Marr, K. A., OstroskyZeichner, L., et al. (2016). Clinical practice guideline for the management ofcandidiasis: 2016 update by the Infectious Diseases Society of America. Clin. Infect. Dis. 62, e1–e50. doi: 10.1093/cid/civ1194
Pfaller, M. A., & Diekema, D. J. (2012). Progress in antifungal susceptibility testing of Candida spp. by use of Clinical and Laboratory Standards Institute broth microdilution methods, 2010 to 2012. *Journal of clinical microbiology*, *50*(9), 2846-2856.
Pfaller, M. A., Andes, D. R., Diekema, D. J., Horn, D. L., Reboli, A. C., Rotstein, C., ... & Azie, N. E. (2014). Epidemiology and outcomes of invasive candidiasis due to non-*albicans* species of *Candida* in 2,496 patients: data from the Prospective Antifungal Therapy (PATH) registry 2004–2008. *PloS one*, *9*(7), e101510.
Pfaller, M. A., Castanheira, M., Messer, S. A., Moet, G. J., & Jones, R. N. (2010). Variation in Candida spp. distribution and antifungal resistance rates among bloodstream infection isolates by patient age: report from the SENTRY Antimicrobial Surveillance Program (2008–2009). *Diagnostic microbiology and infectious disease*, *68*(3), 278-283.
Pfaller, M. A., Messer, S. A., Hollis, R. J., Boyken, L., Tendolkar, S., Kroeger, J., & Diekema, D. J. (2009). Variation in susceptibility of bloodstream isolates of *Candida* glabrata to fluconazole according to patient age and geographic location in the United States in 2001 to 2007. *Journal of clinical microbiology*, *47*(10), 3185-3190.
Shinwari, M. I., & Khan, M. A. (1998). Indigenous use of medicinal trees and shrubs of Margalla Hills National Park, Islamabad. *Pakistan Journal of Forestry*, *48*(1/4), 63-90
Tendencia, E. A. (2004). Disk diffusion method. Laboratory manual of standarized methods for antimicrobial sensitivity tests for bacteria isolates from aquatic animals and environment. Tigbauan, Iloilo. Philippines: Aquaculture Department. Southeast Asian Fisheries,

Development Center,13-29
Wachtler, B., Citiulo, F., Jablonowski, N., Förster, S., Dalle, F., Schaller, M., ... & Hube, B. (2012). *Candida albicans*-epithelial interactions: dissecting the roles of active penetration, induced endocytosis and host factors on the infection process. *PloS one*, *7*(5), e36952.
Welter, S., Bracho-Nuñez, A., Mir, C., Zimmer, I., Kesselmeier, J., Lumaret, R., ... & Staudt, M. (2012). The diversification of terpene emissions in Mediterranean oaks: lessons from a study of Quercus suber, Quercus canariensis and its hybrid Quercus afares. *Tree Physiology*, *32*(9), 1082-1091.
Zghair, FS (2020) First record of Cyberlindnera fabianii that causes Vulvovaginitis in Iraq. Eurasia J Biosci 14: 1253-1255