Antifungal Impact of Some Medicinal Plants and Natural Products on *Candida albicans* and Its Biofilm Formation Capability

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Abstract: The yeast *Candida albicans* is one of the most common pathogens of medical concern. It can cause diseases ranging from superficial and topical infections to systematic disseminated medical illnesses. Eradication of biofilms and its disruption is a great medical challenge to treat infections caused by *Candida albicans*. The yeast could develop antifungal resistant throughout decades, which is mainly due to their dimorphic phenomenon and biofilm construction. The use of medicinal plants has been used by ancient populations of humanity civilizations for the cure of fungi. Many chemical ingredients have been proved to have impact on the growth and biofilms of fungi. In this investigation, certain medical plants such as Galls of *Quercus infectoria (Q. infectoria)*, *Cyprus rotundus*, and Alum extract against of *Candida albicans* atcc10231 *in vitro* and biofilm development. The well diffusion method was used to determine the sensitivity and Minimal Inhibitory Concentration (MIC). Zones of inhibition were recorded in concentration of 200% of the extracts. The ethanolic extract of *Cyprus rotundus* and interaction of and Alum showed best inhibitory zone of 23 mm. While the watery extract *Q. infectoria* showed an inhibition zone of 22 mm. The MIC records were 20000 µg/ml for *Cyprus rotundus* was, 1250 µg/ml for *Q. infectoria*, 15000 µg/ml for Alum, and 80000 for an interaction of Alum and *Q. infectoria* extracts respectively. Biofilm disruption were assessed. Extracts of *Quercus* gall, *Cyprus rotundus*, and alum extract were found to have a substantial inhibitory effect on *Candida albicans* ATCC10231 growth inhibition, as well as the production of biofilms. This study is the first approach to study the biofilm of *Candida albicans* in RPMI medium in the Kurdistan Region of Iraq. The combination of the used extracts can enlighten the synergetic impact of those extracts and other medications to be used for treatment of fungal infections.

Keywords: Antifungal Activity, *Q. infectoria, Cyprus Rotundus,* Alum Extract, *Candida Albicans* ATCC10231, Well Diffusion Method, Disrupt

1. Introduction

The yeast of *Candida albicans* (*C. albicans*) is a human opportunist pathogen which may flourish *in vivo* and *in vitro* as yeast, pseudo hyphae, or actual hyphae based on the environment (Mukaremera et al.., 2017). In a comprehensive study from January 2012 to October 2017 in hospitals in Shenyang, China, Zhang and other in (2019) showed that over 6000 Patient were recorded as adult hospitalized cases of candidaemia. Abirami and others in (2020) stated that the pathogenicity of the yeast *C. albicans* is greatly influenced by the expression of virulence factors. Quindós and other (2018) reveled that *Candida albicans* is the most diffuse cause of severe Candida infections in compare to other species.

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The yeast *Candida* have two unique ways of life: planktonic and biofilms. The latter are colonies attached to the surface. Biofilms are comprised of several of cell types that are held together by an extracellular substrate (Zeise et al.., 2021). A biofilm is a colony of microbial that are permanently affix to a surface, inert material, or living tissues and produce polymers found outside of cells as a matrix structure (Kurzbaum et al.., 2019). El Kareh (2014) demonstrated the *Candida albican* biofilms in RPMI-1640 medium, He proved that biofilms of *Candida* are to be a stumbling block in the removal of *Candida* infections. Biofilms of the yeast are more resistance to antifungal stresses (Lohse et al.., 2018). Efflux pump are target proteins in the ergosterol biosynthesis pathway, play a role in antifungal resistance (Lee et al.., 2020). Medical plants have been a great source of natural products for sustaining human health. More recently, plant extracts for medicinal use have advanced, medical herbal extracts have been shown to be anti-Candida (Sivapalan, 2013). Koshak and Koshak (2020) showed that medicinal plants contained more active compounds with strong antibiotic were used against many diseases like *Mycobacterium.* The *Cyprus rotundus* and *Q. infectoria* are two medicinal plants that are used because of their anti-Candida and anti-fungal properties. Alum is the natural products that has been commonly used as anti-fungal element. Babadi (2019) in the findings of a study claimed that white alum has a positive effect on inhibiting the growth of C. albicans. This study stated a problem of which patients with C. albicans infection experiencing difficulties of recovery due to the biofilm nature of infection along with emergence of antifungal resistance.

The aims of the presented work are to assess the sensitivity of the yeast toward some commonly used medicinal plants, determining the minimal inhibitory concentration of plant extracts to be used against the yeast, and demonstrating the impact of medicinal and natural herbs on the Candida biofilm.

2. Methodology, Research Design

2.1 Candida Strain

The Candida strain used in this study was *C. albicans* ATTC strain 10231 which was obtained from Media Diagnostic center- Erbil, Iraq.

2.2 Medicinal Plants and Natural Product

The plants used in this study were *Cyprus rotund*, *Q. infectoria* (*Q. infectoria)* Botanical Name *Q. infectoria veneri* identified by (Townsend and Guest, 1966, 1980 and 1985). The alum as a natural product was obtained from local market.

2.3 Methodology

2.3.1 Plant Extract

The *Cyprus rotundus* (roots) was collected from Daquq near Kirkuk city and *Q. infectoria* Olivier galls (roots) (was collected from district near Sulaimani) in September 2020.The plants were collected with their roots. The obtained plants and parts were cleaned and dried with room temperature. Grinding was performed to obtain the powder (Abdurrahman, 2014).

2.3.2 Plant Extract Preparation

Two solvents have been used (Watery extraction) and (Ethanoic extraction) of the medicinal plants. Precisely, fifty gram of plant powder added to conical flask. Amount of 250ml of (double distilled water) and (ethanol solvent) was taken in a conical flask, which was then placed on a magnetic stirrer

and allowed to mix at room temperature by a magnetic bara for 48 hours the solution was filtered. The extraction was subjected to rotary evaporation (Abdurrahman, 2014).

2.3.3 Phytochemical Compound

The phytochemical qualitative tests were carried out for root extract of *Q. infectoria* and *Cyprus rotundus* to screen for the presence of tannins, saponins, Resins, alkaloids, Phenols using standard procedure by (Hasan, 2001). These active compounds were commonly reported in plant extract with bioactivity.

2.3.3.1 Detection of Tannins

Amount of 10 mL of plant extract was divided into two equal parts, and then drops of 1% lead acetate (BDH-England) were added to the first part. The appearance of white pellets indicates a positive result. Drops of 1% FeCl3 (Fluka-Germany) were added to the second part, and the formation of a green bluish color indicates a positive result (Abdurrahman, 2014).

2.3.3.2 Detection of Saponin

Amount of 5 ml of the extract was shaken vigorously for 30 seconds before being placed in a vertical case for 15 minutes. The presence of foam indicates the presence of saponin (Hasan, 2001).

2.3.3.3 Detection of Resins

Total of 10 mL acidify water that has been distilled. If turbidity appears after adding HCl to 10ml of plant extract, this indicates a positive reaction (Abdurrahman, 2014).

2.3.3.4 Detection of Phenols

Total of 3 ml of plant extract was mixed with 2 ml of potassium hexacyanofrrate and 2 ml of FeCl3, and the green bluish color indicates a positive result (Abdurrahman, 2014).

2.4 Preparation of Stock Solution

Four different types of stock solutions were produced by utilizing a solvent:

1-Serile D.W. for *Q. infectoria* extract and *Cyprus rotundus* (Ethanolic/aqueous) (25, 50, 100, 200) g/mL

2.5 McFarland Turbidity Standard Solution

The McFarland 0.5 standard corresponds to a suspension of 1.5*108 CFU/mL.

For candida. At 530 nm, the absorbance should be 0.11 to 0.14 (Woods *et al.*., 2019).

2.6 Preparation of Candida Albicans Suspension

The ATCC strain obtained from Media Diagnostic Center-Erbil

2.7 Determination Minimum Inhibitor Concentration (MIC) by Tube Dilution and Microplate Dilution Method

Plant extracts in trial extracts 4 is diluted in different dose %200, %100, and % 50, % 25 and 100 µl

volumes containing 100 µl of plant extraction dilution for each well. The MIC was determined using dilution method. The MIC of medicinal plant extracts were (312, 625, 1250, 2500, 5000, 10000, 15000, 20000, 25000, 30000, 35000, 40000, 45000, 50000µg/ml). Preparation of cell suspensions for biofilms were adapted from (Chandra et al.., 2001 and Pierce et al.., 2008).

Using of RPMI-1640 (containing L-glutamate but not sodium bicarbonate) *C. albicans* (ATCC 10231) isolates were taken from -80°C freezer stocks and streaked on Sabouraud dextrose agar (SDA) plates. The plates incubated for 2 full days in 37°C. A single colony from a SDA plate was suspended in 20 ml of Yeast Peptone Dextrose broth (YPD) in 40ml tubes. Cultures were grown in a shaking incubator overnight (max 16h) at 37°C. The YPD medium was removed by centrifuging the tubes at 3000g for 5 min at 4°C. The cells were washed twice in 20 ml PBS, pH 7.4, and then finally the cells suspended in 20 ml of PBS. The cells were counted using a hem cytometer. Dilution of 1:10 was used and a cell suspension was made to a final concentration of 1.0 x 10^6 CFU/ml in an appropriate volume of RPMI-1640 (containing L-glutamate but not sodium bicarbonate .1 ml of suspension was added to each well plate (Costar) into which Thermanox cover slips (Thermanox) had been placed previously. The plates were sealed with parafilm and incubated for 24 hours at 37°C.

3. Results and Discussion

3.1 Anti-Candida Activity of *Q. infectoria* Watery Boiling Extract. *Cyprus rotundus* Ethanol and Alum Extraction by Well Diffusion Assay

In Table 1, Figures 1 and 2 the records showed that the ethanolic extract of *Cyprus rotundus* and interaction of and Alum showed best inhibitory zone of 23 mm. While the watery extract *Q. infectoria* showed an inhibition zone of 22 mm, Alum extract with distil water inhibited the growth by 21 mm zone. The result may indicate that the plant contains tannins, phenols, saponins, and alkaloids. The role of alum in changing the pH of the medium, as well as its effect on the availability of cell wall precursors could be the reason for the fungus's inability to grow and nourish in such conditions.

Table 1: Zone of inhibition of anti-*Candida albicans* activity of plant extracts against selected *Candida* by (S D A) well diffusion

Zone of inhibition inconcentration	%200
Interaction between *Q. infectoria*	23 mm
C. rotundus	23 mm
Q. infectoria	22 mm
Alum extract	21 mm

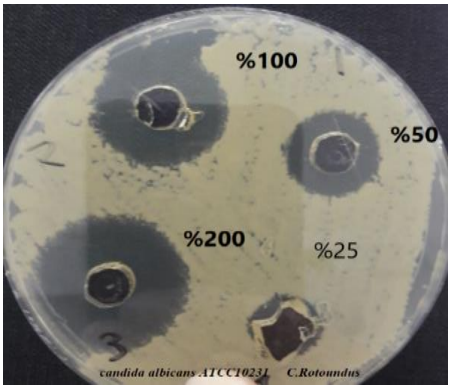

Figure 1: Antifungal activity of *Cyprus rotundus* Ethanol extract against *Candida*

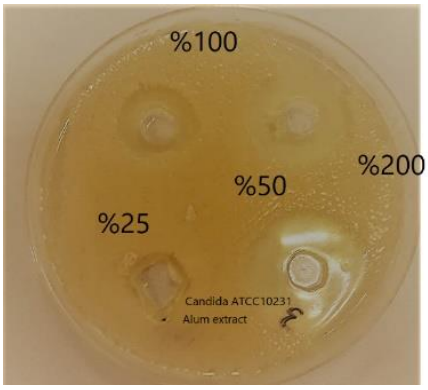

Figure 2: Antifungal activity alum aqueous extract against Candida ATCC 1023

3.2 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of Plant Extracts for *C. albicans* ATCC10231

The MIC for *Cyprus rotundus* was 20000 µg/ml, for *Q. infectoria* was 1250 µg/ml, while for Alum it was 15000 µg/ml. In addition, the MIC for an interaction of Alum and *Q. infectoria* extracts was 80000 µg/ml. Plant and alum extracts including the phytochemical groups or biochemical impact was able to perform changes in the cell wall and cell membrane permeability or influential reaction (Medda et al.., 2015)

Table 2: (MIC) and (MFC) of *C. albicans* ATCC 10231 By spectrophotometer and grown on SDA

Extracts	MIC	MFC
Cyprus rotundus	20000 µg/ml	25000 µg/ml
Q. infectoria	(1250 µg/ml	(2500 µg/ml),
Alum extract	15000 µg/ml	(20000 µg/ml)
Alum extract interaction with *Q. infectoria* extract	80000 µg/ml.	90000 µg/ml

Biofilm formation and biofilm disrupted by the sub-MICs of used plant extracts and natural products (Chandra et al.., 2001 and Pierce et al.., 2008). The MIC values were used to observe the effect of such

extracts on the disruption of the biofilms. Concentration of alum extract 15000 g/ml, *Q. infectoria* 1250 g/ml, *Cyprus rotundus* Ethanol (l20000 g/ml), combination alum and *Q. infectoria* extract 80000 g/ml were used in this study.

Results showed promising effect of these extracts on the biofilm intensity and integrity. Disruptions were noticed when biofilms were checked with naked eye (Figure 3) in compare to the positive control of the yeast grown in RPMI (Figure 3). The penetration if the extract to the complex structure of the biofilm and ability to halt their networking and growth can be an encouraging outcome for future directions and further thorough investigations. The chemical contents of the extracts specifically the phenolic compounds may alter the cell membrane activity and pseudo hyphal and generation tube tips. These parts of the fungus are prone to chemical impacts due to their sensitivity during elongation as cell wall lyse to pave the way for elongation (Kong et al.., 2010).

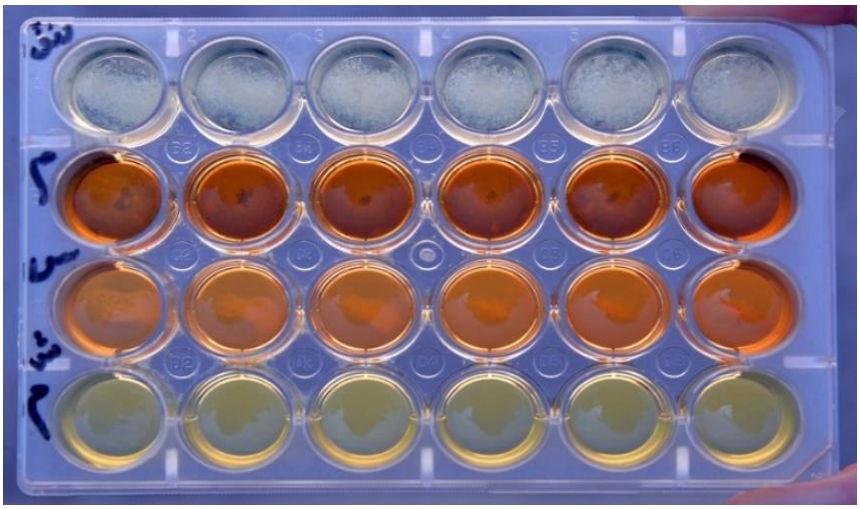

Figure 3: Formation of biofilm and disrupt of biofilm by plant and natural extract

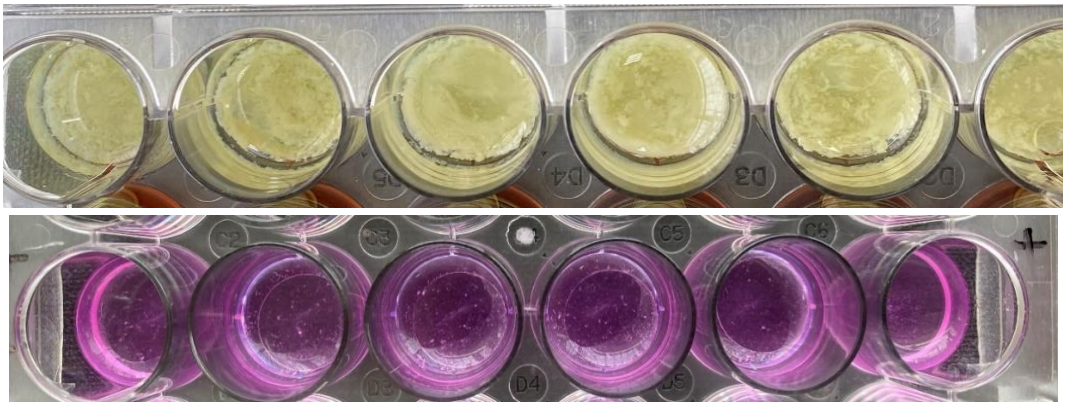

Figure 4: +VE: positive control C. albicans ATCC10231, -v negative control

Note:

-Positive control is plant extract, with solvent +broth. Blank: is prepared by plant extract and broth for each concentration. Negative controls were prepared by using YPD broth medium

-Negative control is solvent (RPMI-1640 media (containing L-glutamate but not sodium bicarbonate

4. Conclusion and Future Directions

The present study offers an alternative way of treating fungal infections specifically when reports of antifungal resistance is emerging globally. The findings of this investigation are a novel trial to inhibit and disrupt the biofilm formation of C. albicans using natural medicinal plants and their extracts. It is a first study in this region to use the standard methodologies for biofilm formation and studying the disruptive impact of tested extracts

The principal findings of the present work can pave the way toward using the recorded concentrations in vivo to see the side effects and impacts of the extracts on human body.

Future directions can lead the investigation toward the followings:

1. More strains of *C. albicans* to be used specifically from medical samples and patients suffering from this yeast.
2. Further investigation to be efforted to understand the molecular mechanism of antifungal effect of these extracts. Determining the genes involved and the proteins halted.
3. More medicinal plants can be involved to study interactive and synergetic impacts of those extracts on the fungal growth and biofilm formation.
4. Cell viability test to be performed after biofilm disruption to indicate real impact of those extracts on the vitality of cellular enzymes and proteins.

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