

## In Vitro Inhibitory Effect of Alcoholic Extract of Inner Stratum of Oak Fruit (Jaft) on Candida Albicans

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**Running title:** alcoholic extract of inner stratum of oak fruit (Jaft)

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### ABSTRACT

**Background and objective:** Candidiasis is one of the most common opportunistic fungal diseases in humans. This disease is caused by a fungus, yeast called candidial. Candida vaginitis is the most important fungal disease in women. Traditionally, in western parts of Iran different plant extracts are used for treatment of vaginal infections. This study aims to evaluate alcoholic extract of inner stratum of oak fruit (Jaft) on Candida albicans isolated from vaginitis.

**Methods:** the fruits of this Plant were collected from Ilam Mountain then they were dried in shadow, the alcoholic extraction was carried out. Inhibitory effect was studied by disk diffusion and agar well diffusion assay and the statistical analysis was done with SPSS 16 with used of repeat measure examination.

**Result:** in disk diffusion method, high inhibition zone was in 80 µg/disk and in agar well diffusion method 80 mg/ml has the highest inhibition zone.

**Conclusion:** alcoholic extract of jaft contains some metabolites that have inhibitory effect on Candida albicans. We suggest determining effective components of this extract.

**KEY WORDS:** inhibitory, Candida albicans, disk diffusion, phenolic extract

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### INTRODUCTION

Breadth of opportunist fungal disease in inclined person on the one hand and increasing drug resistance and their effect on other hand are the cause of the research about anti fungal effect of plants[1]. These traditional treatments are cheaper and more effective than the modern one. Actually the risk of getting resistant pathogen infection in this region is less than other regions that antibiotic is used for treatment of their infection[2]. Still about 75% of people in the world use plant drugs for supplying their needs [3] . People in west regions of Iran use the extract of different plants such as extract of inner layer of oak for treatment of infection from ancient times[4, 5]. In this project we will study the effect of alcoholic extract the inner layer of oak (Jaft) on Candida albicans isolated from virginities in vitro. Candida albicans is normal flora of human bodies that rarely creates disease in healthy persons. But an opportunist yeast can (create infect) in inclined person such as persons affected by AIDS, persons who extensively use Antibiotic and persons who transplant [6, 7]. This opportunist fungus can create different clinical demonstration such as thrush, virginities, skin infection, endocarditic, meningitis, and arthritis in human host. C.albicans is one part of normal flora of human bodies that colonize in mouth pituitary surface, digestion system and vagina and majority of those infections create disease in those regions [8, 9, 10]. Drug resistance to this fungus is increasing and further more increasing the dose of using common drugs and subsequently enhancing the side effects of clinical drugs are the main causes of using plant drugs [11, 12].

### MATERIAL AND METHODS

Fruits of oak trees were collected from Ilam Mountains of in west of Iran and inner stratum of it was isolated and dried in 25 °C in shadow away from direct sunlight and then dried plant was pestled and mixed with 80% ethanol and the alcoholic extraction was done with soxhlet [13].

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**Microorganisms and media:**

30 Clinical isolates of *Candida albicans* isolated from vaginitis of referring to obstetrics clinics and *C. albicans* ATCC 2091 Strain as standard strain was used.

**Agar well diffusion assay:**

The agar diffusion and Filter paper disk embedding sink methods were used to determine the antifungal activities of the medicinal plant extracts against the opportunistic fungi [12]. The agar diffusion assay was carried out with slight modifications [14, 15]. Using the micropipette, 100  $\mu$ l of 0.5 McFarland solution of *C. albicans* culture in 0.85% sterile distilled water (SDW) was placed over the surface of an agar plate, and was spread using a sterile inoculation loop. The Using sterile pipettes Pasteur, four holes (5 mm in diameter) were punched in each of the culture plates. In the first hole 35  $\mu$ l of the corresponding extract solvent was added as a negative control, in the second hole; 35  $\mu$ l of the plant extract was added. Each test was duplicated. The culture plates were then incubated at 37 °C, and the results were observed after 24 h. The clear zone around each hole was measured in mm, indicating the activity of the plant extract against the fungal organisms [15, 16].

**Filter paper disk embedding:**

Filter paper disks (whatman NO 1:5mm diameter) were impregnated with crude extract (100 $\mu$ g/disk-3.2 mg/disk) or standard drugs (20 $\mu$ g/disk clotrimazol) the disk was overlaid on SDA plates and incubated at 37 c for 24 h. the disk was tested in triplicate including one with a solvent blank and 3 for the standard drugs. Inhibition zones were calculated as the difference between disk diameter and the diameters of inhibition the mean inhibition zone was used to calculate. Activity index was calculated as the mean inhibition zone for test samples divided by the mean inhibition zone for the standard drugs [16-18].

**Statistical analysis:**

The obtained result was analyzed by SPSS 16 with use of repeated measure statistical examination.

**RESULT****Result of Filter paper disk embedding and Agar well diffusion assay:**

Filter paper disk method, high inhibition zone was seen in 80 $\mu$ g/disk and clotrimazol disk didn't have any inhibitory but had inhibitory effect on ATCC 2091 Strain. Moreover In the agar diffusion method the concentration of 80mg/ml had higher inhibition zone than others concentrations. ( $P < 0.01$ ), (Table-1)



Figure1. inhibition effect of alcoholic extract by agar well diffusion

Table-1: inhibitory effect of alcoholic extract of inner stratum of oak fruit (Jaft) on *Candida albicans*

| 35 $\mu$ l of Concentration mg/ml | Inhibition zone(10mm hole size) | Filter disk impregnated |       |
|-----------------------------------|---------------------------------|-------------------------|-------|
| 5 mg/ml                           | 5 mm                            | 100 $\mu$ g/disk        | 5 mm  |
| 10 mg/ml                          | 10 mm                           | 200 $\mu$ g/disk        | 10 mm |
| 20 mg/ml                          | 12 mm                           | 400 $\mu$ g/disk        | 15 mm |
| 40 mg/ml                          | 18mm                            | 800 $\mu$ g/disk        | 22 mm |
| 80 mg/ml                          | 25mm                            | 1600 $\mu$ g/disk       | 26 mm |
| <i>Candida albicans</i>           | 25 mm                           | 1600mg/disk             | 26mm  |
| ATCC 2091 , 80 mg/ml              |                                 |                         |       |

### Result of MIC and MFC:

This Result that we obtained indicated that in 40mg/ml concentration of extract colony count was less than 2000 that this concentration is MIC and in 50mg/ml concentration, colony count was less than 200(MFC).this result indicates the effect of this extract on C.albicans. Effect of this extract on ATCC (MIC&MFC) was the same to clinical samples. Anti fungal disk didn't have any effect on ATCC this result was knot of this experiment and time also didn't have any effect on increasing inhibitory effect on C.albicans.

### DISCUSSION

The fruit of oak (*Quercus coccifera*) was used in traditional medicine for treatment of microbial infection[19]. The antibacterial evaluations of different extracts obtained from *Quercus* spp. indicate potential against Bacteria[20, 21]. This fruit has effective content such as tannin, oil, gersit, nitosan and amidan. The antibacterial effect of hydroalcoholic extract of this fruits was due to lavanoidas [22]. Since these classes of metabolites have been proved to have antibacterial activity which confirm the obtained results and further validated in antifungal evaluation. So far, no research about inhibitory effect of alcoholic extract of inner stratum of oak fruit (Jaft) has been done on *Candida albicans*. In this study, we evaluated the inhibitory of alcoholic extract of inner stratum of oak fruit (Jaft) on *Candida albicans* in vitro. Antibacterial activity may involve complex mechanism like the inhibition of the metabolism and the inhibition of the synthesis of cell walls, cell members, nucleic acid and protein. it seems likely that substance in the alcoholic extract contain polios and non polios and act separately of in content to exert those, in other hands *Quercus* spp, such as *Quercus dilatata*, *Quercus coccifera*, and others ,have antibacterial activity against *E.coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Micrococues leutus*, *Bordetella branchiceus*, this indicate *Quercus* spp such as *Quercus coccifera* could have inhibition effect on other organism such *Candida albicans*. Karimi Poor fard et al did one research about Antibacterial Activities of *Thymus Denaensis*, Jaft and Hydro-Alcoholic Extract of Green hull *Pistacia atlantica* on *Listeria monocytogenes* [23]. Their study indicates that Hydro-Alcoholic extract of jaft has the most antibacterial effect compared with other extracts. The results of their study indicate alcoholic extract of Jaft has significant inhibitory effect on *Candida albicans*. MIC and MFC of this extract was 40  $\mu\text{g/ml}$  and 50 $\mu\text{ g/ml}$  respectively, this results shows alcoholic extract of Jaft contain higher amount of effective metabolites. In other hands nowadays *Candida albicans* isolated from clinical samples is resistant to some antifungal drugs. Kurrum et al indicated water extract of *Quercus infectoria* has MIC and MBC ,500 ,1000 microgram/ml respectively against bacteria. Comparing this extract to *Candida albicans* and *sapnoligia* to bacteria indicates that extract has high inhibitory effect on *Candida albicans*, But alcoholic extract of oak has more antibacterial effect compared with antifungal[24] . Inhibitory effect of aqueuse extract against *Candida* spp. is under investigation.

**Conclusion:** alcoholic extract of jaft contain some metabolites that has inhibitory effect on *Candida albicans*.

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