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Efficacy of Hot Alcoholic *Peganum harmala* seed Extract Against Oral *Candida albicans* and *Candida tropicalis* isolates from childrenin Karbala Governorate, Iraq

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Abstract

Background: Communicative microorganisms contribute to maintaining the balance of the oral environment, which is an open environment to the external environment, making it a vital and diverse environment that allows for the colonization of different types of microbes Candida, in particular, is one of the most common types of fungi and is considered a normal flora of the skin. Its overgrowth leads to the development of oral candidiasis, an opportunistic fungal infection that includes inflammation of the tongue and parts of the oral mucosa. **Methodology:** The study included obtaining 51 samples from children without genetic diseases from the wards of Karbala Teaching Hospital for Children, ranging in age from 16 days to 10 years. The samples were collected using cotton swabs containing a sample collection medium and transferred to the laboratory of the Imam Hussein Center for Manuscripts and Research. The samples were examined morphologically and microscopically, and some virulence factors were studied, and the inhibitory ability of the hot alcoholic extract of harmal seeds against the yeasts used in the study was tested. **Results:** The results of the study showed that 33 out of 51 samples were positive for Candida growth, representing 65%. *C. albicans* was recorded at 80% (24 isolates), and *C. tropicalis* numbered 6, representing 20%. These two Candida species were identified through the study of some morphological and microscopic characteristics, which showed that *C. albicans* appeared green color and *C. tropicalis* blue color on chromogenic agar. *C. albicans* was the most common species in the current study, characterized by its ability to produce germ tubes, hyphae, and Chlamydospore, unlike *C. tropicalis*, which was only capable of forming pseudo hyphae. The inhibitory activity of harmal seeds was tested, with the highest inhibitory activity recorded at a concentration of 100 mg/ml for both *C. tropicalis* and *C. albicans*, reaching inhibition rates of 20.4 and 15.9 mm, respectively. The lowest inhibitory activity was recorded at a concentration of 20 mg/ml for both *C. tropicalis* and *C. albicans*, reaching inhibition rates of 12.3 and 8.5 mm, respectively. The inhibition rate of the antifungal nystatin was 13.5 and 14.7 mm, respectively. **Conclusions:** *C. albicans* was one of the most common species in this study, as this species was distinguished by possessing a set of virulence factors .The plant (Peganum harmala L) is a medicinal plant with pharmaceutical properties and has proven its inhibitory efficiency against the species under study

Keywords: Candida Spp. , Oral candidiasis, virulence factors, *Peganum harmal seeds ,alcoholic extract.

1. Introduction

The symbiotic microorganisms contribute to maintaining the balance of the environment inside the mouth, which is an environment open to the external environment, making it a vital and diverse environment that allows for the colonization of different types of microbes (Şenel, 2021). In particular, yeasts of the Candida genus, which are among the most common types of fungi and are considered a normal flora in the skin and mucous membranes of the normal human body at a rate of 31-50% (Khalaf, 2016). With increased colonization, they lead to the development of oral candidiasis, which is considered an opportunistic fungal infection known as "thrush," which includes inflammation of the tongue and parts of the oral mucosa, characterized by excessive growth of fungi and superficial invasion of the tissue (Hellstein & Marek, 2019). This appears as a white patch on the tongue and mucous membranes, generating ulcers, a feeling of pain and burning, and problems in swallowing (Kushnir, 2021). The extent of colonization of this disease increases in cases of weakened immunity and various diseases such as cancer and devices used inside the mouth, including orthodontic appliances and dentures (Patel, 2022). One of the most common types of Candida causing oral candidiasis is *C. albicans*, which is the most frequent cause of mucosal and systemic infections and is responsible for approximately 70% of fungal infections worldwide (Morad et al., 2018). Other Candida infections, such as *C. glabrata* and *C. krusei*, can also occur (Kumar et al., 2022).

Due to the side effects of antifungal drugs, interest has grown in medicinal plants as alternative treatments. One such plant is harmal (Peganum harmala), a perennial herb widely cultivated in warm regions from the Mediterranean to parts of America (Zhu *et al*., 2022; Al-Essa *et al*., 2023). This plant contains a number of active compounds, most notably harmine and harmaline, which constitute approximately 6% of the total dry weight of the seeds (Fahmy *et al.,* 2021). These compounds give the plant various medicinal properties, including antispasmodic, antimicrobial, and psychoactive effects (Shatarat et al., 2020).

2. Methodology

- Collection of Pathological samples**:** Oral swabs were collected from fifty-one pediatric patients admitted to the Children's Teaching Hospital, ranging in age from 16 days to 10 years old, and the samples were transferred to the Manuscripts and Investigation Laboratory at the Imam Hussein Center.

- Isolation and Identification: All samples were cultured using the streak method, with three replicates per sample, on saproidodextrose agar medium. All samples were incubated at 37°C for 48 hours, and then the samples were characterized morphologically and microscopically.

- Morphology examination: The method of (K.Al-Khafaji & Z.Al-Maamouri., 2013) was followed in the morphological diagnosis of fungal growth on the saproid medium to which an antibacterial agent was added to prevent contamination.

- Microscopic examination: A portion of the growing colonies was spread on a glass slide, stained with lactophenol blue, and then examined under a 40x light microscope to visualize the oval, budding yeast cells (Hospenthal et al., 2006) .

- Germ tube formation: The method of (Bhargava, 2019) was followed, which is to mix a small part of the colony with half a milliliter of serum and place the mixture in the incubator for 3 hours at a temperature of 37°C, then a small drop of this suspension was examined under the microscope with a power of 40 X.

- Chromogenic agar : This medium was used to differentiate between types of yeast based on color. After the isolation and morphological and microscopic diagnosis, the samples were cultured on this medium and incubated at a temperature of 37°C for 48 hours (Hospenthal et al., 2006).

- Chlamydospore and Pseudohyphae Formation Test: The formation of chlamydospores and pseudohyphae was examined under a 40X microscope using a sterile glass slide. A portion of the Candida colony was cultured on cornmeal medium. The surface of the medium was marked and covered with this sterile glass slide. The plates were incubated at 28°C for 48-72 hours (Forbes et al., 1998) **.**

- Plant Sample Collection**:** The parts were collected from the plant species whose inhibitory activity against isolated Candida species was studied. They were sourced from local markets in Karbala Governorate. The plant parts were washed to remove dirt and impurities, first with ordinary water and then with distilled water. They were left to dry at room temperature and then ground separately using an electric grinder to obtain a plant powder. This powder was stored in clean, dry glass containers until use and kept in the laboratory.

- Preparation of the hot alcoholic extract of *Peganum harmala:* weigh 20 grams of harmala seeds and mix them with 200 ml of 70% ethyl alcohol in a 1000 ml glass flask, heat for half an hour, and let it stand for 24 hours at room temperature. Then, filter the mixture using several layers of gauze to remove impurities. Next, use a centrifuge at 2500 rpm for 10 minutes. Filter the extract using 0.1 Whatman NO filter paper and place the filtrate in clean, sterile glass containers. Incubate at 40°C for 2-3 days until the extract is dry. Scrape the dried extract with a clean, sterile knife and store the dry powder in clean, airtight plastic containers until use. This preparation is called a dry alcoholic extract (Khanzada & Akram, 2006).

- Testing the inhibitory efficacy of the hot alcoholic extract of harmal seeds against some Candida species: The method of spreading by the wells mentioned in (Balouiri *et al.,* 2016) was followed, whereby the yeast suspension was prepared by transferring a number of colonies grown on SDA medium to a test tube containing 5 ml of sterile saline solution with a concentration of 0.85% and shaking the solution well to obtain a suspension with a concentration of 1×10^6 to 5×10^6 cells by comparing its turbidity with the turbidity of McFarland solution (Lee *et al.,* 2009) , and 0.1 ml of this suspension was distributed uniformly

onto SDA medium using a sterile cotton swab. The plates were allowed to dry at room temperature for 10–15 minutes. Six evenly spaced holes were then made using a 7 mm diameter cork drill in each of the cultured yeast plates. The stock solution of the extract was prepared by dissolving 1 g in 10 ml of sterile distilled water. The required concentrations for the study (20, 40, 60, 80, 100) were prepared using the dilution ,100 µL of each of the above concentrations was then inoculated into each hole sequentially and in duplicate for each Candida species. A positive control hole was also prepared, and 100 µL of the antifungal agent was added to it. The fungal nystatin, at a concentration of 100,000 IU, was used as the control. For the negative control, pits were prepared in separate dishes, and 100 microliters of sterile distilled water were added. The dishes were then incubated for 24-48 hours at 37°C. The inhibitory activity of the extracts against the isolated species was then observed by measuring the areas of inhibition in millimeters using a ruler.

3. Results

3.1. Isolation and Identification

This study Growth results showed that 33 out of 51 samples were positive for growth (65%). The most common species, and the focus of this research, was *C. albicans*, with 24 samples (80%). Six *C. Tropicalis* colonies, representing 20% of the total, were identified. These two Candida species were diagnosed by studying certain morphological and microscopic characteristics, as follows:

3.2. Morphological and Microscopic Identification of Candida spp.

Morphological diagnosis revealed that *C. albicans* and *C. Tropicalis* appear white or cream-colored, disc-shaped or round, with a smooth, convex surface. They grow on SDA agar at 37°C for 48 hours, as shown in Figure 1. Microscopic diagnosis of the Candida colonies showed single or budding spherical or oval cells, as shown in Figure 1.

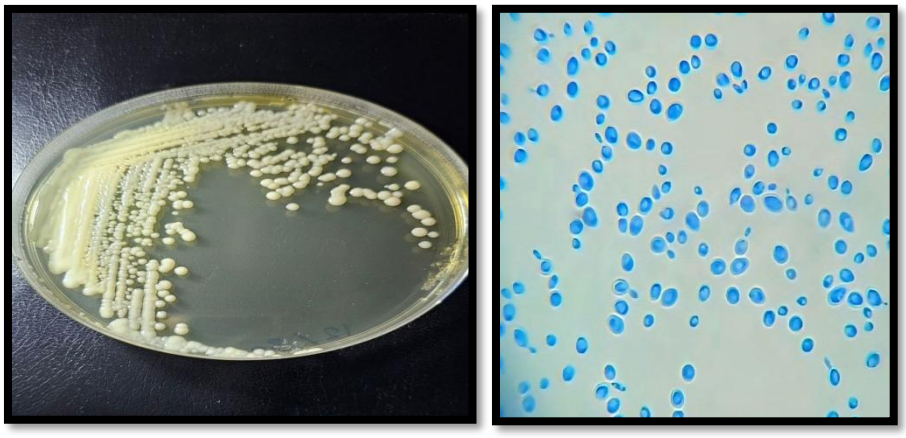

Figure1. Morphological and Microscopic Identification of colony Candida spp

3.3. Growth on Chromogenic agar medium

Chromogenic agar medium showed Candida yeasts with different colors specific to each species, as all isolates of *C. albicans* appeared in a light green color, and isolates of *C. tropicals* appeared in a blue color as shown in Figure (2).

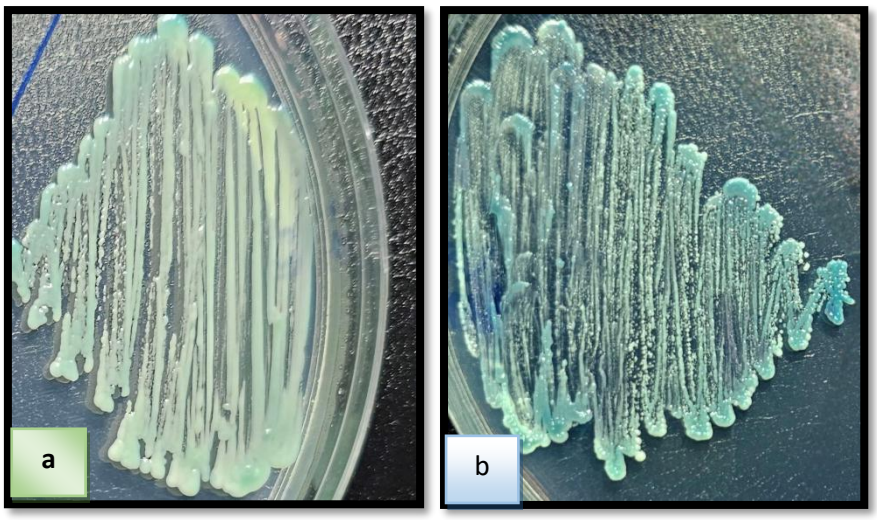

Figure 2. Candida species (a) *C.albecans*, (b) *C. tropicalis,* growing on Chromogenic agar medium at 37 °C and an incubation period of 48 hours.

3.4. Germ tube formation: The test results showed that all *C. albicans* isolates were able to produce germ tubes, as shown in Figure (4). All other isolates of *C.Tropicals* did not produce germ tubes under the same conditions.

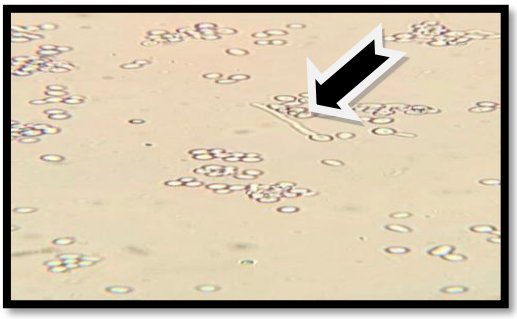

Figure3. Germ tube formation in *C. albicans*

3.5. Chlamydospore Formation and Pseudohyphae

The results showed that *C. albicans* was able to produce fungal hyphae with large, thick-walled, spherical spores known as chlamydospores, as illustrated in Figure 4. In contrast, *C. tropicalis* isolates were unable to produce chlamydospores but were capable of producing pseudohyphae only.

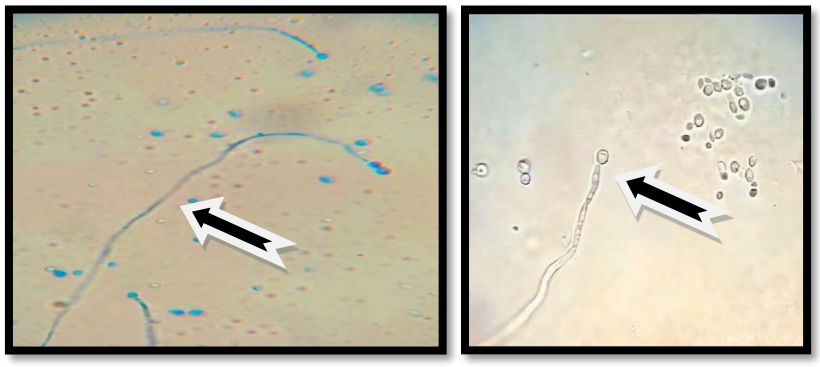

Figure4. Chlamydospore Formation and Pseudohyphae of *C. albicans*.

3.6. Testing the efficacy of the alcoholic extract of harmal seeds against certain Candida species

The efficacy of the alcoholic extract of harmala seeds was tested against Candida species isolated from the mouths of children with oral candidiasis, as shown in Table (1). Most of the tested concentrations showed a clear effect in inhibiting all Candida species compared to the control treatment. This effect increased with increasing concentrations, with the highest inhibitory activity recorded at a concentration of 100 mg/ml for each , The inhibition rates for *C. tropicalis* and *C. albicans* were 20.4 and 15.9 mm, respectively. The lowest inhibitory activity was recorded at a concentration of 20 mg/ml for both *C. tropicalis* and *C. albican*, with inhibition rates of 12.3 and 8.5 mm, respectively. The inhibition rates for the antifungal nystatin were 13.5 and 14.7 mm, respectively.

Table 1: Average inhibition zones of the alcoholic extract of harmal seeds on *C. albican* and *C. tropicalis* isolated from the mouths of children .

*P C = Nystatin antibiotic (positive control)

*N C = Distilled water (negative control)

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species	N C	P C	Concentration mg\ml					Mean of inhibition zones of all concentration(mm)
			20	40	60	80	100	
C. Tropicalis	0	13.5 mm	12.3 mm	14.2 mm	16.9 mm	19.9 mm	20.4 mm	16.7 mm
C. albican	0	14.7 mm	8.5 mm	11.9 mm	13.9 mm	15.4 mm	15.9 mm	13.1mm

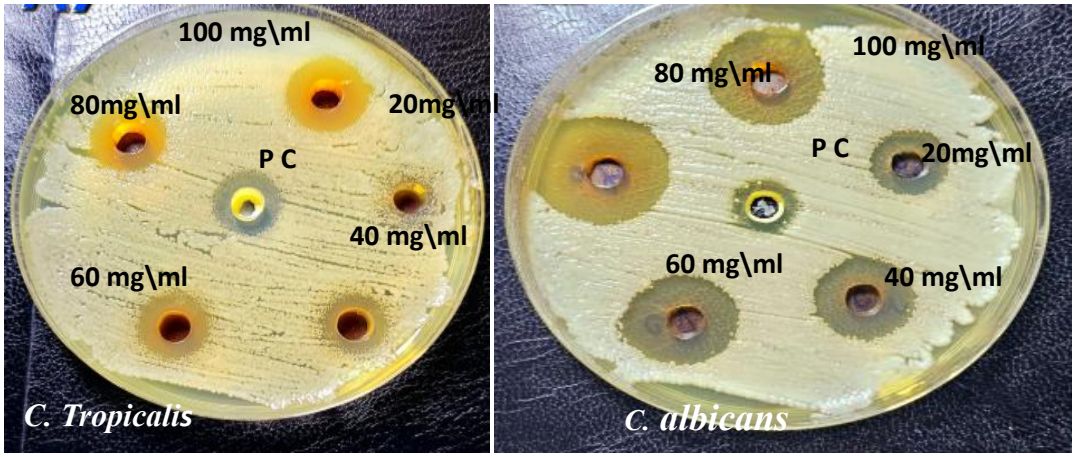

Figure 5. Effect of concentrations of alcoholic extract of the harmal seed plant on *C. albican* and *C. tropicalis* isolated from the mouths of children

4. Discussion

This study involved the isolation and identification of yeasts taken from the mouths of children with oral candidiasis. Samples were obtained from 51 children, ranging in age from newborns to ten years old, who were not genetically predisposed to any disease. The prevalence of *C. albicans* in this study is consistent with previous studies e.g (Salah *et al.,* 2023), which indicate that this type of yeast is among the most common isolates. This is attributed to the fact that it is a naturally occurring organism in the body, but changes in its environment can cause it to become pathogenic. These changes include immune system dysfunction, excessive antibiotic use, and formula feeding, particularly in young children.

The current results are also consistent with the findings of (Salem *et al.,* 2023).regarding the smooth, creamy appearance of colonies when cultured on SDA medium. This reinforces the reliability of this medium for the initial accurate diagnosis of yeasts. Furthermore, the cells appear spherical, oval, single, or budding under the microscope, with bluish-green margins resulting from stain accumulation on the Gram-positive cell wall of most Candida species after lactophenol staining.

The identification of Candida isolates on chromogenic agar is achieved through the enzymes secreted by each Candida species upon reaction with the substrate in the medium. Consequently, the colors vary depending on the species (Hassan, 2024) this result was also consistent with (Al-Aref, 2023). One of the most important virulence factors is the formation of germ tubes in serum after incubation for 2-3 hours at 37°C. This test is used to differentiate between *C. albicans* and other species, as germ tube formation is a crucial factor that plays a significant role in adhesion and biofilm formation, which is essential for colonization, disease initiation, and infection (Jalal *et al.,* 2019). Chlamydial spore production is an indicator of virulence, and *C. albicans* exhibits a clear advantage in spore formation due to its role in infecting the fungus compared to other species. Furthermore, cornmeal is a starvation medium, a condition conducive to spore production (Ali, 2021) This result was consistent with (Al-Halfi, 2025). The alcoholic extract of rue (Peganum harmala L.) showed significant effects against Candida species, particularly at higher concentrations, specifically 100 mg/ml. This is attributed to the fact that rue (Peganum harmala L.) is a medicinal plant with numerous

documented pharmacological properties (Jalali *et al.,* 2021). It is rich in beta-carboline alkaloids, especially harmine and harmalin, which possess antifungal properties. These alkaloids interact with microbial cell structures and synthesize reactive oxygen species (ROS), disrupting cell integrity and viability of fungal pathogens (Zhu *et al.,* 2022). The results also showed a significant decrease in inhibition activity at lower concentrations, with inhibition rates at 20 mg/ml being considerably lower than those at 100 mg/ml. This concentration-dependent activity underscores the need for optimal dosage determination when using rue (P. harmala) extract in various applications. Therapeutic against fungal infections.

5. Conclusions

C. albicans was the most common species in the current study. This species was characterized by its ability to produce germ tubes, form hyphae and fungal spores, unlike *C. tropicalis*, which was characterized by its ability to form only fungal hyphae. The plant (Peganum harmala L) is considered a medicinal plant with pharmacological properties and has proven its inhibitory efficacy against the species under study.

Recommendations

Experimenting with different concentrations of various medicinal plants to identify potential alternatives to antifungal medications, testing other yeasts to determine their effects, and using different extraction methods to determine which is most effective.

A acknowledgments

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Ethical Approval

The ethical guidelines for the research were followed, with approvals obtained from the Karbala Health Department before sample collection began, and permission sought from the families of the sick children before taking the sample.

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