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Antifungal effect of *Heracleum persicum* on standard *Candida dublinensis* strain: An in Vitro study

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Abstract

Background and Objective: The aim of this study was to investigate the antifungal effects of aqueous, alcoholic, acetone extracts, and essential oil of *Heracleum persicum* seeds (*Golpar*) against standard *Candida dublinensis (C.d)* strain.

Materials and Methods: The antifungal effects were evaluated using the agar well diffusion method. Additionally, the *Minimum Inhibitory Concentration* (MIC) and *Minimum Fungicidal Concentration* (MFC) were determined using the serial dilution method. Standard strain *C. d* was investigated, and Nystatin was used as the positive control.

Results: The mean diameter of growth inhibition zones for the alcoholic and acetone extracts and essential oil of *Golpar* seeds were reported in millimeters as follows: 13 mm and 10 mm and 15 mm for *C.d*, respectively. Based on the results, the MIC and MFC of the alcoholic extract of Golpar seeds against *C.d* were reported as 40 mg/ml and 40 mg/ml. Additionally, the MIC and MFC of the acetone extract of Golpar seeds against *C. d* were reported as 14 mg/ml and 28 mg/ml, respectively. Moreover, MIC of the essential oil of Golpar seeds against *C.d* was 0.5 mg/ml and the MFC was 1 mg/ml.

Conclusion: According to the results, the essential oil and the alcoholic and acetone extracts of Golpar seeds demonstrated promising antifungal and anti- Candidal activity against *C.d*. Therefore, based on the findings of this study and further research on the extracts and essential oil of Golpar, their antifungal and anti-Candidal effects could be utilized in the formulation of mouthwashes for patients with removable dentures suffering from Oral Candidiasis.

Keywords: *Heracleum persicum*, Golpar, *C. dublinensis*, Mouthwash, Removable Dentures

1. Introduction

The use of complete removable dentures poses challenges for both patients and clinicians. Issues arising from the use of complete dentures for patients and clinicians, particularly in relation to oral hygiene neglect leading to microbial proliferation in the denture, are highlighted as the main cause of denture stomatitis (1, 2). This introduction also addresses the prevalence of denture stomatitis in Iran and the contributing factors to this condition (3, 4). Various treatments, including antifungal therapies and denture replacement or adjustment, are examined for controlling the symptoms of denture stomatitis (5, 6, 7).

This section of the article explores the main problem associated with denture stomatitis and its causative factors (3, 8). Among the causative factors of denture stomatitis are candidal infections, particularly with Candida species. Various factors such as poor denture hygiene, improper use of antifungal medications, and compromised immune system can lead to the development of denture stomatitis (9, 10). It has been investigated that various species of Candida, especially Candida albicans, are the primary cause of denture stomatitis (3, 11, 12, 13). Additionally, other

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species of Candida such as Candida glabrata and Candida dubliniensis are also discussed (14). These species may exhibit greater resistance to antifungal treatments (13, 15).

Furthermore, this research examines the antimicrobial and antifungal effects of the extract and essential oil of *Heracleum persicum* (commonly known as Golpar). This plant contains compounds that can effectively control the growth of fungal species, especially Candida. Additionally, this plant possesses antioxidant and immunomodulatory properties that could be effective in inhibiting and preventing the occurrence of denture stomatitis (16, 17, 18).

Given the limited information available regarding the efficacy of Golpar extracts and essential oil in inhibiting and controlling the growth of fungal species, further laboratory studies are proposed to evaluate the antifungal properties of this plant and its use as a therapeutic option for denture stomatitis.

In summary, this study focuses on the antifungal effects of Golpar seed extracts and essential oil on different fungal species and emphasizes their importance in controlling denture stomatitis and reducing resistance to chemical antifungal agents. This research introduces innovation in examining the different effects of herbal products on fungi and the impact of processing variations on their properties.

This disease poses many challenges for patients and clinicians using complete removable dentures (3). Many symptoms and factors, including poor oral hygiene and continuous denture use, are associated with denture stomatitis (19). Treating this disease requires a comprehensive treatment plan and identifying all predisposing factors (20).

Candida dubliniensis:

C. dubliniensis is an opportunistic pathogen first identified in AIDS patients (21). This fungus is commonly found alongside C. albicans in immunocompromised patients but differs morphologically (22). Cases of C. dubliniensis identification in oral samples of diabetic patients or those using insulin have been reported (23). *C. dubliniensis* is sensitive to fluconazole but resistance to this drug has also been reported (24).

Heracleum persicum (Golpar):

Heracleum persicum, commonly known as Golpar, is a member of the Apiaceae family and is native to Iran. Its seeds have long been used as a spice, and its young blossoms in pickles and traditional medicine. Important furanocoumarins extracted from the roots of this plant include Pimpinellin, Isopimpinellin, bergapten, isobergapten, and sphonodin. The main components of Golpar seed extracts include hexyl butyrate, octyl acetate, hexyl 2-methyl butanoate, and hexyl isobutyrate (25, 26). This plant has various properties in the field of medicine and traditional medicine, including inhibiting oxidative stress processes and possessing antioxidant properties (27, 28, 29, 30). Additionally, its anti-inflammatory and antimicrobial properties are noteworthy (31, 32). Some terpene compounds and alkaloids present in Golpar exhibit anticonvulsant and analgesic properties (33, 34). The use of Golpar seeds can help stimulate the human humoral and cellular immune systems, but attention should be paid to the amount of consumption and its limitations (35).

2. Materials and Methods

2.1. Research method

This study was conducted as a laboratory experiment using the well diffusion and macrobroth dilution methods (serial dilution method) to investigate the anti-candidal effect of Golpar seed alcohol and acetone extracts (36).

2.2. Preparation of essential oil

The essential oil was prepared using the water distillation method. To prepare the oil, 100 grams of dried Golpar seed petals were ground using an electric grinder and placed in the balloon of the distillation device. Then, 700 milliliters of distilled water was added to the balloon, and the distillation process continued for two hours. Finally, the collected oil volume was determined and stored in the refrigerator for preservation (37).

2.3. Preparation of extract

Extraction was performed using the immersion method (maceration) with ethanol and acetone solvents. A quantity of 100 grams of the plant material, after grinding, was placed in extraction vessels, and 100% acetone and 80% ethanol solvents were added. The solutions were then stored at laboratory temperature for 3 to 4 days (37).

2.4. Preparation of aqueous extract

The Seed of plant (100 g) was cleaned and powdered using a mechanical grinder. The powder (100 g) was added to 400 mL hot water, boiled for 15 minutes and filtered through a Whatman paper (No. 42). The filtrate was evaporated to dryness under reduced pressure to obtain a viscous residue. The residue was suspended in normal saline (37). It is necessary to mention that the plant extract solutions were sterile.

2.5. Well diffusion assay

In this assay, wells with a diameter of approximately 6 millimeters were created on Sabouraud dextrose agar plates, and the experimental solutions of Golpar seed extracts were poured into the wells. After 24 to 48 hours, areas of fungal growth inhibition were

examined. Finally, the macrobroth dilution assay steps were completed (37).

2.6. Study population

Standard strain *C. dubliniensis* ATCC = CD60 was selected for the study.

2.5. Statistical analysis

Using SPSS software and One-Way ANOVA test followed by Tukey's post-hoc test, the results were analyzed. The tests indicated that the results of the experiments on Golpar seed water extract were ineffective and were not included in the statistical analysis.

3. Results

3.1. Results on *C. dubliniensis* strain

Investigation showed that the diameter of the growth inhibition zone of Golpar seed essential oil was significantly higher compared to its acetone extract. However, no significant difference was observed compared to its ethanol extract. Furthermore, the diameter of the growth inhibition zone for both extract and essential oil was significantly lower than nystatin. This was also true for MIC and MFC.

These results demonstrate that Golpar seed extracts and essential oil can be considered as effective options in controlling candidal pathogens.

4. Discussion

Candida species are part of the normal flora of the oral cavity, but under conditions such as immunodeficiency and malnutrition, they can cause opportunistic superficial skin and mucosal infections. C. albicans is the main causative agent of oral candidiasis, but nowadays, other Candida species are also observed as causative agents. In this study, we investigated the antifungal effects of aqueous, ethanol, and acetone extracts, as well as the essential oil of Golpar seeds against C. dubliniensis.

Golpar seed has been traditionally used as an anti-inflammatory and analgesic drug in traditional medicine and its antioxidant and antimicrobial effects have been recognized in laboratory settings. Studies have shown that Golpar seed essential oil has moderate antifungal effects (38). Additionally, in other studies, the inhibitory effect of Golpar seed essential oil on the growth of Candida Zeylonoides has been reported (39).

In the present study, the antifungal effect of Golpar seed essential oil in inhibiting the growth of C. dubliniensis fungi was greater than its extract form. The stronger activity of essential oils in inhibiting fungal growth may be due to the synergistic effects of active components of the essential oil compared to the individual compounds that make up the extract (40, 41).

The findings of these investigations have shown that the aqueous Golpar extract due to its abundant polar groups ionizes the surrounding environment of the fungus, minimizing the interference of fungal compounds with the surrounding environment, which does not have a measurable effect on the growth inhibition of C. dubliniensis (42).According to studies, C. dubliensis have shown the highest resistance to Golpar seed extracts, which is consistent with our findings. Additionally, the fungal species C. dubliniensis quickly develops resistance to antifungal drugs and compounds, which may be due to its ability to survive in harsh environmental conditions (43).

In other research, it has been shown that the antioxidant compounds present in Golpar plant stimulate the proliferation of B lymphocytes and macrophages, playing an important role in strengthening the humoral and cellular immune system (44). Golpar seeds contain a variety of compounds including alkaloids, triterpenes, and terpenoids responsible for the plant's anticonvulsant effects (45, 46).

Conclusion

Based on the findings of the present study, it can be concluded that the seed extract of the Golpar plant can be used effectively in combating candida species in denture stomatitis infections, given its efficacy comparable to that of nystatin. Moreover, the efficacy of both alcoholic and acetone forms of Golpar seed extract against *C. dubliensis* is similar, and the choice between these two can be made based on other parameters such as side effects and cost-effectiveness.

Limitations and recommendations

One of the limitations of this study is its laboratory-based nature. The growth and colonization conditions of microbial species in the oral cavity and under dentures may differ from laboratory conditions. Although denture stomatitis-causing species are highly diverse, only four species were investigated in this study. Future studies are recommended to explore more parameters in this regard.

Since most studies have focused on *C. albicans* strains and the mechanism of action of Galper extract has been examined, it is suggested to investigate the effects of these plant derivatives on non-albicans strains in further studies. Typically, the efficacy of extracts and essences in converting these derivatives into mouthwashes and their interaction with other auxiliary substances or existing oxidants in mouthwashes may vary. Clinical trials in the future are recommended to examine the antifungal effects of the mentioned compounds in the form of mouthwashes under controlled conditions.

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