Evaluation of the Antifungal Effect of Chicory Extracts on *Candida Glabrata* and *Candida Krusei* in a Laboratory Environment

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**ABSTRACT**

**Aim:** This research has evaluated the extract’s antifungal effects on *Candida glabrata* and *Candida krusei* in a laboratory environment.

**Materials and methods:** In this research, to evaluate the antifungal effect and the minimal inhibitory concentration (MIC) determination of chicory extract, the Clinical and Laboratory Standards Institute (CLSI) was used. *Candida glabrata* and *C. krusei* funguses were procured from the Tehran Pasteur Institute; they were grown in the relative growing environment according to the required conditions. Also for further assurance about the macrodilution method reality, the agar well diffusion method was used. Finally, the obtained results were analyzed using the Statistical Package for the Social Sciences version 16 software.

**Results:** The MIC for the chicory extract was 50 µg/mL for *C. krusei* and 100 µg/mL for *C. glabrata*. On the contrary, in the evaluation of different concentrations of the chicory extract by the agar well diffusion method, *C. krusei*’s lack of growth in similar concentrations was greater than that of *C. glabrata*. As a result, the findings related to both the methods of agar well diffusion and MIC prevention concentration maximization proved that *C. krusei* sensitivity to the chicory extract is more compared with the sensitivity of *C. glabrata*.

**Conclusion:** Chicory extract has the benefits of low price, accessibility, and proper taste as compared with nystatin. It also has fewer side effects, and after a clinical test, it could be considered a proper candidate as an antifungal drug against infections caused by *C. krusei* and *C. glabrata*.

**Clinical significance:** The results obtained from this research have shown that chicory extract has antifungal features and is the best choice as an antifungal drug because of its low price, accessibility, and proper taste as compared with nystatin.

**Keywords:** Candida, Chicory extract, Fungal infection, Glabrata, Krusei.


**Source of support:** Nil

**Conflict of interest:** None

**INTRODUCTION**

Oral candidiasis is one of the most prevalent opportunistic fungal infections of the mouth made by a yeast fungal microorganism named candida; approximately 50% of the population carry this fungus naturally. Although one of the most common types of candidiasis infection is the albicans type, the widespread use and long-term application of antibiotics, corticosteroids, and immunity-suppressing drugs, as well as underlying diseases such as diabetes, acquired immunodeficiency syndrome (AIDS), etc., have caused the rate of fungal infections to increase; in particular, *C. glabrata* and *C. krusei* types have increased more as compared with the past. Furthermore, the infections due to *C. glabrata* have become more complex, especially in people with an immunity system deflection, because of its capability of producing biofilm and an increase of medical resistance to it. *Candida krusei* also occurs
especially in patients of neutropenic, hematologic malignancies and patients receiving bone marrow implants who are resistant toward systemic fungal treatments such as fluconazole and other antifungal treatments. The possibility of living is very low in patients who suffer from fungal infections. So today, the use of plants and plant compositions is regarded as a potential source for producing new drugs for treating illnesses such as fungal infections. Chicory extract is obtained from Cichorium intybus, which is one of the Asteraceae plants. It is one of the compositions that has been considered significantly in recent years in medical studies and treatments. In general, the origin of the chicory plant is in the Western regions, Central Asia, and North Africa. It is widespread in different regions of Iran, especially in the north of Iran, Azerbaijan, and the mountainous areas. This plant usually needs cool weather and a weak sun or some shade, and it cannot tolerate high temperatures. In the performed studies, the seed, root, and leaves of this plant have effects on the different microorganisms that were evaluated. For many years, this plant has been used for the treatment of AIDS, cancer, sexual incapability, insomnia, and spleen swelling. Some previous studies proved that C. intybus seeds have antioxidant and antimicrobial features.

The toxicology studies which were performed on the chicory extract proved the low toxicity of this plant; evaluations made on laboratory mice showed that the chicory root extract in the comestible format of about 70 to 100 mg/kg/day has no toxic effect. The lethal dose of this drug is more than 2,244 mg/kg (median lethal dose) in mice, so as a medical treatment, it has no damaging effect. In a study, the chicory extract’s anti-inflammation features were evaluated and the important role of chicory in osteoarthritis was clarified. The antibacterial features of this plant were also realized in different studies.

The antibacterial and antioxidation activities of the chicory plant have been proven before, but limited studies were performed on the antifungal features of this plant. In the present research, we have attempted to evaluate the antifungal effects of this extract on C. glabrata and C. krusei in a laboratory environment.

**MATERIALS AND METHODS**

**Preparing the Fungal Strains**

In this study, the target population was the standard strains of C. glabrata (BSM 11226) and C. krusei (BSM 70079) that were procured from the Tehran Pasteur Institute. These funguses were grown in a fungus dextrose environment at a maximum temperature of 30°C for 4 days. The sample volume was determined based on the CLSI.

**Chicory Extract Preparation Method**

To prepare the chicory extract, C. intybus leaves were dried according to the standard condition. After being dried completely, the leaves were grained; then, 1,000 gm of grained powder was drenched in a water and ethanol composition for 72 hours. Then, the solvent was passed through a Whatman paper filter; then, with the use of a Heidolph Rotary Evaporator made in Germany, the water was vaporized slowly, and a concentrated liquid was obtained. Then, the liquid was frozen in a freeze dryer (Christ alpha 1–4, Germany) and kept in a freezer below 20°C up to the time of use.

**Solvent Type Selection**

Based on some previous studies, alcoholic solvents, especially ethanol, has better results compared with acetone. In justification about the solvent type selection, we can declare that the ethanol solvents and methanol could dissolve the nonpolar compositions in the C. intybus plant and reveal the antimicrobial features of the plant more effectively. Usually, compositions such as flavonoids and flavonols, which are of the aromatic type, are dissolved in methanol properly. Under standard condition, it is dried, and after complete drying, 1,000 gm of grained powder is drenched in 70% water and ethanol solvents for 72 hours. Then, the solvent is passed through a Whatman paper-1 filter, and with the use of a Heidolph Rotary Evaporator (Germany), it is vaporized slowly, and the liquid gets concentrated. Then, the obtained dense liquid is frozen in a freeze dryer (Christ alpha 1–4, Germany) and kept in the freezer at a temperature below 20°C up to the time of application.

**MIC Determination by the Macrodilution Method**

To evaluate the effect of chicory extract on these funguses and for the determination of the MIC, the macrodilution method was used based on the CLSI protocol.

For this purpose, some amount of fungal colony was taken beside the flame under the ventilator in order to avoid pollution from the saprophytic fungi. We prepared a suspension in a testing pipe containing a sterile physiological serum whose concentration was equal to 0.5 standard of McFarland. Then, to prepare the chicory extract, we dissolved the prepared powder in the ethanol solvent, and based on the CLSI protocol, we prepared different concentrations of the C. intybus for each candida in a total of 20 pipes, and for each of candida, we chose two positive control pipes and two negative controls as the witness, making it a total of 24 pipes. After the suspension was prepared, to avoid vaporization, the testing pipe doors were closed, and we put them inside the incubator for 48 hours at a temperature of 35°C. After
48 hours had passed, we evaluated the transparency and opacity of each sample, and for assurance about the fungal growth or lack of growth in each pipe, we cultivated them. It should be noted that this process was performed with a 24-hour strain for both C. krusei and C. glabrata.

**Agar Well Diffusion Method**

The agar well diffusion method is widely used for the evaluation of antimicrobe activities in plants or extracts. This method is similar to the disk diffusion method and has simple benefits and low cost. The difference in the agar well diffusion method is that we can prepare adequate and proper space for understanding the material. Furthermore, all antimicrobial and antifungal materials will not fit on the disk. Some disks may be very expensive, or preparing some of them may be really difficult. In this study, to evaluate the chicory extract’s antifungal effect, the agar well diffusion was also used, but for the chicory extract solution, the results obtained from the macrodilution method were documented more. In this method, after preparing a 0.5 concentration of McFarland from the fungal dilution, we reduced the concentration to $1.5 \times 10^6$ CFU/mL, and 500 µL of the prepared suspension was transferred to a Mueller–Hinton agar environment and were cultivated by the sterile cotton swab in three directions. Then, wells with a diameter of 6 mm and an approximate distance of 2.5 cm were prepared for pipes numbered 2 to 9 according to the chicory extract concentration in the agar level. Then, 100 µL of each prepared chicory extract concentration was injected into each well (Figs 1 and 2); then, the plates were incubated for 24 hours at a temperature of 37°C. Finally, they were evaluated for lack of growth and this action was repeated twice.

![Figs 1A and B](image1.png)

**Figs 1A and B:** The sample of well diffusion method plates related to C. krusei method. (A) Wells number 2 to 5 orderly including concentrations of 1,600, 800, 400, and 200 µg/mL of chicory; (B) Wells number 6 to 9 orderly including concentrations of 100, 50, 25, and 12.5 µg/mL of chicory

![Figs 2A and B](image2.png)

**Figs 2A and B:** The sample of well diffusion method plates related to C. glabrata. (A) Wells number 2 to 5 orderly including concentrations of 1,600, 800, 400, and 200 µg/mL of chicory; (B) Wells number 5 to 10 orderly including concentrations of 100, 50, 25, 12.5, and 6.25 µg/mL of chicory
RESULTS

Based on the results obtained, we can say that \textit{C. krusei} in concentrations of 100 µg/mL has been stopped (Tables 1 and 2).

According to Tables 1 and 2, chicory extract is 50 µg/mL in \textit{C. krusei} and it is 100 µg/mL for \textit{C. glabrata} and this shows more sensitivity on \textit{C. krusei} compared with chicory extract and in comparison with \textit{C. glabrata}.

Comparison of the different concentrations of chicory extract by the well diffusion method on \textit{C. glabrata} and \textit{C. krusei} with two-way analysis proved that regardless of growth prevention of candida, the halo diameter of \textit{C. krusei} is greater than that of \textit{C. glabrata}; so, it demonstrates that \textit{C. krusei} has more microbial sensitivity to chicory extract, while \textit{C. glabrata} shows lower sensitivity (Table 3 and Graph 1). The results related to the MIC preventive maximum concentration (Table 1) also approve the above findings.

DISCUSSION

In this study, the antimicrobial effect of the chicory plant extract on two types of candidiasis—\textit{C. glabrata} and \textit{C. krusei}—was evaluated and the obtained results showed that the chicory extract is effective on \textit{C. glabrata} and \textit{C. krusei} according to a two-directional result, but \textit{C. krusei}'s sensitivity to the chicory extract is more than the sensitivity of \textit{C. glabrata}, as \textit{C. krusei}'s lack of growth halo diameter is more than that of \textit{C. glabrata}, and the \textit{C. intybus} growth preventive lowest concentration was lesser for \textit{C. krusei} compared with \textit{C. glabrata}.

The widespread and long-run application of antibiotics, corticosteroids, and immunity-suppressing drugs, as well as basic illnesses such as diabetes and AIDS, etc., has caused an increase in the rate of fungal infection, especially \textit{C. glabrata} and \textit{C. krusei}, as compared with the past.\textsuperscript{3} On the contrary, in addition to the side effects of common antifungal drugs, the previous studies have proved the defeat of these antifungal drugs on different clinical infections of candidiasis and their medical resistance as the thiazole (like fluconazole) in long-run usage, especially in patients with deficiency of immunity system in two types of mentioned candidas (\textit{C. glabrata} and \textit{C. krusei}).\textsuperscript{1,2,23}

In recent years, many studies have been performed; these evaluations have shown that some plants have the same effects as medical drugs or even more than them. One such plant is from the Asteraceae family and it is one of the compositions that has been considered in many studies and treatments in the medical field recently.\textsuperscript{6} Previous studies have shown that chicory extract has antibacterial, antimalarial, anti-inflammatory, painkiller, anticancer, antidiabetic, digestion, and liver protective effects.

\begin{table}[h!]
\centering
\caption{The average and standard deviation for halo diameter lack of growth based on the millimeter for chicory extract fungal two-directional analysis of chicory extract in different concentrations}
\begin{tabular}{l|c|c|c|c|c|c|c|c|c}
\hline
\textbf{Candida type} & \textbf{Chicory concentration (µg/mL)} & 1,600 & 800 & 400 & 200 & 100 & 50 & 25 & 12.5 & 6.25 \\
\hline
\textit{Candida glabrata} & 24 ± 0.08 & 20 & 14 ± 0.14 & 13 ± 0.98 & 12 ± 0.42 & 10 ± 0.84 & 9 & 8 ± 0.7 & 8 ± 0.8 \\
\hline
\textit{Candida krusei} & 14 ± 0.8 & 13 ± 0.98 & 11 ± 0.84 & 11 & 11 ± 0.8 & 10 & 9 ± 0.14 & 8 ± 0.98 & 8 ± 1.41 \\
\hline
\end{tabular}

Data are reported as mean ± criterion deviation and the obtained results are outcome of two-time test repetition.
\end{table}

\begin{table}[h!]
\centering
\caption{The results of growth in two types of evaluated candida based on the growth condition in different chicory concentrations}
\begin{tabular}{l|cccccccc}
\hline
\textbf{Candida type} & \textbf{Candida growth condition in different chicory concentrations (µg/mL)} & 3.12 & 6.25 & 12.5 & 25 & 50 & 100 & 200 & 400 & 800 & 1,600 \\
\hline
\textit{Candida glabrata} & + & + & + & + & − & − & − & − & − & − & − \\
\hline
\textit{Candida krusei} & + & + & + & + & + & − & − & − & − & − & − \\
\hline
\end{tabular}

+: Microorganism growth; −: Lack of growth in microorganism
\end{table}

\begin{table}[h!]
\centering
\caption{The antifungal sensitivity points evaluation in \textit{C. glabrata} and \textit{C. krusei} types in well diffusion method}
\begin{tabular}{l|c|c|c}
\hline
\textbf{Well diffusion} & \textbf{Sensitive (mm)} & \textbf{Sensitive depending on the dose (mm)} & \textbf{Resistance (mm)} \\
\hline
\textit{Krusei candida} & 24 (>20) & 10–20 & <10 \\
\hline
\textit{Glabrata candida} & 14 (>11) & 11 & <10 \\
\hline
\end{tabular}
\end{table}

Graph 1: The comparison of chicory extract’s antibacterial effect against \textit{C. krusei} and \textit{C. glabrata}
effects. Some studies evaluated the antimicrobial effect of the chicory extract on the positive pathogen bacteria such as Bacillus subtilis, Staphylococcus aureus, and Micrococcus luteus and the Gram-negative pathogenic bacteria such as Escherichia coli and Salmonella typhi. The results of these studies proved that the chicory root extract has a stronger preventive effect on the Gram-positive bacteria compared with the Gram-negative types. Also, the chicory root extract hexane ethyl acetate has a stronger antibacterial effect compared with the chloroform extract and the watery extract of the chicory root.

The coffee–chicory’s antibacterial effect on Streptococcus mutans that exists in the dental plaque has been evaluated, and the result of this study has shown that chicory has an antibacterial effect and coffee has an antiadhesive effect, leading to a reduction of bacteria for connecting to the surfaces and shaping biofilms.

Furthermore, the antiviral features of the chicory extract on the adenovirus and subcategories of the herpes virus are specified. In contrast to the previous study results, Ghaderi et al’s study in 2012 showed that the chicory extract does not have an antibacterial effect on the Gram-positive Streptococcus aureus, Streptococcus pyogenes, and Enterococci bacteria. The reason for this difference could be due to the following issues:

- The part of the chicory plant was used to prepare the extract: The previous study results have shown that different parts of the chicory plant such as the leaf, seed, root, and crust have antibacterial features, but the antibacterial features of the root and leaves in this plant are more than other parts. Studies have often used either the chicory root or the leaves to prepare the chicory extract. The root and leaf of the chicory plant have differences in their secondary components and these differences could be a reason for the differences in the extract’s antibacterial effects and the results obtained from previous studies. In this study, the plant leaf was used to prepare the chicory extract.

- The chicory plant growing region type: This plant usually needs cool weather or sunny weather with some shade and it does not tolerate intensive heat. The secondary components of the various parts of the chicory plant can be different depending on the region that the plant was grown and even the time of the plant’s harvest.

- The type of solvent used for preparing the chicory extract: The type of solvent used could have an effect on the extract, causing different features to emerge. Based on the results obtained from previous studies, alcoholic solvents such as ethanol are more suitable than other solvents to prepare the chicory extract and it presents the chicory extract’s antimicrobial features better than others.

- Different concentrations based on the applied methods for the evaluation of the antimicrobial features of the chicory extract: There are different protocols for evaluating the antimicrobial features of drugs and extracts. The suggested method is different for each of them as per the CLSI protocol. These include different methods that are suggested according to the microorganisms or the drugs that are being evaluated, such as the agar well diffusion method and the determination of MIC through macrodilution.

- The studying microorganism type and differences in the rate of their medical resistance: For example, there are different types of candidias and the medical resistance of common candidas is different in various conditions.

Some of the previous studies have evaluated the chicory root extract’s antifungal features and the results of these studies have shown that this extract has antifungal effects on Aspergillus niger, Saccharomyces cerevisiae, Pellicularia sasakii, and Alternaria kikuchiana fungi and that the antifungal effect of the chicory hexane extract on A. niger is approximately similar to that of S. cerevisiae. According to the performed studies, the secondary components in the chicory extract, especially in the sesquiterpene lactones such as lactucin and lactycopirin, are responsible for the plant’s antifungal effect.

One of the most important studies is the Mares et al study that evaluated the chicory extract’s effect on the phytopathogenic fungus (plant infecting fungus) and the dermatophyte (skin infecting fungus). The results of this study showed that the effect of this extract in preventing the dermatophyte fungus is more than the phytopathogenic fungus, and the main part of the chicory extract’s antifungal activity is due to the presence of two main sesquiterpene lactones including deoxylactucin and β,13-dihydrolactucin.

The results of previous studies proved that the acetone and ethyl acetate chicory extract in composition with the antibiotics could show synergism effects.

Similar to the previous studies, the present research shows the antimicrobial and antifungal effects of the chicory extract; but in relation to the chicory extract’s antifungal effects, previous studies were performed mainly on noncandidiasis fungi. Hence, in this study, we evaluated the extract’s antifungal effects on C. glabrata and C. krusei as these are the types with more prevalence and medical resistance compared with the albicans type, especially in patients who suffer from immunosuppressive diseases. In the present research, to evaluate the extract’s antifungal effects, the two methods of agar well diffusion and MIC were used based on the protocol determined by the CLSI. As per the CLSI, the results of both tests are the same and show a higher sensitivity of C. krusei toward the chicory extract compared with C. glabrata.
According to the side effects, low cost of chicory extract, and the considerable effect of this extract on dermatophyte funguses, C. kruzei and C. glabrata, and by regarding up to this point that most of the candidiasis infections involve the skin and oral mucosa, it is hoped that such extract will be substituted for chemical drugs in fungal infection treatment in future years. Of course, to generalize this concept, more widespread studies as clinical trials are inevitable. Furthermore, it is advised that the clinical trials are planned to evaluate the chicory extract antifungal effects in oral fungal infection treatments.

**CONCLUSION**

The results obtained from the present research have shown that the chicory extract has antifungal features. The results obtained from both the methods of agar well diffusion and the MIC as the macrodilution with the maximum preventive concentration proved that C. kruzei has more microbial sensitivity compared with C. glabrata, regardless of microbial sensitivity of both funguses to the chicory extract.

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