

The antifungal effect of milk thistle (*Silybum marianum*) extract: An *in vitro* study using the agar dilution method

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Article info

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Karbala, 56001, Iraq**Citation:** Hameed, M. A. K. (2025). The antifungal effect of milk thistle (*Silybum marianum*) extract: An *in vitro* study using the agar dilution method. *Scientific Progress & Innovations*, 29(1), 117–121. doi: 10.31210/spi2026.29.01.19

Silybum marianum, commonly known as milk thistle, is a widely recognized plant for its medicinal properties, especially its hepatoprotective and antioxidant activities, which have been mainly attributed to silymarin. Recently, an increasing number of studies have reported the antimicrobial, including antifungal, activity of milk thistle. This study was designed to examine the antifungal activity of milk thistle extract against two common fungal pathogens, *Candida albicans* and *Aspergillus niger*. The crude extract of milk thistle was prepared in three different concentrations (25 %, 50 %, and 100 %), which were directly incorporated into Sabouraud Dextrose Agar (SDA) using the agar dilution method. The results clearly indicated a concentration-dependent inhibition of fungal growth. At a 25 % concentration, moderate inhibition of fungal growth was observed for *Candida albicans* (35.2±3.1 %), while *Aspergillus niger* showed minimal inhibition (12.5±2.4 %). At a 50 % concentration, the inhibition increased significantly for both fungal species, with *Candida albicans* showing 62.8±4.5 % inhibition and *Aspergillus niger* showing 38.7±3.6 % inhibition. At a 100 % concentration, the maximum growth inhibition was achieved, reaching 85.4±5.2 % for *Candida albicans* and 64.3 ± 4.8% for *Aspergillus niger*. Additionally, disk diffusion susceptibility testing confirmed that both the 50 % and 100% extract solutions displayed clear zones of inhibition, categorizing both isolates as sensitive. These results strongly indicate that *Silybum marianum* possesses substantial inherent antifungal properties. The ability of milk thistle to act as a natural antifungal agent supports its potential application in fields beyond hepatoprotection. Further studies are needed to identify the active antifungal compounds from the crude extract, determine their spectrum of activity against a broader range of fungal pathogens, and assess their possible therapeutic or preservative applications in medicine and agriculture.

Keywords: *Silybum marianum*, milk thistle, antifungal activity, agar dilution method, *Candida albicans*, *Aspergillus niger*.

Протигрибковий ефект екстракту розторопші плямистої (*Silybum marianum*): дослідження *in vitro* з використанням методу серійних розведень в агарі

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Silybum marianum, відома як розторопша плямиста, є широко визнаною рослиною завдяки своїм лікувальним властивостям, зокрема гепатопротекторній та антиоксидантній активності, які переважно пов'язують із силімарином. Останнім часом у дедалі більшій кількості досліджень повідомляється про антимікробну, зокрема протигрибкову, дію розторопші. Враховуючи вищевказане, це дослідження здійснено з метою встановлення протигрибкової активності екстракту розторопші проти двох поширених грибкових мікроорганізмів – *Candida albicans* та *Aspergillus niger*. Екстракт розторопші готували у трьох різних концентраціях (25 %, 50 % та 100 %), які безпосередньо вносили в середовище агару Сабуро (SDA) методом серійних розведень в агарі. Результати чітко вказали на концентраційно-залежне пригнічення росту грибів обох видів. Зокрема, за концентрації 25 % спостерігалось помірне пригнічення росту грибів для *C. albicans* (35,2±3,1 %), тоді як *A. niger* виявив мінімальне пригнічення (12,5±2,4 %). За концентрації 50 % рівень пригнічення значно зріс для обох видів грибів. У тесті з *C. albicans* зафіксовано, що затримка росту становила 62,8±4,5 %, а у *A. niger* – 38,7±3,6 %. За 100 % концентрації було досягнуто максимального пригнічення росту, яке становило 85,4±5,2 % для *C. albicans* та 64,3±4,8 % для *A. niger*. Крім того, тестування чутливості методом дифузії на дисках підтвердило, що розчини екстракту як 50 %, так і 100 % концентрації утворювали чіткі зони затримки росту, що дозволило класифікувати обидва ізоляти як чутливі. Отримані результати переконливо свідчать про те, що *S. marianum* має виражені протигрибкові властивості. Здатність розторопші діяти як природний протигрибковий засіб обґрунтовує її потенційне застосування в сферах, що виходять за межі гепатопротекції. Необхідні подальші дослідження для ідентифікації точних протигрибкових сполук із екстракту, визначення спектра їхньої активності проти ширшого кола грибкових патогенів, а також оцінки можливостей їхнього терапевтичного чи застосування у гуманній і ветеринарній медицині та сільському господарстві.

Ключові слова: *Silybum marianum*, розторопша плямиста, протигрибкова активність, метод розведення в агарі, *Candida albicans*, *Aspergillus niger*.**Бібліографічний опис для цитування:** Хамед М. А. К. Протигрибковий ефект екстракту розторопші плямистої (*Silybum marianum*): дослідження *in vitro* з використанням методу серійних розведень в агарі. *Scientific Progress & Innovations*. 2026. № 29 (1). С. 117–121.

Introduction

Fungal infections represent an important and growing public health challenge worldwide, especially among immunocompromised individuals, such as patients with HIV/AIDS, cancer patients undergoing chemotherapy, and organ transplant recipients [1]. *Candida* and *Aspergillus* species are responsible for a wide range of infections. These range from superficial conditions, such as oral thrush and superficial candidiasis, to life-threatening systemic infections, such as invasive aspergillosis and candidemia [2]. The global burden of fungal infections is exacerbated by the increasing spread of antifungal resistance, which has become a major concern in clinical settings. For example, the emergence of azole-resistant *Candida* species and multi-drug-resistant *Aspergillus* strains has severely limited the efficacy of traditional antifungal therapies [3].

Traditional antifungal medications, such as azoles (e.g., fluconazole), polyenes (e.g., amphotericin B), and echinocandins (e.g., caspofungin), remain the cornerstone of treatment for fungal infections. However, these therapies are often associated with significant limitations, including toxicity, high costs, and the rapid development of resistance. For instance, amphotericin B, although highly effective against a wide range of fungi, is notorious for its nephrotoxic side effects. Similarly, the extensive use of fluconazole has led to the emergence of resistant *Candida* strains, particularly *Candida glabrata* and *Candida krusei*. These challenges underscore an urgent need for alternative antifungal agents that are effective, safe, and less prone to inducing resistance [4].

In recent years, there has been a resurgence of interest in natural products as potential sources of antimicrobial agents. Plants, in particular, are a rich source of bioactive compounds with diverse medicinal properties, including antimicrobial, antioxidant, and anti-inflammatory effects. Among these, milk thistle (*Silybum marianum*), native to the Mediterranean region, has been extensively studied for its hepatoprotective and antioxidant properties, which are primarily attributed to its main active complex, silymarin [5]. Silymarin is a complex mixture of flavonolignans, including silybin, silychristin, and silydianin, which have been shown to demonstrate a wide range of biological activities [6].

Emerging evidence indicates that milk thistle may also possess significant antimicrobial properties, including antifungal activity. Initial studies have shown that silymarin and its components can inhibit the growth of various fungal pathogens, including *Candida albicans* and *Aspergillus niger*, by disrupting fungal cell membranes and inhibiting biofilm formation. These findings are particularly promising given the role of biofilms in fungal virulence and resistance to traditional agents [7]. In addition, the antioxidant properties of milk thistle may help mitigate the oxidative stress associated with fungal infections, thereby potentially enhancing the host's immune response and promoting tissue repair [8].

Despite these promising findings, the antifungal capacity of milk thistle remains insufficiently characterized, particularly regarding its mechanism of

action and efficacy at different concentrations. Most studies to date have focused on the agar well diffusion method, which may not fully capture the volatile or lipophilic antifungal activity of plant extracts [9]. In contrast, the agar dilution (mixing) method, which involves incorporating extracts directly into the growth medium, provides a more comprehensive evaluation of antifungal effects by ensuring direct and continuous interaction between fungal cells and bioactive compounds [10]. By incorporating the extracts directly into the medium, we aimed to simulate conditions that align closely with potential real-world applications, such as topical formulations or antifungal coatings. The findings from this study may provide valuable insights into the potential of milk thistle as a natural antifungal agent and contribute to the development of alternative therapies for fungal infections.

The aim of the study

The purpose of this study was to evaluate the in vitro antifungal activity of milk thistle extracts at 25 %, 50 %, and 100 % concentrations using the agar dilution method in Sabouraud Dextrose Agar (SDA).

Materials and methods

Preparation of Milk Thistle Extract

Milk thistle seeds were obtained and ground into a fine powder. The extract was prepared using a solvent extraction method with ethanol (70 %) as a solvent. The mixture was filtered, and the solvent was evaporated under low pressure to get a concentrated extract. The extract was then diluted to create 25 %, 50 % and 100 % (weight/volume) concentrations.

Preparation of Sabouraud Dextrose Agar (SDA) with Milk Thistle Extract

Sabouraud dextrose agar (SDA) was prepared according to the standard protocol. The milk thistle extract was included in the concentration of 25 %, 50 % and 100 % (weight/volume) before autoclaving. The mixture was well mixed to ensure equal distribution of extracts. The control plates were produced without the addition of milk thistle extract.

Fungal Strains and Culture Conditions

The antifungal activity of milk thistle extract was tested against *Candida albicans* and *Aspergillus niger*, two common fungal pathogens. In order to ensure viability and purity, the strains on SDA plates without milk thistle extract were cultivated. For the experiment, the fungal suspensions were prepared in sterile saline solution and adjusted to a turbidity of 0.5 McFarland standard ($\sim 1.5 \times 10^6$ CFU/ml) [8].

Inoculation and Incubation

Each concentration of milk thistle extract (25 %, 50 %, and 100 %) was tested in triplicate. Fungal suspensions (10 μ L) were spot-inoculated onto the surface of the SDA plates containing milk thistle extract. Control plates were inoculated in the same manner. The

plates were incubated at 25°C for 48–72 hours, and fungal growth was assessed visually and microscopically.

Assessment of Antifungal Activity

Fungal growth inhibition was evaluated by measuring the diameter of the colonies and comparing them to the control plates. The percentage of inhibition was calculated using the formula:

$$\text{Inhibition (\%)} = (1 - \text{Colony diameter on treated agar} / \text{Colony diameter on control agar}) \times 100$$

Antifungal sensitivity test (Application of Discs)

Antifungal discs were placed on the surface of the inoculated Mueller Hinton agar plate. Each disc was gently pressed into the agar using sterile, flamed, and cooled forceps to ensure full contact with the agar surface. The plates were then inverted and incubated at 37°C for 48 hours. The results obtained from the disc diffusion method were interpreted and categorized as susceptible, intermediate, or resistant according to the criteria established by the Clinical and Laboratory Standards Institute [11].

Statistical Analysis

The data were analyzed using one-way ANOVA, and the results were expressed as mean ± standard deviation (SD). A p-value of <0.05 was considered statistically significant.

Results and discussion

The results demonstrated a concentration-dependent antifungal effect of milk thistle extract. At 25 % concentration, moderate inhibition of fungal growth was observed for *Candida albicans* (inhibition = 35.2±3.1 %), while *Aspergillus niger* showed minimal inhibition (inhibition = 12.5±2.4 %). At 50 % concentration, the inhibition increased significantly for both fungal species, with *Candida albicans* showing 62.8±4.5 % inhibition and *Aspergillus niger* showing 38.7±3.6 % inhibition. At 100 % concentration, the maximum inhibition was observed, with *Candida albicans* showing 85.4±5.2 % inhibition and *Aspergillus niger* showing 64.3±4.8 % inhibition. The control plates showed no inhibition, confirming that the antifungal effect was due to the milk thistle extract (Figure 1a, b, c).

Results of Antifungal Susceptibility Testing by Disk Diffusion Method

The isolates of *Candida albicans* and *Aspergillus niger* were tested using the disk diffusion technique to determine the susceptibility to 4 different antifungal drugs and milk thistle extract (50 % and 100 %). The results are presented in Table 1.

The disk diffusion testing revealed varying susceptibility profiles of the tested fungal isolates to traditional drugs and milk thistle extract. *Candida albicans* demonstrated sensitivity to fluconazole, amphotericin B, and clotrimazole, but was found to be resistant to voriconazole.

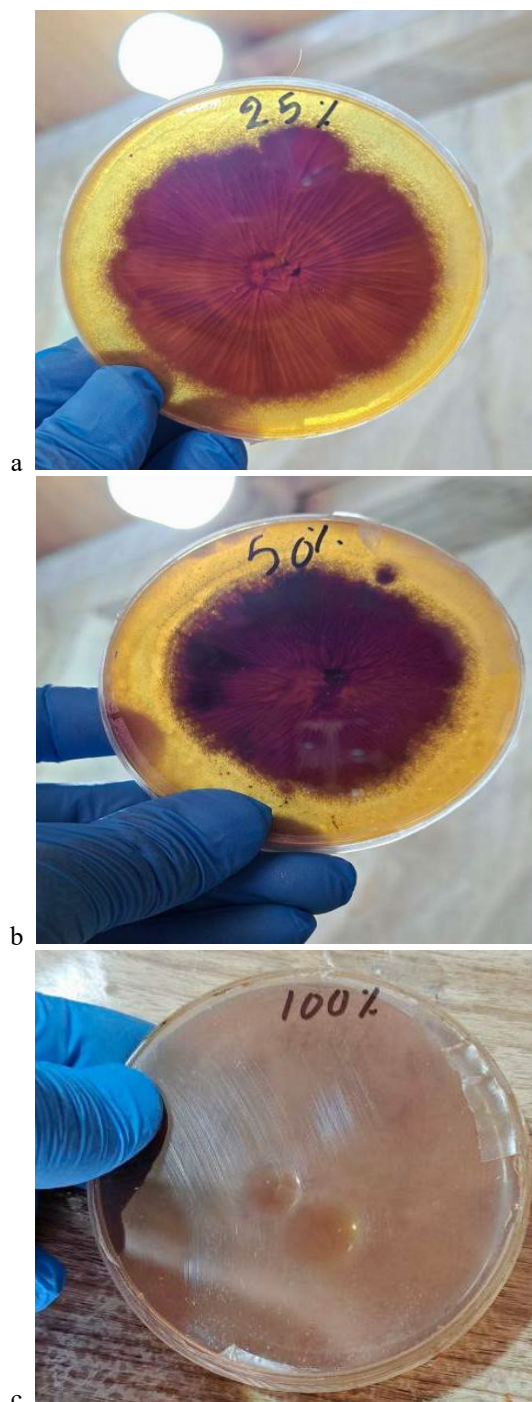


Figure 1. (a, b, c). Antifungal activity of *Silybum marianum* extract at 25 %, 50 %, and 100 % concentrations in Sabouraud Dextrose Agar (SDA)

Table 1

The results of the disk diffusion method

Antifungal disc	<i>Candida albicans</i>	<i>Aspergillus niger</i>
Fluconazole (25 µg)	S	R
Voriconazole (1 µg)	R	S
Amphotericin B (10 µg)	S	R
Clotrimazole (10 µg)	S	R
Milk thistle extract 50%	S	S
Milk thistle extract 100%	S	S

Note: S – Susceptible (Sensitive); R – Resistant.

In contrast, *Aspergillus niger* showed resistance to fluconazole, amphotericin B, and clotrimazole, while remaining sensitive to voriconazole. Notably, both milk thistle extract concentrations (50 % and 100 %) demonstrated effective antifungal activity, with both *Candida albicans* and *Aspergillus niger* isolates categorized as sensitive to the plant extract. These findings indicate that milk thistle extract exhibits a broad-spectrum antifungal effect, capable of inhibiting strains that show resistance to conventional antifungal agents.

The findings of this study indicate that milk thistle extract exhibits significant antifungal activity when incorporated directly into Sabouraud Dextrose Agar, with higher concentrations showing greater inhibition of fungal growth. The observed concentration-dependent inhibition suggests that the active compounds in milk thistle, such as silymarin, may disrupt fungal cell membranes or interfere with fungal metabolic processes. Previous studies have reported that silymarin can inhibit ergosterol synthesis, a key component of fungal cell membranes, which aligns with our findings [12–14].

The differences observed between *Candida albicans* and *Aspergillus niger* may be due to the variation in the structure of the fungal cell wall and the sensitivity of these species to the active compounds in milk thistle. While *Candida albicans* showed higher sensitivity, the inhibition of *Aspergillus niger* at high concentrations suggests that milk thistle can have a wide-spectrum antifungal capacity [15, 16].

The agar dilution method used in this study allowed for direct interaction between fungal cells and milk thistle extract, giving a more accurate evaluation of its antifungal activity than the agar well diffusion method. This approach mimics potential real-world applications, such as incorporating milk thistle into topical formulations or antifungal coatings. These results are in line with previous studies that highlight the antimicrobial properties of milk thistle [17, 18].

The *Candida albicans* isolate was tested for its susceptibility to a panel of standard antifungal agents and *Silybum marianum* extracts; the isolate was found to be susceptible to the polyene agent amphotericin B and the topical azole clotrimazole, as evidenced by the zones of inhibition. However, resistance to fluconazole, a first-line triazole, was also observed, which indicates the expression of acquired resistance. Acquired resistance among *C. albicans* has increasingly been reported, which often results from the upregulation of efflux pumps, for example, CDR1, MDR1, or mutations in the ERG11 gene encoding the azole target enzyme [19]. However, the response of the pathogen to voriconazole, another triazole, implies that the resistance mechanism expressed might be specific to fluconazole, since even against fluconazole-resistant strains, voriconazole has shown efficacy due to its higher affinity for the target enzyme [20]. Most notably, both the 50 % and 100 % milk thistle extract solutions displayed clear evidence of antifungal activity, revealing zones of inhibition. As such, these study results echo more recent investigations which have shown the anticandidal efficacy of silymarin, milk thistle flavonolignans, and their related compounds. For example, a study performed by researchers [21] described

how *Silybum marianum* seed extract possessed a marked ability to inhibit the growth of clinical *C. albicans* isolates. The mechanisms of effectiveness relied on disrupting the *C. albicans* membranes, alongside inhibiting virulence factors such as biofilm formation and hyphal morphogenesis. Clearly, this tested antifungal activity implies that compounds present in milk thistle have a potential means to bypass common mechanisms of azole resistance, thus necessitating further investigations of the synergistic value of this compound alongside traditional drug treatments.

A specific antifungal susceptibility pattern was recognizable in the case of the *Aspergillus niger* isolate. Intrinsic resistance of the organism to fluconazole was verified because it failed to produce a zone of inhibition. This species of *Aspergillus* possesses universal resistance due to its inherent insensitivity to the aforementioned antifungal drug. This is because of the inherent insensitivity of the cytochrome P450 14 α -demethylase enzyme of *Aspergillus* species to fluconazole [22]. In contrast to the usual susceptibility pattern of the majority of *Aspergillus* species, the results revealed the resistance of the *Aspergillus* species involved in the study to both amphotericin B and voriconazole [23]. The resistance of the pathogen to amphotericin B is significant, and this could be an actual display of resistance, perhaps related to altered ergosterol membrane content or increased catalase production counteracting the oxidative effects of the drug. It is interesting that the pathogen was demonstrated to be susceptible to the topical azole compound clotrimazole, although this agent would be of no use in treating systemic aspergillosis. Most important, however, is the fact that both concentrations of the milk thistle compound were demonstrated to be effective in inhibiting the pathogen *A. niger*. This is a very significant display of effectiveness, supported by scientific literature. For instance, it was shown that silibinin [24], a component of the active complex of silymarin, demonstrated a significant ability to inhibit *Aspergillus* by producing apoptotic-like cell death and disrupting the structure of the pathogen's cell wall.

Conclusions

This experiment proved that milk thistle extract has a considerable antifungal effect on *Candida albicans* and *Aspergillus niger* when added directly to Sabouraud Dextrose Agar at concentrations of 25 %, 50 %, and 100 %. The results indicate that milk thistle may serve as a valuable source of natural antifungal agents, especially where standard treatment has been constrained by resistance or toxicity. Furthermore, *S. marianum* extract demonstrates significant potential as a natural antifungal compound against both yeasts and molds of clinicopathological relevance, including drug-resistant isolates. These findings strongly support the notion that constituents of milk thistle offer great potential for the management of drug-resistant pathogens, either as monotherapeutic agents and/or in a synergistic capacity to re-establish sensitivity to conventional antifungal agents.

Future research should focus on the isolation of the exact bioactive compounds responsible for these

antifungal effects, as well as the evaluation of their prospects regarding clinical usage.

DECLARATIONS

Ethical Statement

Not applicable. This study was conducted entirely in vitro using fungal isolates and did not involve any live animal subjects or human tissues.

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Conflict of interest

The authors state that there is no conflict of interest.

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Declaration of AI and AI-assisted technologies

The author declare that no artificial intelligence or AI-assisted technologies were used in the preparation of this manuscript.

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