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## Original Research Article

### Antifungal Activity of Ethanol Extracts of *Ganoderma resinaceum* against Vaginal Fungal Isolates

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#### ABSTRACT

Reishi mushrooms, particularly *Ganoderma resinaceum*, contain bioactive compounds with antifungal, antibacterial, and antiviral properties. Traditionally used for longevity, they show promise in treating chronic ailments and vaginal infections. The present study investigated the antifungal activity of *Ganoderma resinaceum* Ethanol extract against vaginal fungal pathogens commonly associated with vaginitis. Vagina samples were obtained from 213 female patients for fungal isolation and identification. Ethanol extract of *G. resinaceum* was prepared and fractionated by Gas Chromatography-Mass Spectrometry analysis. The antifungal effects of varying concentrations (2–250 mg/mL) of the extract were evaluated. Also, the effect of the extract was assessed on fungal sporulation. Fungal isolates, primarily *Candida albicans* (41.38%), *Candida glabrata* (34.48%), and *Candida pichia* (24.14), were obtained from the vaginal samples. The results revealed that the Ethanol extract of *G. resinaceum* exhibited dose-dependent antifungal activity, with the highest concentration (250 mg/mL) showing significant ( $p \leq 0.05$ ) inhibition of fungal growth. Inhibition zones for *C. albicans*, *C. glabrata*, and *C. pichia* were  $23.33 \pm 0.33$ ,  $22.00 \pm 0.00$ , and  $30.00 \pm 0.00$  mm, respectively. Additionally, the extract significantly reduced fungal sporulation, particularly for *C. pichia*, which showed the greatest reduction in spore count. Gas chromatography-mass spectrometry analysis identified active compounds such as oleic acid and  $\beta$ -sitosterol, likely contributing to the observed antifungal activity. The study revealed that *G. resinaceum* as a potential, less toxic alternative for treating vaginal fungal infections, especially those caused by *Candida* species. However, further clinical trials are required to validate its effectiveness and establish proper treatment guidelines.

**Keywords:** *Ganoderma resinaceum*, antifungal activity, *Candida* species, vaginal infections, Ethanol extract, fungal sporulation, GC-MS analysis.

#### Introduction

The reishi mushrooms contain diverse bioactive compounds that may have several health benefits. Mushrooms have become a potential source of natural health cures due to their wide range of bioactive compounds.<sup>1</sup> Carbohydrates, proteins, fatty acids, vitamins, minerals, and phenolic compounds are among the numerous essential components found in reishi mushrooms. An increasing number of individuals are attracted to natural products due to their ability to provide health benefits with fewer adverse reactions compared to conventional drugs.<sup>1</sup> *Ganoderma*, often referred to as reishi, is a medicinal fungus that is currently gaining popularity. It has been revered in Asian cultures for over two millennia due to its medicinal properties, which include increasing longevity and combating several ailments. The benefits of this mushroom are attributed to its unique composition, which is high in polysaccharides, triterpenoids, and polyphenols. The bioactive compounds contained in the mushroom have shown antifungal, antibacterial, and antiviral activities.<sup>2</sup>

Traditionally, *Ganoderma* has been administered independently. In contrast, a new study indicates that chronic illnesses, such as bronchitis and hepatitis could potentially be improved by using a combination of *Ganoderma* and conventional treatment. Meanwhile, the specific mechanisms by which it eliminates viruses and bacteria remain unknown. Research has shown that it can impede the proliferation of many bacteria in laboratory environments.<sup>3</sup> *Ganoderma resinaceum*, a distinct species, has demonstrated potential in enhancing the functionality of essential organs, such as the stomach, spleen, kidneys, and lungs.<sup>4</sup> Cardiovascular and liver diseases are two ailments that could benefit from its anti-inflammatory and antioxidant properties.<sup>5</sup> Vaginitis is a medical condition characterized by abnormal vaginal discharge, soreness, itching, and unpleasant odour, or a burning feeling. A considerable proportion of women will develop vaginitis at least once in their lifetime.<sup>6,7</sup>

The present study aimed to investigate the antifungal activity of ethanol extract of *Ganoderma resinaceum* against selected fungal species associated with vaginal infections.

#### Materials and Methods

##### Sample collection

The study was conducted at Al-Kadhimiyah Teaching Hospital in Baghdad from November 2021 to April 2022 and included 213 female patients. The age range of these individuals was 21–60 years. Vagina samples were collected from the participants using sterile cotton swabs and placed in containers with a preservative gel to maintain the viability of the fungi for an extended period. Subsequently, the samples were subjected to laboratory analysis.

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**Ethics statement**

Ethical approval was obtained from the College of Science, University of Baghdad, Baghdad, Iraq (Ref. No.: CSEC/0225/0035). All participants were permitted to provide the researchers with specimens. Informed consent was obtained from all participants according to the Declaration of Helsinki.

**Isolation and identification of fungal pathogens from the samples**

Swabs suggestive of fungal development were inoculated on Sabouraud Dextrose Agar (SDA). This specialized media provided an ideal environment for fungal growth. The cultures were incubated for 2-5 days at 37°C, an optimal temperature for fungal development. Microscopic examination was conducted on the cultures after detecting fungal growth on the SDA plates for diagnosis. Multiple fungal isolates were collected from different regions of the fungal growth on the plate. The samples were carefully placed on a clean glass slide. Lactophenol cotton blue was used to stain the samples. This pigment makes fungal spores more visible, which is important for identification. The slide was gently heated over a spirit lamp to improve the staining process and remove any air bubbles that may interfere with observation. Any excess stain was removed using tissue paper to ensure clear visibility of the fungal structures. The specific fungus in the sample was identified by combining observed colony morphology on the SDA with the microscopic characteristics of the stained spores.

**Preparation of *Ganoderma resinaceum* Ethanol extract**

The thimble was filled with 15 g *Ganoderma resinaceum* powder before being inserted in the Soxhlet chamber. Five hundred milliliters (500 mL) of ethanol were poured into a round-bottom flask and assembled for the Soxhlet extractor. The extraction was performed for 5-6 hours before beginning the distillation process. After completing the extraction process, the solvent and extractor were placed under reduced pressure (rotary evaporator) to obtain the dried Ethanol extracts.<sup>8</sup>

**Gas chromatography-mass spectrometry of *Ganoderma resinaceum* Ethanol extract**

The *Ganoderma resinaceum* Ethanol extract was fractionated using Gas Chromatography-Mass Spectrometry (GC-MS) analysis according to the method described by Taofiq *et al.* (2019).<sup>9</sup>

**Antimicrobial sensitivity testing**

The antifungal activity of various concentrations (2, 4, 8, 16, 31.25, 62.5, 125, and 250 mg/mL) of the *Ganoderma resinaceum* Ethanol extract was tested against the vaginal fungal isolates using the agar well diffusion method.<sup>10</sup> Each of the vaginal fungal isolates was prepared as a suspension and standardized using the colony suspension method by comparing it with 0.5 McFarland standards to give a resultant concentration of  $1.0 \times 10^6$  cfu/mL. The modified Kirby-Bauer diffusion method was used to investigate the isolates' sensitivity to the various *Ganoderma resinaceum* Ethanol extracts by swabbing the SDA plates with the saline suspension of each vaginal fungal isolate. A 6-mm heat-sterilized cork borer was used to bore wells into the agar medium. Each extract was prepared with care to prevent solution spilling onto the agar surface, and 100  $\mu$ L of each concentration (2, 4, 8, 16, 31.25, 62.5, 125, and 250 mg/mL) were dispensed into the wells. The cultures were let to stand for at least 30 minutes and then incubated for 24 hours at 37°C. The experiments were replicated thrice. After a 24-hour incubation period, the plates were observed and recorded for zones of inhibition.

**Sporulation assay on the *Ganoderma resinaceum* Ethanol extract**

The effect of the *Ganoderma resinaceum* Ethanol extract on sporulation of the test pathogens were examined using the method described by Cai *et al.* (2021).<sup>11</sup> A 5 mm agar plug of the vaginal fungal isolate was obtained from the margin of an actively growing colony and placed at the center of each SDA plate. The Ethanol extract of *Ganoderma resinaceum* was prepared into various concentrations (2, 4, 8, 16, 31.25, 62.5, 125, and 250 mg/mL). An aliquot (1 mL) of each extract

concentration was spread evenly across the fungal cultures. The plates were incubated at 28°C ± 2°C for 7 days in a dark environment. After the incubation period, each plate was flooded with 10 mL of sterile distilled water containing 0.1% Tween 80 to extract the spores. A sterile L-shaped glass spreader was used to gently scrape the surface of the plate to remove the spores. To get rid of mycelia particles, the spore suspension was filtered through sterile gauze. A hemocytometer was used to count the spores under a light microscope with a 40× magnification. Sporulation was expressed as spores per milliliter of suspension. All the experiments were replicated thrice to ensure reliability and reproducibility of results.

**Statistical analysis**

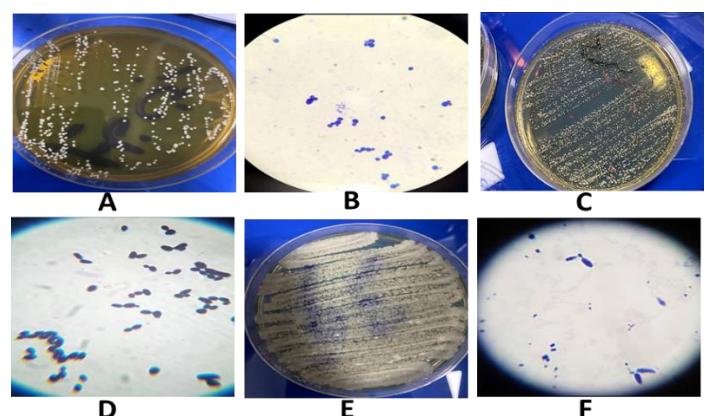
The data were analyzed using the Statistical Package for Social Sciences (SPSS; version 21), and the values are expressed as means ± standard errors of the mean (SEM). Differences between groups were evaluated by analysis of variance (ANOVA) with the Bonferroni post hoc test. Statistical significance was set at  $p < 0.05$ .

**Results and Discussion**

*Ganoderma resinaceum*, commonly known as the red reishi, is commonly used to treat chronic infectious diseases, such as hepatitis and bronchitis.<sup>9</sup> The present study investigated the antifungal activity of *Ganoderma resinaceum* Ethanol extract against vaginal fungal pathogens, specifically *Candida* species (*C. albicans*, *C. glabrata*, and *C. pichia*), commonly associated with vaginitis. The findings provide significant insights into the potential use of *Ganoderma resinaceum* as a natural antifungal agent against these pathogens and its effectiveness in reducing both fungal growth and sporulation.

**Identification of *Candida* species**

Based on morphological characteristics and microscopic examination (Figure 1), three *Candida* species were identified in female patients. The identified species included *Candida albicans*, found in 60 samples (41.38%), *Candida glabrata* with 50 isolates (34.48%), and *Candida pichia* with 35 isolates (24.14%), as shown in Table 1. These findings are consistent with previous reports, which have identified *C. albicans* as the predominant cause of vaginal candidiasis, while *C. glabrata* and *C. pichia* have been recognized as emerging pathogens with increasing clinical relevance.<sup>6</sup> The distribution of these species in the present study reflects their prevalence in vaginal infections, reinforcing the importance of addressing these pathogens in antifungal research.



**Figure 1:** Cultivation of *Candida* spp. on Sabouraud Dextrose Agar following 2-5 days of incubation at 37°C.

A: A culture of *Candida albicans* showing different colonies; B: Microscopic features of *Candida albicans* (40X); C: A culture of *Candida glabrata* showing different colonies; D: Microscopic features of *Candida glabrata* (40X); E: A culture of *Candida pichia* showing different colonies; F: Microscopic features of *Candida Pichia* (40X).

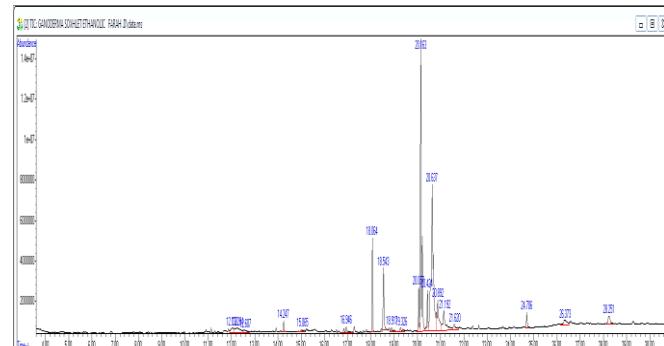
**Table 1:** Percentage occurrence of fungal isolates in vagina samples.

| Isolate                 | No of isolates | Percentage |
|-------------------------|----------------|------------|
| <i>Candida albicans</i> | 60             | 41.38      |
| <i>Candida glabrata</i> | 50             | 34.48      |
| <i>Candida pichia</i>   | 35             | 24.14      |
| Total                   | 145            | 100.00     |

*Active compounds detected in the Ganoderma resinaceum Ethanol analysis*

Several active compounds (Figure 2 and Table 2) in the Ethanol extract of *Ganoderma resinaceum* were detected using GC-MS analysis. The results revealed a high content of fatty acid ester compounds, including 9-octadecenoic acid (E) and oleic acid (31.04%), as well as an increased retention time for steroid compounds, such as  $\gamma$ -Sitosterol,  $\beta$ -Sitosterol, and Cedran-diol (8S,14), with a retention time of 28.250. In contrast, there was a low content of 2-nonenoic acid, 9-(dimethylamino), 7-hydroxy-2-methyl-9-oxo-, methyl ester, 3-butenamide, and arginine

(0.69%), while compounds like silane, trimethyl [(1-methyl hexyl) oxy], silane, trimethyl [(1-methyl pentyl) oxy], and 4-methyl-2-pentanol, trimethylsilyl ether exhibited a low retention time of 12.034.

**Figure 2:** Gas chromatography-mass spectrometry analysis of *Ganoderma resinaceum* Ethanol extract**Table 2:** Active compounds of Ethanol extract of *Ganoderma resinaceum* analysis by GC-Mass technology.

| SN | Constituent   | Retention time | Peak area (%) |
|----|---|----------------|---------------|
| 1  | Silane, trimethyl[(1-methylhexyl)oxy]<br>Silane, trimethyl[(1-methylpentyl)oxy]-<br>4-Methyl-2-pentanol, trimethylsilyl ether                                   | 12.034         | 1.36          |
| 2  | Silane, trimethyl[(1-methylpentyl) oxy]<br>beta.-Alanine, trimethylsilyl ester<br>Butane, 2,3-bis(trimethylsiloxy)  | 12.282         | 2.33          |
| 3  | Galactitol, 1,3,5-tri-O-methyl-riacetate<br>beta.-Alanine, trimethylsilyl ester   | 12.606         | 0.71          |
| 4  | Silane, [[(3.alpha.,5.beta.,20S)pregnane-3,20-diy]bis(oxyl)bis[tri-methyl<br>Dodecanoic acid, 1-methylethyl ester<br>Isopropyl myristate<br>n-Hexadecanoic acid | 14.246         | 0.71          |
| 5  | Glycine, N-[N-(N-acetyl-L-alanyl)glycyl]-, methyl ester<br>Allantoin<br>1,3-Dioxolane, 4-methyl   | 15.066         | 0.72          |
| 6  | 5-Acetoxytridecane<br>Butyraldehyde, semicarbazone<br>Lactose   | 16.943         | 1.18          |
| 7  | Hexadecanoic acid, methyl ester   | 18.065         | 5.42          |
| 8  | n-Hexadecanoic acid   | 18.540         | 5.35          |
| 9  | 2-Nonenoic acid, 9-(dimethylamino)<br>7-hydroxy-2-methyl-9-oxo-, methyl ester, 3-Butenamide<br>Arginine   | 18.917         | 0.69          |
| 10 | N-Acetylneuraminic acid,synthetic<br>N-(3,5-Dinitropyridin-2-yl)-L-aspartic acid<br>Amidephrine   | 19.327         | 0.86          |
| 11 | 9,12-Octadecadienoic acid (Z, Z)- methyl ester<br>9,12-Octadecadienoic acid (Z, Z)-, methyl ester<br>9,12-Octadecadienoic acid (Z,Z)                            | 20.061         | 3.31          |
| 12 | 9-Octadecenoic acid, methyl ester (E)<br>9-Octadecenoic acid, methyl ester<br>cis-13-Octadecenoic acid, methyl ester  | 20.158         | 26.62         |
| 13 | Methyl stearate   | 20.428         | 3.29          |
| 14 | Oleic Acid  | 20.633         | 31.04         |
| 15 | 9-Octadecenoic acid, (E)<br>Oleic Acid<br>cis-13-Octadecenoic acid  | 20.892         | 5.65          |
| 16 | cis-9-Hexadecenal<br>9-Tetradecenal, (Z)<br>Z,E-2,13-Octadecadien-1-ol  | 21.194         | 5.09          |
| 17 | 9,12-Octadecadien-1-ol, (Z,Z)<br>9,12-Octadecadienoic acid (Z,Z)<br>(R)-(-)-14-Methyl-8-hexadecyn   | 21.615         | 1.31          |
| 18 | Phthalic acid, di(2-propylpentyl ester<br>Bis(2-ethylhexyl) phthalate   | 24.711         | 1.12          |

|    |  |        |      |
|----|--|--------|------|
| 19 | Bis(2-ethylhexyl) phthalate<br>2-Methyl-Z,Z-3,13-octadecadienol<br>Oleic Acid<br>Cyclobarbital | 26.372 | 1.56 |
| 20 | gamma.-Sitosterol<br>beta.-Sitosterol<br>Cedran-diol, (8S,14)-                                 | 28.250 | 1.66 |

**Antifungal effect of the *Ganoderma resinaceum* Ethanol extract on the test pathogens**

The antifungal activity of *Ganoderma resinaceum* Ethanol extract was evaluated through both inhibition zone diameters and its effect on fungal sporulation. The effect of *Ganoderma resinaceum* Ethanol extract on the inhibition zones of the three *Candida* spp. using the agar well diffusion method is shown in Figure 3 and Table 3. The results showed that at the lowest concentration of 2 mg/ml, the inhibition zone diameters for *Candida albicans*, *Candida glabrata*, and *Candida pichia* were  $14.33 \pm 0.33$  mm,  $13.67 \pm 0.33$  mm, and  $9.00 \pm 0.57$  mm, respectively. As the concentration increased, there was a significant ( $p \leq 0.01$ ) increase in the inhibition zone diameters for all three species in a dose-dependent manner. At 250 mg/ml, the inhibition zones were the largest:  $23.33 \pm 0.33$  mm for *C. albicans*,  $22.00 \pm 0.00$  mm for *C. glabrata*, and  $30.00 \pm 0.00$  mm for *C. pichia*. The highest concentration (250 mg/ml) exhibited the greatest inhibitory effect, with *C. pichia* showing the largest inhibition zone across all tested concentrations. The findings demonstrated a clear dose-dependent increase in antifungal activity with higher concentrations of the extract. At a concentration of 2 mg/ml, the inhibition zones for *C. albicans*, *C. glabrata*, and *C. pichia* were modest. However, at 250 mg/ml, the inhibition zones increased significantly, with the largest inhibition

observed for *C. pichia* ( $30.00 \pm 0.00$  mm). These findings indicated that *Ganoderma resinaceum* possesses potent antifungal activity, particularly against *C. pichia*, which exhibited the largest growth inhibition among the tested species. Also, *Ganoderma resinaceum* may be more effective against certain *Candida* species, potentially due to differences in their cellular structure or metabolic pathways that influence their susceptibility to the extract. The findings of the present study are consistent with the study conducted by Tamilselvan *et al.* 2019,<sup>12</sup> where they reported the antifungal activity of *Ganoderma lucidum* ethanol extract against some fungi such as *Aspergillus niger*, *Fusarium* sp., *Candida* sp., and *Penicillium* sp. It was observed that the diameter of the inhibition zone of *Aspergillus niger* was 12 mm, *Fusarium* sp. was 25.5 mm, *Candida* sp. was 09 mm, and *Penicillium* sp. was 10 mm. Also, the study's findings align with previous research on *Ganoderma* species, which have demonstrated broad-spectrum antimicrobial properties.<sup>13</sup> The active compounds identified in the GC-MS analysis, including fatty acid esters like 9-octadecenoic acid and oleic acid, along with steroid compounds such as  $\beta$ -sitosterol, are likely responsible for the observed antifungal effects. These compounds have been reported in other studies to exhibit antifungal and antimicrobial properties, supporting the potential of *Ganoderma resinaceum* as an alternative therapeutic agent for managing fungal infections.<sup>14</sup>



**Figure 3:** The effect of *Ganoderma resinaceum* extract using ethanol on three *Candida* spp.

On MHA medium after 1-2 days of incubation at  $37^{\circ}\text{C}$  : A: *Candida albicans* (2, 4, 8, 16) mg/ml; B: *Candida glabrata* (31.25, 62.5, 125, 250) mg/ml; C: *Candida pichia* (2, 4, 8, 16) mg/ml; D: *Candida glabrata* (31.25, 62.5, 125, 250) mg/ml; E: *Candida pichia* (2, 4, 8, 16) mg/ml; F: *Candida pichia* (31.25, 62.5, 125, 250) mg/ml.

**Table 3:** The effect of *Ganoderma resinaceum* ethanol extract on the growth inhibition zone of test pathogens.

| Concentration (mg/ml) | Inhibition zone<br><i>C. albicans</i> | Inhibition zone<br><i>C. glabrata</i> | Inhibition zone<br><i>C. pichia</i> |
|-----------------------|---------------------------------------|---------------------------------------|-------------------------------------|
| 2.00                  | $14.33 \pm 0.33$ ef                   | $13.67 \pm 0.33$ g                    | $9.00 \pm 0.57$ g                   |
| 4.00                  | $13.66 \pm 0.33$ f                    | $11.67 \pm 0.33$ f                    | $17.00 \pm 0.00$ f                  |
| 8.00                  | $14.67 \pm 0.33$ e                    | $14.00 \pm 0.00$ e                    | $20.00 \pm 0.00$ e                  |
| 16.00                 | $16.00 \pm 0.00$ d                    | $15.00 \pm 0.00$ d                    | $21.00 \pm 0.00$ d                  |
| 31.25                 | $18.00 \pm 0.00$ c                    | $15.33 \pm 0.33$ cd                   | $21.00 \pm 0.00$ d                  |
| 62.50                 | $20.00 \pm 0.00$ b                    | $15.67 \pm 0.33$ bc                   | $23.00 \pm 0.00$ c                  |
| 125.00                | $20.33 \pm 0.33$ b                    | $16.00 \pm 0.00$ b                    | $25.00 \pm 0.00$ b                  |
| 250.00                | $23.33 \pm 0.33$ a                    | $22.00 \pm 0.00$ a                    | $30.00 \pm 0.00$ a                  |
| LSD value             | 0.790 **                              | 0.612 **                              | 0.612 **                            |
| P-value               | 0.0001                                | 0.0001                                | 0.0001                              |

This means that the different letters in the same column differed significantly. \*\* ( $P \leq 0.01$ ).

**Effect of *Ganoderma resinaceum* Ethanol extract on the sporulation of the test pathogens**

In addition to its inhibitory effects on fungal growth, *Ganoderma resinaceum* Ethanol extract also demonstrated a significant reduction in

fungal sporulation. Table 4 displays the effect of *Ganoderma resinaceum* Ethanol extract on the sporulation of three *Candida* species (*C. glabrata*, *C. pichia*, and *C. albicans*) at different concentrations. At the control level (0 mg/ml), *C. albicans* exhibited the highest

sporulation number at  $140.00 \pm 0.00 \times 10^2$ , followed by *C. glabrata* ( $77.00 \pm 0.00 \times 10^2$ ) and *C. pichia* ( $40.00 \pm 0.00 \times 10^2$ ). As the concentration of the Ethanol extract increased, the sporulation numbers for all three species significantly ( $p \leq 0.01$ ) decreased, with the lowest values observed at the highest concentration of 250 mg/ml. At 250 mg/ml, *C. glabrata* had a sporulation count of  $6.33 \pm 0.33 \times 10^2$ , *C. pichia* showed  $2.67 \pm 0.33 \times 10^2$ , and *C. albicans* had  $42.33 \pm 1.85 \times 10^2$ , indicating a substantial reduction compared to the control group. The results demonstrated that *Ganoderma resinaceum* Ethanol extract has a dose-dependent inhibitory effect on fungal sporulation, with higher concentrations leading to greater inhibition. *Candida pichia* exhibited the most significant reduction in sporulation compared to the other two species across the concentrations tested.

At the highest concentration (250 mg/ml), the sporulation of all three *Candida* species was markedly reduced, with *C. pichia* showing the

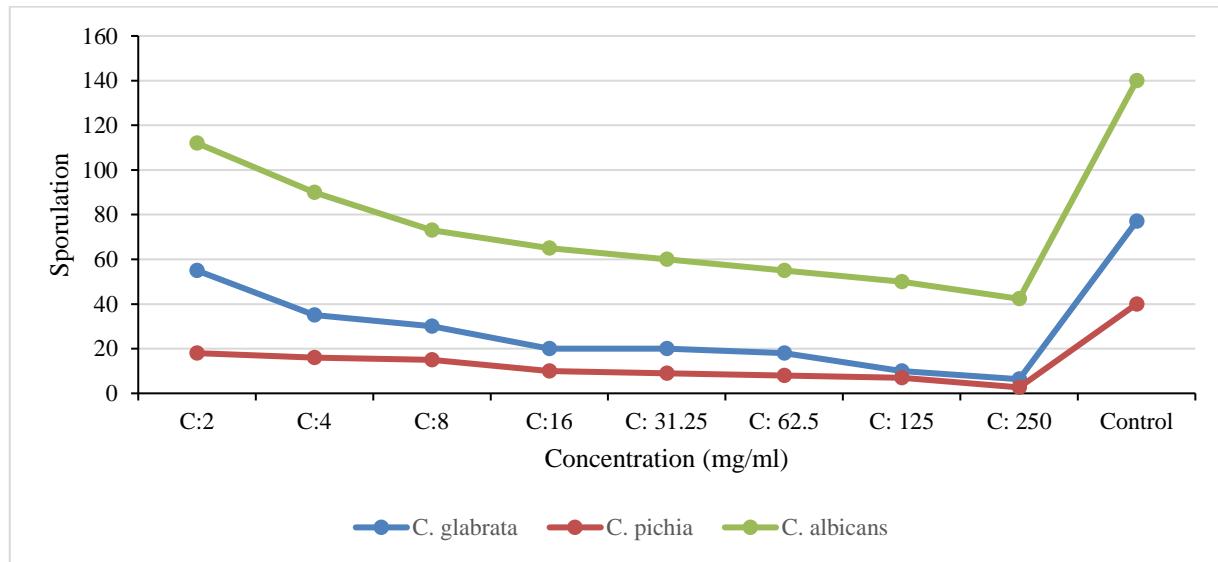
greatest reduction. This suggests that the extract not only inhibits fungal growth but also disrupts the reproductive mechanisms of the fungi, potentially reducing their ability to spread and infect. These findings are important, as controlling fungal sporulation is crucial for preventing the recurrence of infections, particularly in clinical settings where recurrent *Candida* infections are common. The reduction in sporulation observed in this study further highlights the efficacy of *Ganoderma resinaceum* as a potential therapeutic agent. This effect could be attributed to the bioactive compounds in the extract, which may interfere with cellular processes involved in spore formation, such as cell wall synthesis and membrane integrity.<sup>11</sup> The dose-dependent nature of the inhibition of sporulation mirrors the observed inhibition zones, indicating a consistent pattern of activity across both measures.

**Table 4:** The effect of *Ganoderma resinaceum* Ethanol extract on fungal sporulation

| Concentration (mg/ml) | Sporulation number ( $\times 10^2$ ) |                    |                     |
|-----------------------|--------------------------------------|--------------------|---------------------|
|                       | <i>C. glabrata</i>                   | <i>C. pichia</i>   | <i>C. albicans</i>  |
| Control               | $77.00 \pm 0.00$ a                   | $40.00 \pm 0.00$ a | $140.00 \pm 0.00$ a |
| 2.00                  | $54.66 \pm 1.76$ b                   | $15.33 \pm 0.33$ b | $112.00 \pm 2.51$ b |
| 4.00                  | $33.33 \pm 1.45$ c                   | $11.67 \pm 0.33$ c | $89.67 \pm 1.20$ c  |
| 8.00                  | $29.00 \pm 0.57$ d                   | $11.00 \pm 0.57$ c | $73.33 \pm 2.40$ d  |
| 16.00                 | $20.67 \pm 0.66$ e                   | $8.33 \pm 0.33$ d  | $67.33 \pm 0.66$ e  |
| 31.25                 | $20.00 \pm 0.57$ ef                  | $8.33 \pm 0.88$ d  | $61.00 \pm 1.73$ f  |
| 62.50                 | $17.66 \pm 0.33$ f                   | $6.67 \pm 0.33$ e  | $54.67 \pm 0.88$ g  |
| 125.00                | $8.67 \pm 0.67$ g                    | $5.33 \pm 0.33$ f  | $51.00 \pm 0.57$ g  |
| 250.00                | $6.33 \pm 0.33$ g                    | $2.67 \pm 0.33$ g  | $42.33 \pm 1.85$ h  |
| LSD value             | 2.620 **                             | 1.320 **           | 4.598 **            |
| P-value               | 0.0001                               | 0.0001             | 0.0001              |

This means that the different letters in the same column differed significantly.

\*\* ( $P \leq 0.01$ ).



**Figure 4:** The sporulation number of *Candida* spp. on *Ganoderma resinaceum* Ethanol extract ( $\times 10^2$ )

#### Comparative effectiveness against different *Candida* species

The findings revealed notable differences in the antifungal effectiveness of the extract across the three *Candida* species. *Candida pichia* demonstrated the highest susceptibility, showing the largest inhibition zone and the most significant reduction in sporulation. This is consistent with earlier studies indicating that *C. pichia* may be more susceptible to certain antifungal agents compared to other *Candida* species.<sup>14</sup> On the other hand, *C. albicans* and *C. glabrata* exhibited relatively lower sensitivity to the extract, which may reflect inherent differences in their cell wall compositions and resistance mechanisms. *Candida albicans*, in particular, is known for its ability to form biofilms, which can

contribute to its resistance to antifungal agents.<sup>15</sup> These species-specific differences are important considerations when evaluating the clinical potential of *Ganoderma resinaceum* as a treatment option for fungal infections. There are another reports record antifungal activity of hot alcoholic extraction of algae<sup>16</sup>.

#### Implications for vaginal fungal infections

The findings of this study are particularly relevant for the treatment of vaginitis, a common condition caused by fungal infections. As more individuals turn to natural products for their therapeutic potential,

*Ganoderma resinaceum* presents a promising candidate for inclusion in antifungal treatments, particularly in managing *Candida*-induced vaginitis. Given the growing concerns over the side effects and resistance associated with conventional antifungal drugs, *Ganoderma resinaceum* may offer a safer alternative with fewer adverse effects. The dose-dependent activity observed in this study underscores the need for further clinical evaluation to establish the optimal concentrations and formulations for therapeutic use. Recommend the application of molecular techniques, such as PCR, to detect the expression of resistance genes in *Candida* species in response to alternative antifungal drugs. The molecular method due to give accuracy results, it was utilized in numerous studies to detect resistance genes and quantify their expression levels in various bacteria.

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## Conclusion

The findings of the present study revealed that *Ganoderma resinaceum* Ethanol extract demonstrated significant antifungal activity against *Candida albicans*, *Candida glabrata*, and *Candida pichia*, with the greatest efficacy observed at higher concentrations. The extract not only inhibited fungal growth but also reduced fungal sporulation, highlighting its potential as a natural antifungal agent for the treatment of vaginal infections. Further studies, including *in vivo* experiments and clinical trials, are needed to confirm these findings and explore the full therapeutic potential of *Ganoderma resinaceum* in managing fungal infections.

## Conflict of Interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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