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Antifungal Activity of Ginger Extract Against *Aspergillus Spp* In Vitro Study

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Abstract

Zingiber officinale is widely used in traditional Asian medicine. Ginger may be used medicinally to treat a variety of ailments. Ginger contains active ingredients such as phenolics (like zingerone) and sesquiterpenoids (like gingerol and shogaol). That shows antibacterial properties against a variety of bacteria, fungus, and parasites. Ginger has numerous medicinal uses for a wide range of illnesses, such as growth problems, atherosclerosis, TB, vomiting, rubella, and inflammatory diseases. To evaluate the anti-fungal activity of *Z. officinale* against *Aspergillus fumigatus* Results of the study indicate that *Z. officinale* extracts, both aqueous and ethanolic, have strong antifungal activity against the *A. fumigatus*. The concentration and kind of extract affected the antifungal action. The alcoholic extract worked better than the aqueous extract. Possibly because ethanol extracts contain stronger antifungal compounds. Antifungal effectiveness was evaluated in the current study using the poisoned food method. Results of the "disc diffusion method" on the growth of *A. fumigatus* using ethanolic and aqueous extracts of *Z. officinale.* The inhibition zones for the tested fungus were (28.6 mm) at a concentration of 25 % ethanolic extracts and (16.2 mm) at a concentration of 25 % aqueous extracts when compared to the control. The result demonstrates that ethanolic extracts were more effective than aqueous extracts, and the effect increased with increased concentration.

Keywords: Biocontrol, *Aspergillus* species, *Zingiber officinale*.

1. Introduction

Aspergillus species are among the most frequent fungus-caused diseases that can be lethal, especially in individuals with compromised immune systems [1]. It has also been demonstrated that *Aspergillus*

species other than fumigatus, such *A. flavus, A. niger,* and *A. terreus*, may also cause infections. *A. fumigatus* is responsible for many illnesses with *Aspergillus* products. [1, 2].

In industrialized countries, *A. fumigatus* is the most prevalent airborne fungal infection. It may cause invasive aspergillosis in people with weak immune systems, which is usually fatal [3]. One feature of this fungus species' extremely basic life cycle is its strong sporulation capacity, which causes significant concentrations of conidia to be present in the air both indoors and outdoors. Indeed, humans continuously inhale *A. fumigatus* conidia, but they rarely cause any harm because of the immune system. However, the rise in immunosuppressed patients and the intensification of immunosuppressive treatments.

The innocuous status of *A. fumigatus* has evolved over the last ten to twenty years. The most common airborne fungal pathogen today is *A. fumigatus,* which in industrialized nations generates invasive infections in immunocompromised hosts that are severe and frequently fatal [4]. Spice ginger, or *Zingiber officinale roscoe* is often used. Many chemical substances are included such as, lipids, organic acids, polysaccharides, phenolic chemicals, terpenes, and raw fibers. The main source of ginger's health advantages are its phenolic compounds, which include shogaols and gingerols. According to various studies, ginger has a wide range of biological benefits, such as anti-inflammatory, antimicrobial, anti-cancer, protective of nerves, pulmonary, cardiovascular, anti-obesity, anti-diabetic, anti-nausea, and antiemetic effects. [5].

Active substances found in ginger include phenolic compounds (like zingerone) and sesquiterpenoids (like gingerol and shogaol), demonstrating the effectiveness of antibacterial against various parasites, fungi, and bacteria [6, 7]. According to another study, with an aqueous extract, the most potent antifungal activity against *Aspergillus fumigatus, A. niger, A. oryzae,* and *A. flavus* was demonstrated by the methanolic extract of Z. *officinale* [8].

2. Materials and Methods

2. 1 Fungal Isolates' Source

Aspergillus fumigatus isolate was kindly supplied by the Mycology Laboratory, College of Science, Department of Biology. *Aspergillus* was cultured on PDA medium and maintained under routine laboratory conditions for future experiments.

A mycology specialist morphologically identified the isolate as *A. fumigatus*. The identification was based on macroscopic and microscopic characters including the formation of colonies with a powdery to velvety texture developing quickly, whitish turning greenish gray on aging, and the development of columnar conidial heads.

Septate hyphae microscopically, uniseriate phialides along the upper two-thirds of the vesicle, and the development of typical conidia into round shapes were visible. All characters agreed with the typical morphological features of *A. fumigatus*, ensuring the uniformity of the isolate used in this study.

2. 2 Source of Ginger Plants

After obtaining ginger rhizomes from the country market, they were cleaned with tap water. Allowed to dry at room temperature in the shade, and then blender into a powder before being brought to the lab.

2.3 Preparation of *Zingber Officinale* Extract

2.3.1 Preparation of Aqueous Extracts

In conical flasks, 50 grams of the powdered *rhizomes* were combined with 200 mL of distilled water to create aqueous extracts. The mixture was filtered using Whatman filter paper number one and stored in a shaded container at 4 °C until used [9].

2.3.2 Preparation of Ethanolic Extracts

Fifty grams of the powdered *rhizomes* were combined with 200 mL of 70 % ethanol to create ethanolic extracts. After 30 minutes of concentration in Rotavapor (Buchi Rotavapor type R-210) at 40 °C, the filtrate is utilized to extract the solvent, leaving behind a residue that is stored in a shaded container at 4 °C until used [9].

2.3.3 Effect of Aqueous and Ethanolic Extracts on *Aspergillus* Growth by Food Poisoning Technique

Using the poisoned food technique, under controlled laboratory settings (25 ± 2 °C, relative humidity 65–70 %). The effect of *Z. officinale* extract on *Aspergillus* growth was measured by mixing varying amounts of dry extracts with distilled water (aqueous extract). In the case of ethanolic extracts, Dimethyl Sulfoxide (DMSO) to achieve concentrations of 25 (2 gm per 80 mL), 12.5, and 6.25 mg/mL. Ten milliliters

of each concentration were then combined with 90 milliliters of PDA medium that had previously been autoclaved. Then, poured into petri dishes 9 cm. The Petri dishes used as a control had medium devoid of extract. Seven-day old culture of the targeted fungus was injected into each plate using mycelial discs (5 mm in diameter). Then, incubated for the allotted 7 days at 25 °C, and results of measuring the growth's diameter have been documented [10].

2.3.4 Effect of Aqueous and Ethanolic Extracts on *Aspergillus* Growth by Disc Diffusion Method

For assess how *Zingiber officinale* extract, both ethanolic and aqueous, affects *Aspergillus* growth using the discs diffusion method. After being autoclave sterilized, 5 mm diameter paper discs were immersed in 6.25, 12.5, and 25 mg/mL of *Z. officinale* (aqueous and ethanolic) solution.

Then, placed on a petri dish containing PDA that had already been inoculated with the target fungus. After two hours, agar plate was maintained at room temperature to facilitate the diffusion of the fluid. Following that, each plate was incubated for a predetermined amount of time at 25 °C. Millimeter measurements of the inhibitory zones were calculated [11].

2.4 Statistical Analysis

Analysis of variance (ANOVA) and statistical interpretation were performed on the data using the Statistical Package for the Social Sciences (SPSS) software. The Least Significant Difference (LSD) test was used to assess treatment differences at the < 0.05 probability level [12], and every value is a mean of three replicates.

3. Results and Discussion

3.1 Effect of Aqueous, and Ethanolic Extracts of *Zingiber officinale* on *Aspergillus* Growth

3.1.1 Effect of *Zingiber* Extracts by Food Poisoning Technique

Antifungal activity of *Zingiber officinale* extract in *Aspergillus* radial growth is listed in (table 1). At all test concentrations, *Z. officinale's* aqueous and ethanolic extract inhibited *A. fumigatus's* radial growth and markedly slowed it down in comparison to the control. As the extract concentration grew, correspondingly increased the effects of both extracts. The instance of *Z. officinale,* percentages of growth inhibition vary from 52.27 to 10.39 % for the aqueous extract and from 59.48 to

22.69 % for the ethanolic extract. The concentration of 25 mg/mL of alcohol extract showed the highest percentage of growth inhibition of *A. fumigatus* (59.48 %). While the concentration of 6.25 mg/mL of water extract showed the minimal percentage of growth inhibition (10.39 %).

Table 1: The effect of extracts on *Aspergillus* growth by food poisoning technique

Zingiber extract	**Concentration mg/mL**	**Radial growth (mm)**	**Growth Inhibition (%)**
Alcohol extract	25	32.6 g	59.48 a
	12.5	39.5 e	50.90 c
	6.25	62.2 c	22.69 e
Water extract	25	38.4 f	52.27 b
	12.5	44.6 d	44.56 d
	6.25	71.8 b	10.39 f
Control	-	80.5 a	-
LSD 0.05		0.53	0.68

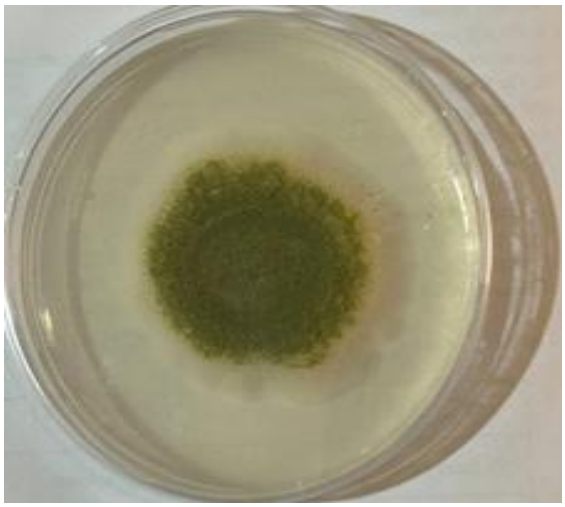

Figure 1: Effect of ethanolic' extracts on Aspergillus growth using food poisoning technique (25mg/mL).

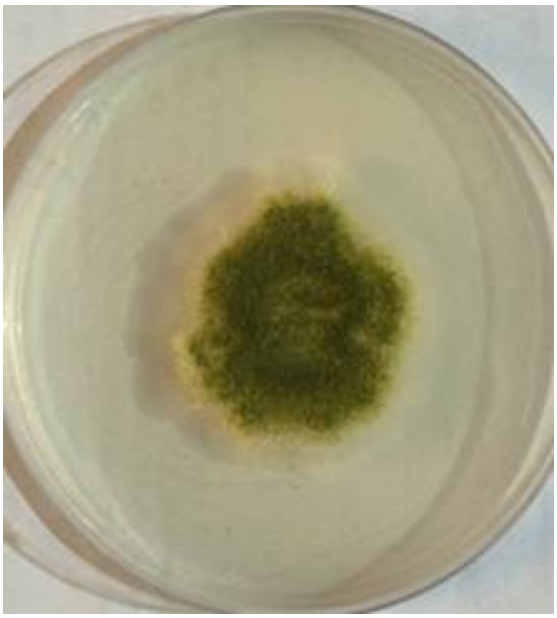

Figure 2: Effect of ethanolic' extracts on *Aspergillus* growth using food poisoning technique (12.5mg/mL).

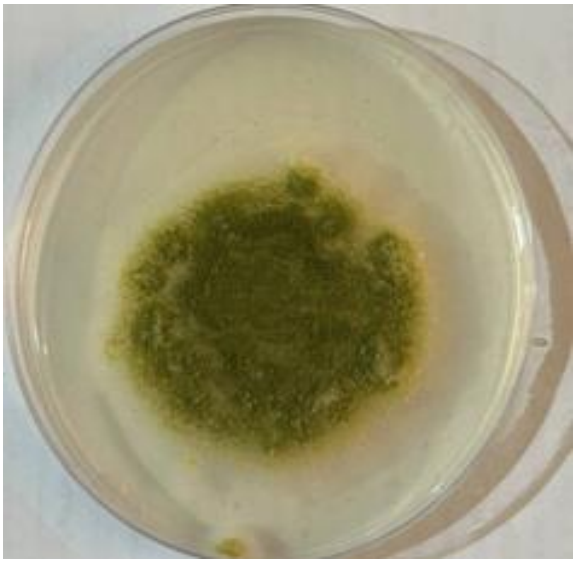

Figure 3: Effect of ethanolic' extracts on *Aspergillus* growth using food poisoning technique (6.25mg/mL).

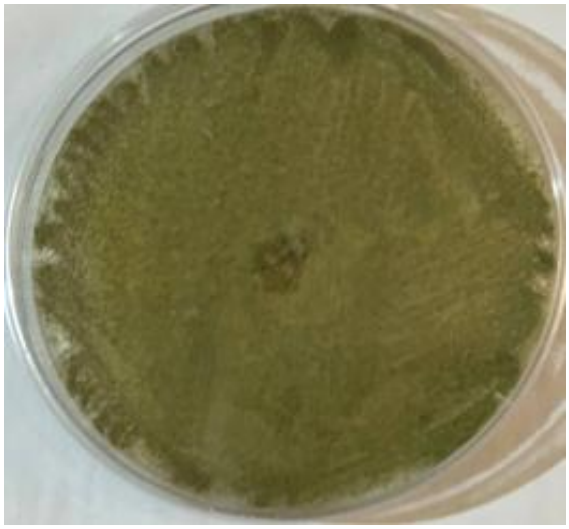

Figure 4: Effect of ethanolic' extracts on Aspergillus growth using food poisoning technique (control).

Based on the investigation's findings, aqueous and ethanolic extract of Z. *officinale* have significant antifungal activity on the *A. fumigatus* microorganism. Antifungal effect was depending on the type of extract and the concentration. Perhaps because ethanol extracts more potent antifungal materials, the alcoholic extract outperformed the aqueous one. The current study used the poisoned food approach to assess the antifungal efficacy of 22 plant extracts against 10 food-associated fungus isolates.

Ginger ethanolic extracts had the highest inhibitory effect against all test fungal strains out of the 22 plant extracts [13]. The outcome also demonstrated that the effect grew as the concentration increased, which was consistent with the findings of Alwan [14]. Alwan reported that the effective concentrations of *Z.officinale* extract against *A. fumigatus* and *A. niger* were 1, 3.1, and 5.2 cm when the extract was applied at 15 %, 20 %, and 25 %, respectively. Findings of Gowri [15], that ethanolic extract was more efficient against *Aspergillus fumigatus*, and agrees with the findings of this investigation. Additionally, our findings outperformed those of Sirilak [16], that reported ethanol extract of ginger had better antimicrobial effects than the aqueous extract. The antibacterial activity of *Z. officinale Roscoe rhizome* was assessed in another study that revealed outstanding activity against *Aspergillus niger* that is antifungal [17].

The activity of antifungal of *Z. officinale* powder and aqueous extract against *A. niger* was assessed by Baleba [18]. Results showed that the aqueous extract was more effective, that the effect increased with increasing concentration, and that the highest percentage of inhibition was 86.67 %. Another investigation of ginger 'extract's antifungal effectiveness against *A. flavus* reported that the extract's potent bioactive components, including phenolic

compounds and terpenes, inhibited the development of *Aspergillus* hyphae [19].

3.1.2 Effect of *Zingiber* Extracts Using Disc Diffusion Method

Outcomes of the disc diffusion method on the growth of *A. fumigatus* using *Z. officinale* extract (aqueous and ethanolic) are listed in (table 2), and (figures 5-8). Results indicated that ethanolic extracts outperformed aqueous extracts in terms of effectiveness, and the impact grew as concentration rose. The inhibition zones for the tested fungus were (28.6 mm) at a concentration of 25 % ethanolic extracts and (16.2 mm) at a concentration of 25 % aqueous extracts.

According to the results, the fungus *A.fumigatus* had the biggest inhibition zones. Measuring 28.6 mm, in ethanolic extracts at a concentration of 25 % and the lowest inhibition zones, measuring 5.3 mm, in aqueous extract at a concentration of 6.25 %. It is worth noting that aqueous extracts may be less effective due to the solubility of only polar compounds, such as simple sugars and proteins. While non-polar or semi-polar active compounds, such as phenolic and terpenic compounds, are not sufficiently extracted in aqueous media.

This explains the weak inhibitory effect of aqueous extracts compared to ethanolic extracts, as phenolic and terpenic compounds are known for their antifungal properties, inhibiting the growth of fungal hyphae and inhibiting enzymes involved in tissue penetration.

Table 2: Effect of *Zingiber* extract on *Aspergillus* using disc diffusion method.

Treatment	Concentrations (mg/mL)	Zone of Inhibition (mm)
Extract		*A. fumigatus*
Ethanolic	25	28.6 a
	12.5	14.4 c
	6.25	7.3 e
Aqueous	25	16.2 b
	12.5	8.6 d
	6.25	5.3 f
Control		0 g
'LSD'(0.05)		0.32

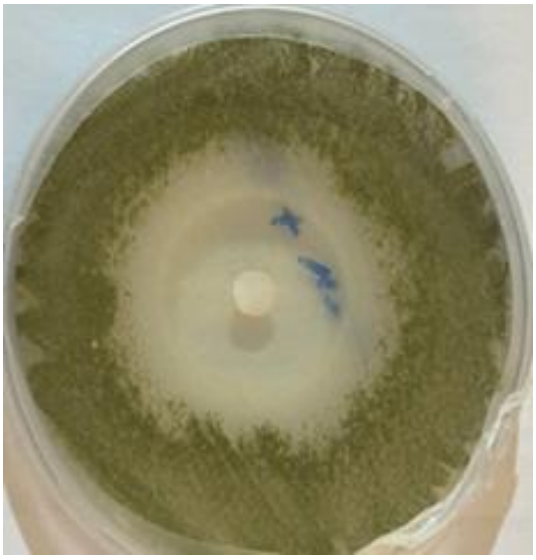

Figure 5: The effect of the ethanolic extract on the growth of *A. fumigatus* by the disc diffusion method (disc diameter 5 mm) (25 mg/mL).

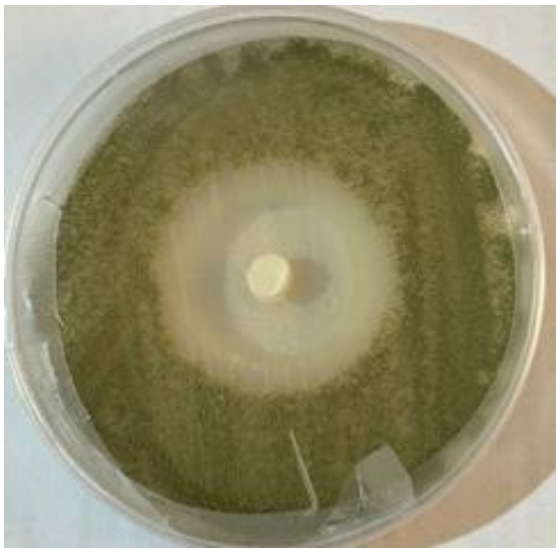

Figure 6: The effect of the ethanolic extract on the growth of A. fumigatus by the disc diffusion method (disc diameter 5 mm) (12.5 mg/mL).

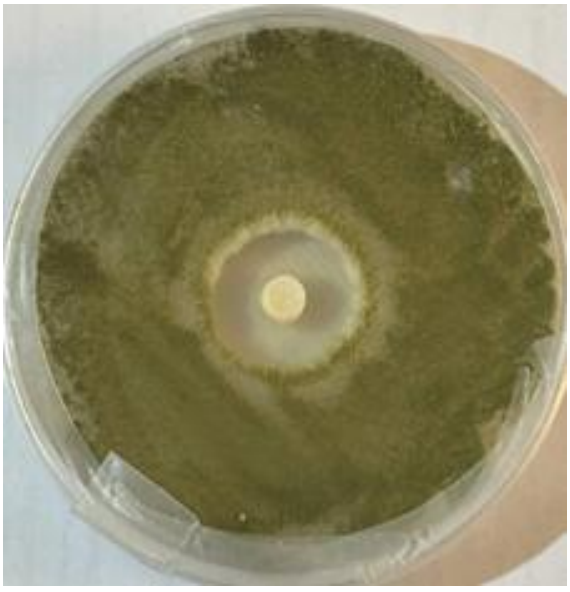

Figure 7: The effect of the ethanolic extract on the growth of A. fumigatus by the disc diffusion method (disc diameter 5 mm) (6 mg/mL).

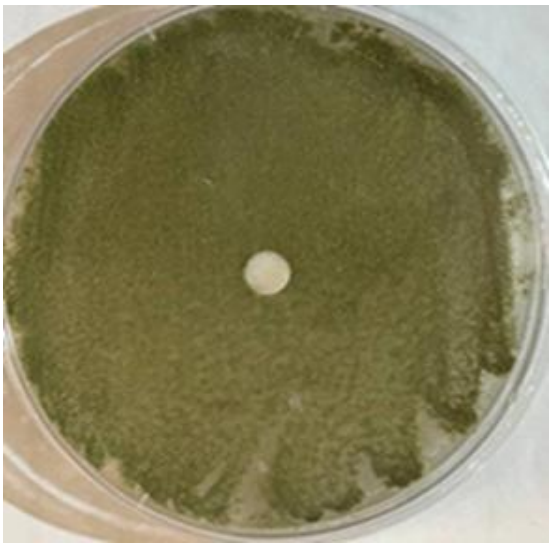

Figure 8: The effect of the ethanolic extract on the growth of A. fumigatus by the disc diffusion method (disc diameter 5 mm) (control).

Concentration of (25 mg/mL) in the dish showed the largest inhibition zone. Followed by (12.5 mg/mL) with a medium diameter. Then, (6 mg/mL) showed a limited diameter, while the control treatment (D) did not show any inhibition zone. Given the extraction solvent, results showed that the ethanolic extract outperformed the aqueous extract in terms of effectiveness.

This might be because the active components of the plant materials were not as well liberated. This indicates that the extracts' level of antifungal activity is influenced by the extraction solvent. The antifungal activity of *Z. officinale*

methanolic extracts was higher than that of aqueous and acetone extracts. According to a similar study, is perhaps due to methanol is an organic solvent that liberates the active ingredient required for antimicrobial action and dissolves organic molecules more easily [20]. The effectiveness of ginger extract in inhibiting fungal growth is attributed to its richness in active constituents, such as phenolic compounds and terminal qualities, that have demonstrated antifungal qualities [21, 22].

Zingiber officinale root extracts showed varying degrees of 'antifungal activity against the tested fungi, zingiber officinale root extracts showed varying degrees of 'antifungal activity against the tested fungi [23]. The antifungal activity of 'Zingiber officinale root' extracts and screened for active phytochemicals against *A. fumigatus, A. flavus*, *A. niger*, and *A. oryzae.* [23].

In contrast to acetone and aqueous extracts, the methanollic extract of *Z. officinale* exhibited the most antifungal efficacy against the investigated fungi. Terpenoids, flavonoids, alkaloids, cardiac glycoside, tannin, and saponin were also detected by the screening. Furthermore, the primary components of ginger, such as diarylheptanoids, gingerol, shogaol , and volatile oil, have shown antimicrobial, and antioxidant qualities [24]. Ginger contains potent bioactive components including terpenes and phenolic substances known for their antifungal properties. Ginger's ethanol extract displayed notable *in vitro* antifungal activity against *Candida albicans*. The existence of antifungal substances like zingerone, shogaols, and gingerols, eugenols, sesquiterpenes, and monoterpenes enables ginger to hinder fungal growth.

Notably, phenolic substances like zingerone, shogaol, and gingerol can disrupt protein bonds within cell membranes, leading to membrane lysis. This disruption leads to cell membrane breakdown, allowing phenolic compounds to infiltrate the cell nucleus, ultimately arresting fungal growth [25, 26]. The well-in-agar technique was used to evaluate methanol, ethanol, ginger extracts, and pasture honey on *A. flavus, A. fumigatus*, and *Candida albicans*. Findings showed that all treatments produced inhibitory zones on the test fungal species, and the phytochemicals found in the ginger sample included cardiac glycoside, paleobotanic, alkaloids, flavonoids, and saponin.

Whereas phytochemicals found in the pasture honey included both substances cardiac glycoside and saponin [27]. Although ginger essential oil (GEO) exhibits

antifungal and anti-aflatoxigenic qualities, it's in situ fumigant use has not yet been investigated [27]. Moreover, investigation of effects of GEO as a fumigant agent on *Aspergillus flavus* in stored maize grains was also reported by Nerilo [28].

4. Conclusion

Results of the current study demonstrated that ginger extract possesses significant activity against *Aspergillus spp*. isolates in vitro, indicating that it contains natural compounds with antifungal properties. The inhibitory activity of the extract also increased with increasing concentration, confirming a direct relationship between concentration and antifungal activity.

Results suggest that ginger extract could be used as a promising natural option or complementary treatment to traditional antifungals, especially considering the growing problem of drug resistance. Accordingly, future studies, including in vivo experiments, are recommended to evaluate the safety and efficacy of the extract before its adoption as a potential treatment.

High performance chromatography analysis (HPLC) is recommended to identify the active chemical compounds in ginger extracts. Compounds include phenolic and terpenic to directly correlate the concentrations of these compounds with antifungal activity. Thus, enabling a deeper understanding of their mechanism of action and their potential use in pharmaceutical applications.

5. References

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