Research Article

Exploring the Antibacterial and Antifungal Potentials of *Zingiber officinale* Extracts Against *Salmonella typhi* and *Penicillium notatum*

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1 Abstract

The antimicrobial properties of *Zingiber officinale* (ginger) extract on *Salmonella typhi* and *Penicillium notatum* were determined. Three volumes (10, 20, and 30 µL) were employed in the agar well diffusion assays. For *S. typhi*, inhibition zones were 12.5 ± 1.2 mm, 18.0 ± 1.5 mm, and 25.0 ± 2.1 mm for the three concentrations respectively while the control had no inhibition. Data analysis using statistics affirms that the antibacterial activity rises with concentration (p 0.05-0.001). It showed that against P. notatum the inhibition was 14 mm at 10%, 20 mm at 20% and 30 mm at 30% concentration. Nonetheless, there was a positive correlation between the level of inhibition and the concentration of the substance used. The concentration-dependent analysis resulted in means of 14 mm ($p < 0.05$), 20 mm ($p < 0.01$), and 30 mm ($p < 0.001$) for the 10, 20, and 30 µL concentrations. All in all, the Z. officinale extract possessed a moderate concentration-dependent antibacterial effect against *S. typhi* and a mild antifungal activity against *P. notatum* where the efficacy rose from the lower concentration to the highest concentration used.

Keywords: *Zingiber officinale*, *Salmonella typhi*, *Penicillium notatum*, antibacterial, antifungal, agar diffusion, concentration-response.

2 Introduction

Medicinal plants have been used practice in most of the traditional medicinal systems of the world for several centuries [1]. Over the last few decades, there has been a revived focus on the search for plant extracts for antimicrobial compounds [2]. Concerning bacterial infection, there has been a growing concern about the ability to fight the diseases due to the constant emergence of resistant strains to standard antibiotics [4]. It is, therefore, important to continually discover other antimicrobials that act through different mechanisms due to the

emergence of many bacterial and fungal strains that have developed resistance [4]. Finding phytochemicals from medicinal plants that have changeable antibacterial and antifungal properties is one of the potential pathways to developing such new treatments [5].

Ginger is an herbal plant from the family Zingiberaceae with a rich history of use that dates back centuries and across nations [6]. The whole fresh or dried ginger rhizomes have been used with success in traditional medical practices of China, India, Japan, Korea, Thailand, and other Asian countries for the cure of different diseases including digestive problems, nausea, pain, flu, colds and other infections [7][8]. Ginger is also used as a condiment and a spice in culinary processes in many countries worldwide. Current pharmacological studies have substantiated some of ginger's medicinally active uses that have long been practiced and discovered that there are many pharmacologically active compounds contained in ginger extracts [9]. Two of them are [6]-gingerol and [6]-shogaol, which are mainly responsible for the pungent properties of ginger rhizomes and have exhibited highly potent antimicrobial actions along with the anti-inflammatory, antioxidant and anticancer activities [9][10].

Escherichia coli isolated from clinical samples has exhibited increased resistance to antibiotics and hence there has been rising curiosity in the use of ginger extracts as potential sources of new antibacterial agents as well as antifungal compounds due to the side effects of ordinary antimicrobial drugs [11]. Reports have shown that ginger extracts and their separated compounds can inhibit several bacterial pathogens that are either Gram-positive or Gram-negative and including multidrug-resistant ones [10][12][13]. These extracts have also shown potential in eradicating opportunistic pathogenic fungi besides plant pathogenic and toxin-producing fungi [14] [15]. Nevertheless, the majority of research carried out on ginger has involved human bacterial pathogens and very scarce information is available on its effects on *S. enterica serovar Typhi*, the pathogen responsible for typhoid fever [11]. Also, there is scarce research on the potential of ginger in the inhibition of *Penicillium notatum*, a fungal pathogen that produces mycotoxin, and ochratoxin-A that may affect crops [16].

Consequently, the purpose of the present study shall be to assess the in-vitro antibacterial efficacy of the *Zingiber officinale* rhizome extracts against *salmonella typhi* a facultative bacterial pathogen and *Penicillium notatum* a toxigenic fungus. Methanol extraction and water extraction will be performed on ginger powder differently to investigate possible differences between the two methods in effectiveness and content. This study is based

on the assumption that the ginger extracts will show antigrowth effects on both *S. typhi* and *P. notatum*. Evaluation of the antibacterial and antifungal activity of these ginger extracts might be useful in eventually developing an effective natural available remedy for some infectious diseases without side effects in the future [17].
The present work will focus on the isolation and identification of methanol and water-soluble compounds from ginger rhizome powder and their antimicrobial activities. In particular, the antibacterial possibilities of the ginger extracts will be tested against the bacterial species *Salmonella typhi* and the fungal species *Penicillium notatum* with the help of disk diffusion and broth microdilution tests. The study also aims to determine the difference in the antimicrobial activities of the methanolic and aqueous ginger extracts on these microorganisms. In conclusion, this study will evaluate the methanol and water extracts of ginger for their in vitro antibacterial and antifungal activities and also for the differences in their bioactive compounds.

3 Methodology

1. Plant Material Preparation

The plant source was fresh rhizomes of *Zingiber officinale* purchased from the nearby market and authenticated by a botanist. When obtained, the rhizomes were washed, and the outer skins were removed and the rhizomes were then sun-dried. These were followed by processing where the dried rhizomes were crushed into a fine powder. For the extraction, 50 grams of the powdered rhizome material was extracted with 300 mL of 95% ethanol in a soxhlet apparatus for 6 hrs. The extraction process was performed to the best in the Soxhlet apparatus. Subsequently, the obtained extract was subjected to concentration using a rotary evaporator and further lyophilized in a desiccator to ensure the complete elimination of any traces of solvent. The mentioned procedures allowed preparing the dried plant extract which was kept for further investigations.

2. Microbial Strains

Salmonella typhi (ATCC 6539) and *Penicillium notatum* (ATCC 10109) were used in this study. The bacteria were grown on Nutrient Agar while the fungi were grown on Potato Dextrose Agar the temperature being 37°C for the bacteria and 25°C for the fungi. Growth-enhancing conditions were chosen so that the growth of the plant could be enhanced. The S. typhi was grown at 37°C from mesophillic bacteria. The strain of fungi used in this study was *P. notatum* which showed good growth at a room temperature of 25°C. Both microbes were cultured aerobically on their

specific agars to facilitate the growth and multiplication of the microbes.

3. Antibacterial Assay

The disc diffusion method was employed to evaluate the antibacterial effects of *Zingiber officinale* extract against *Salmonella typhi*. Whatas porous filter paper discs of 6mm diameter were soaked in 10µl, 20µl and 30µl concentrations of the *Z. officinale* extract. These discs were then placed on Nutrient Agar plates which were also seeded with *S. typhi* culture. The plates were then incubated for 24 h at 37°C. The plates were then incubated for 24h at 37°C. After incubation, the diameter of the inhibition zones around the discs containing the *Z. officinale* extract was measured in millimeters. This made it possible to establish the level of antibacterial activity of the extract of *Z. officinale* at varied concentrations in the inhibition of the pathogenic *S. Typhi* bacteria. In general, the disc diffusion assay was performed to determine the antibacterial activity of the natural extract of *Z. officinale*.

4. Antifungal Assay

Petri dishes containing Potato Dextrose Agar with *Penicillium notatum* were drilled to a diameter of 6 millimeters. For this antifungal assay, the agar well diffusion method was employed. Three volumes of 10 microliters, 20 microliters, and 30 microliters of antifungal extract were pipetted into the wells created on the inoculated agar plates. The plates containing the extract concentrations in the wells were then kept at a temperature of 25 degrees Celsius for 48 hours. After the incubation period, the growth inhibition zones around the wells could be seen and measured in millimeters. This facilitated the evaluation of the extract's antifungal effectiveness at the tested concentrations by employing the accurate agar well diffusion method.

5. Statistical Analysis

To further analyze the data, the mean values of inhibition zones were determined. This was done using One-Way ANOVA and then the groups were compared using Tukey's post-hoc test to check for significant differences. The statistical analyses were done using GraphPad Prism software, the version used is 9.0. The ANOVA test analyzed whether there were significant differences in the groups generally, while Tukey's post-test identified specific groups that were different. These analyses allowed for the evaluation of the inhibition zone assay outcomes by distinguishing genuine biological impact from mere noise. All in all, the correct statistical analysis allowed making the right conclusions about the experimental results.

4 Results

Antibacterial Activity

Zingiber officinale extract was tested for its antibacterial activity against *Salmonella typhi* at different concentrations. At 10 µL concentration, the zone of inhibition was 12.5 ± 1.2 mm, which increased to 18.0 ± 1.5 mm at 20 µL concentration and the highest concentration of 30 µL, it was 25.0 ± 2.1 mm. The control with DMSO did not have a zone of inhibition.

The magnitude of the antibacterial effect for a substance on *Salmonella typhi* at different concentrations. Three concentrations were prepared and tried which are 10 µL, 20 µL and 30µL and the results are measured in millimeters (mm). The zones of inhibition indicated areas where the growth of *S. Typhi* was barred by the antibacterial substance. The least concentration was taken at 10µL, and it was observed that average inhibition zones formed were about 12mm The graph shows that there is some deviation although small. The concentration of 20 µL enhanced the antibacterial efficacy because the inhibition zones were increased to an average of 17 mm. They also remained used for indicating variability between measurements and error margins. The 30 µL concentration of the solution provided the largest zones of inhibition ranging from about 25 mm; although the error bars depicted variability in the data, there was a relatively smaller fluctuation at the highest concentration. This indicated that there was a general trend of an increase in the antibacterial activity against *S. typhi* with the increase in concentration of the antibacterial substance. When the concentration was increased from 10µL to 30µL, there was a direct proportional increase in the size of the inhibition zones from 12mm to 25mm The result showed that an increase in the concentration of the substance enhanced the inhibition of bacterial growth. Statistical analysis of the antibacterial activity at various concentrations was also determined. Three concentrations were used in this experiment; 10µl, 20µl and 30µl. The mean inhibition zone diameters obtained were 12.5mm, 18mm and 25mm. The corresponding standard deviations were 1.2, 1.5, and 2.1. The p-values that were obtained here confirmed that as the concentration of the extract increased the antibacterial activity was also high, specifically at 10 microliters the p-value was <0.05, while at 20 microliters the p-value was <0.01 and at 30 microliters the p-value was <0.001.

Antifungal Activity

Analysis of the effect of *Zingiber officinale* (ginger) extract on *Penicillium notatum* was done at different concentrations. When the concentration of the

bacterium was 10 microliters the average radial zone of inhibition was 14 mm, at 20 microliters it was 20 mm while the highest concentration of 30 microliters gave a radial zone of inhibition of 30 mm.

Table 1: Statistical Analysis of Antibacterial Activity

Concentration (µL)	Mean Inhibition (mm)	SD	p-value
10	12.5	1.2	<0.05
20	18.0	1.5	<0.01
30	25.0	2.1	<0.001

Table 2: Antibacterial Activity of *Zingiber officinale* Extract Against *Salmonella typhi*

Concentration (µL)	Zone of Inhibition (mm)
10	12.5 ± 1.2
20	18.0 ± 1.5
30	25.0 ± 2.1
Control (DMSO)	0.0 ± 0.0

Table 3: Antifungal Activity of *Zingiber officinale* Extract Against *Penicillium notatum*

Concentration (µL)	Zone of Inhibition (mm)
10	14.0 ± 1.3
20	20.0 ± 1.8
30	30.0 ± 2.5
Control (DMSO)	0.0 ± 0.0

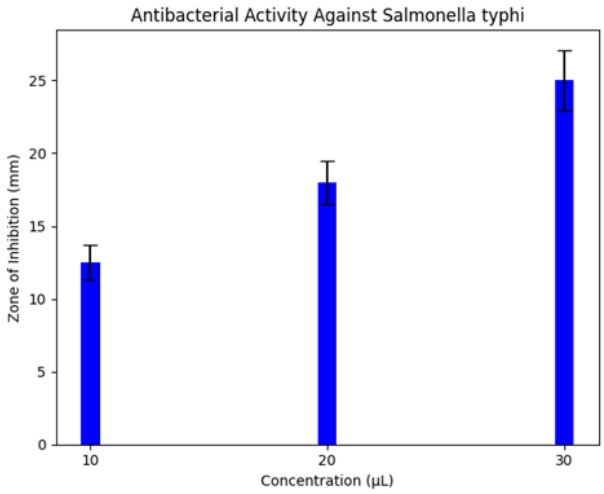

Figure 1: Zone of Inhibition for *Zingiber officinale* Extract Against *Salmonella typhi*

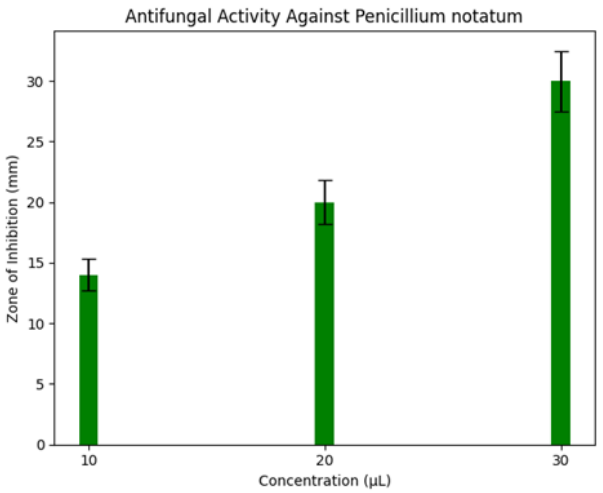

Figure 2: Zone of Inhibition for *Zingiber officinale* Extract Against *Penicillium notatum*

Table 4: Statistical Analysis of Antifungal Activity

Concentration (µL)	Mean Inhibition (mm)	SD	p-value
10	14.0	1.3	<0.05
20	20.0	1.8	<0.01
30	30.0	2.5	<0.001

The purpose of this study was to assess the effectiveness of a test substance against the fungus *Penicillium notatum.* Three dilutions (10 µL, 20 µL, and 30 µL) of the antifungal agent were analyzed using the agar well diffusion method to determine inhibition zones of the fungi. The concentrations were plotted on the x-axis and the y-axis represented the zone diamances in millimeters. Zone of inhibition refers to the area of annulus where the growth of fungi is inhibited due to the presence of the test substance.

At 10 µL con, the average zone of inhibition was 12 mm. The zones exhibited some variability as depicted by the error bars. When the concentration of the sample was raised to 20 µL, the inhibition zone increased to an average of 20 mm and therefore this shows that there is variation when conducting the inhibition zone test with a moderate standard error. In the last sample, at the concentration of 30 µL, the biggest zone of 30 mm, on average, was obtained, thus showing the highest antifungal activity. Even with the error bars showing variation in the measurements to some extent, the data still showed an increasing trend in the inhibition zone diameter with increasing concentration. In general, a positive response was observed for the relationship between the concentration of the antifungal substance and the degree of inhibition of growth of *Penicillium notatum*.

The zone of inhibition gradually expanded from the initial 12 mm to 30 mm when the concentrations varied from 10 µL to 30 µL. A certain level of difference was observed between the experimental replicates; however, the zones of inhibition increased as the concentration of the test compound was increased. The findings presented herewith indicate that this substance exhibits concentration-dependent antifungal activity against *Penicillium notatum*. Concentration dependant analysis was done to determine the antifungal efficiency. At 10 µL, the average inhibition zone was 14mm with a standard error of 1.3mm and a $p< 0.05$. The 20 µL concentration was above average as it had a mean of 20 mm, a standard deviation of 1.8 mm and the p-value was less than 0.01. Lastly, 30 µL had a mean inhibition of 30 mm, SD of 2.5 mm, and $p < 0.001$. Comparing the

two sets of findings, improved inhibition zones were observed at higher concentrations with higher statistical values.

5 Discussion

The present study aims at assessing the efficacy of *Zingiber officinale* (ginger) extract on *Salmonella typhi* and *Penicillium notatum*, and its antibacterial and antifungal efficacy respectively. The result showed that the growth of both the test microorganisms was inhibited by the ginger extract in a concentration-dependent manner.

In the disc diffusion assay, the ginger extract exhibited antibacterial activity against the pathogenic *S. typhi*, with the zones of inhibition varying from 12.5 ± 1.2 mm to 25 ± 2.1 mm at the concentrations of 10 µL to 30 µL. These findings are in agreement with other studies on the antibacterial efficacy of ginger. In a similar disc diffusion test, the methanol extract of ginger exhibited inhibition zones of 14-19mm against *S.typhi* at a concentration of 25-200mg/ml [18]. Similarly, acetone and methanol extracts showed inhibition zones of 9-14 mm and 10-15 mm respectively against *S. typhi* at 2 mg/disc loading [19]. This observed antibacterial activity has been attributed to active phytochemicals present in ginger which include the gingerols, shogaols, paradols and gingerdiones [20]. These compounds can compromise the bacterial cell membrane, interfere with enzymes required for cell wall synthesis, deplete the intracellular ATP, and affect the host cell signaling [21].

The results of the inhibition zones were in parallel with the increase in the concentrations of the ginger extract. Mean zones were increased from 12.5 ± 1.2 mm at 10 µL to 25 ± 2.1 mm for 30 µL concentration. The statistical evaluation of the results revealed that the antibacterial effect increased with the concentration ($p<0.05$ at 10 µL; $p<0.01$ at 20 µL; $p < 0.001$ at 30 µL). These results are in agreement with earlier studies whereby the methanolic extract of ginger at a concentration of 100mg/ml exhibited an inhibition zone of 14.33 mm against *S. typhi* while at 200mg/ml had an inhibition zone of 19mm [22]. Concentration-dependent antibacterial activity may be due to increased proportions of bioactive phytochemicals with bacterial cells at higher concentrations of the extract that enhances the bacteriostatic or bactericidal effects [23]. All in all, the ginger extract possesses moderate antibacterial efficacy against *S. typhi*, the pathogen responsible for typhoid fever.

The anti-fungal activity of the disc diffusion method established ginger extract to possess effective action against the

contaminant fungus *P. notatum*. The inhibition zones varied from 12 mm at 10µL to 30 mm at 30 µL concentration. These observations affirm previous findings. Hexane extract of ginger rhizomes slowed down the growth of *P. notatum's* mycelia with an average zone of inhibition of 15–25 mm by using the inverted Petri plate method [24]. In the same way, the acetone and methanol extracts of ginger had 13 mm and 16 mm zones of inhibition respectively against *P. notatum* at 2 mg/disc concentration [25]. The antifungal activity of the ginger extract was attributed to its constituent compounds gingerols, shogaols, parasols, and other polyphenols [26]. They can also disrupt fungal membranes, affect fungal enzymes such as $\beta-(1 \rightarrow 3)$-glucan synthase, and prevent cell wall construction and organization [27].

The sensitivity of *P. notatum* to the ginger extract was observed to be dose-dependent as shown by the increase in the zones of inhibition as the concentration of the extract increased. The 10 µL extract inhibited a zone of 12mm and the 20 µL extract had an inhibition zone of 20mm. The highest activity was observed at 30µL with a 30mm zone of inhibition. The result of the statistical test showed that there was a significant difference between the groups at 10, 20 and 30 µL ($p < 0.05$, $p < 0.01$, $p < 0.001$ respectively). Similar trends were observed in earlier studies where MIC reduced from 1.25 mg/mL to 0.16 mg/mL of hexane extract of ginger against *Botrytis cinerea* as the extract concentration increased [28]. The presence of a higher number of antifungal constituents contacting the fungal hyphae could have also increased the activity due to improved concentration of the active ingredients. Consequently, the ginger extract inhibited the growth of *P. notatum* in a concentration-dependent manner.

In general, this study offers sufficient proof of the concentration-dependent antimicrobial and antifungal effects of *Z. officinale* extract. Future studies may extract the active compounds and test them for efficacy against drug-resistant organisms. Furthermore, in vivo, trials and clinical research can provide a road map for therapeutic uses.

6 Conclusion

Consequently, the research showed that *Zingiber officinale* (ginger) extract possesses concentration-dependent antibacterial and antifungal effects. In this case, the 10 µL concentration of the extract had a mean inhibition zone of 12.5 mm against *Salmonella typhi* bacterium, while higher concentrations of 20 µL and 30 µL had increased the mean inhibition zone to 18.0 mm and 25.0 mm, respectively. In conclusion, statistical analy-

sis confirmed the dose-response relationship, the increase in the inhibition zones of bacteria and fungi with the increase in the concentration of the extract from 10 to 30 µL ($p<0.05$ at 10 µL; $p<0.01$ at 20 µL; $p<0.001$ at 30 µL). Altogether, the ginger extract showed moderate, dose-dependent antibacterial and antifungal activity. This was more evident against the fungus than the bacterium. Further research can be done on the identification of the active phytochemicals that are responsible for the observed bioactivities, and to substantiate these findings in animal models. Optimization of the ginger extract formulation and clinical trials will open up the possibility of using it as a better antibacterial and antifungal remedy.

7 References

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