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Inhibitory Effects of Ginger (*Zingiber officinale Roscoe*) and Garlic (*Allium sativum* L) extracts on some Clinical Fungi - *Candida albicans*, *Aspergillus flavus* and *Penicillium* sp.

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Abstract

Background: Infectious diseases caused by fungal pathogens are threatening human health all over the world. *Zingiber officinale* and *Allium sativum* are rich in phytochemical compounds and other active constituents which are capable of inhibiting the growth of microorganisms before they produce any toxins. This study evaluated the inhibitory effects of ginger and garlic on clinical isolates of *Candida albicans*, *Aspergillus flavus* and *Penicillium* sp. **Methodology:** The fungal isolates were characterized using standard microbiological techniques. Three different concentrations were used :-100mg/mL, 50mg/mL and 25mg/mL, using disk and agar well diffusion methods. Summary statistics (mean and standard deviation) were calculated. Independent t-test and one way ANOVA were used to ascertain the mean differences for two groups, and for more than two groups respectively. Further analysis with Duncan post hoc test was done accordingly. All tests were conducted at a 5% level of significance. **Results:** The results of this experiment showed that both ginger and garlic extracts inhibited the growth of the known test fungi in a dose-dependent manner. There was a significant difference in the inhibition of the three concentrations of ginger and garlic $p<0.05$. The two medicinal plants showed antifungal activities in varying degrees, but there was no significant difference in their levels of inhibition of the isolates. **Conclusion:** Garlic and ginger could be used for therapeutic purposes in pharmaceutical industries, and for storage of materials to prevent fungal activities.

Keywords: ***Inhibitory* , Dosage, Fungi**, **Garlic**, **Ginger**, **Plant-Extracts**,.

1. Introduction

Fungal infections have been a major health concern causing significantly high morbidity and mortality rates. Infections caused by fungi can be hard to treat and they can occur in any of the body parts,especially, among individuals with low or compromised immunity. Ginger and garlic contain bioactive compounds known for their antifungal properties. Gingerol, shogaol, zingerone, and other compounds are present in ginger, while garlic consists of allicin, ajoene, diallyl disulfide, and other sulfur-containing compounds. The growth of a broad spectrum fungi such as *Candida*, *Aspergillus*, and *Cryptococcus* species was revealed to be restrained by the active compounds present in ginger and garlic (Menghani *et al.,* 2011). The antifungal effect of ginger and garlic, is determined by the susceptibility dosage of the extracts,however, there are varying degrees of susceptibility of the fungi to the extracts at different dosages; although, there is paucity of information concerning the optimal dosage of ginger and garlic extracts required for maximum effect against different strains of fungi (Maryland, 2006).

Aspergillus flavus is a fungus reportedly found in soil and decaying organic matter, well known for its ability to produce Aflatoxins, a mycotoxin that could effectively contaminate food and feed crops, and lead to severe health issues in animals and humans, are also commonly associated with aspergillosis, sinusitis, keratitis and wound infections (Hedayati *et al*., 2007). The severity of the infection could vary widely depending on the patient's immune status and the site of infection, ranging from mild to life-threatening (San-Blas *et al*., 2008). *Candida albicans* is a yeast that can cause severe infections when it overgrows and spreads,though it is known to be part of the normal microflora in many individuals (Brown *et al*., 2007). As reported by Lopez *et al*. (2017), the usual infection caused by *C. albicans* in humans is candidiasis, and it is significantly risky to individuals with low immunity, and the ability of *C. albicans* to switch between different morphological forms, adherence to and penetration of host tissues, production of hydrolytic enzymes and evasion of host immune responses are all attributed to its virulence .*Penicillium spp* belongs to the genus ascomycetous fungi is part of the mycobiome of many species and is of major importance in the natural environment, in food spoilage, and also involved in some health conditions. Superficial infection (keratitis and otomycosis) and allergic pulmonary diseases are commonly caused by Penicillium spp. (Lyratzopoulos *et al*., 2002).

The findings of this study will provide valuable information on the optimal dosage of ginger and garlic extracts needed to exhibit the maximum antifungal activity against strains of fungi, and their potential use as alternative treatments for fungal infections, thereby, providing a baseline for further studies that could potentially lead to the development of new antifungal therapies.

2. Materials and Methods

This study was carried out within the Ikwo Local Government Area of Ebonyi state, Nigeria, and experiment done in the research laboratory of Alex Ekwueme Federal University Ndufu Alike (AE FUNAI).

2.1 Sample Collection

Fresh cloves of garlic (*Allium sativum*) and rhizomes of ginger (*Zingiber officinale*) were purchased from Nwakpu market, Ikwo Local Government Area, Ebonyi State, and transported to the Microbiology Research Laboratory in AE-FUNAI, where they were sorted, washed and kept ready for use.

Three clinical isolates (*Candida albicans, Aspergillus flavus, Penicillium sp*.) were collected from the Microbiology Laboratory, Department of Microbiology, Alex Ekwueme Federal Teaching Hospital Abakaliki, Ebonyi state, into the sterile nutrient broth. The isolates were stored at 18°C for further use

2.2 Extraction of Plant Materials

Fresh plant materials (garlic and ginger) were peeled and sliced into small pieces. The sliced garlic and ginger were dried under primitive conditions at ambient room temperature with air circulation around the plant material, to avoid heat thereby reducing the possibility of chemical reaction. The dried plant materials were ground to fine size powder to increase the extraction efficiency. 100g of each of the powdered plant materials were measured into two different 250 mL stoppered conical flasks. 100 mL of absolute ethanol and distilled water were poured into each of them and labeled. The plant materials soaked in the different solvents were allowed to stand at room temperature for 2–3 days with frequent stirring, to obtain plant

extracts. A sealed extractor was used to avoid solvent evaporation at atmospheric pressure. The mixtures were then pressed or strained after a specific time, and filtered through No.1 Whattman filter paper. A rotary evaporator was used to concentrate the filtrate. Reconstitution of the extract was done by dissolving 10g in 1000 mL of distilled water (i.e 100 mg /mL) to serve as the stock solution of the extract. Also, 50mg/mL and 25mg/mL of the extract were made and stored properly.

2.3 Preparation of Media and Preparation of Sensitivity Disks

All media used were prepared according to the manufacturer's instructions. With the aid of a paper punch, disks of 6mm in diameter were punched out using No.1 whattman filter paper. The disks were then sterilized by autoclaving at 121^{o}C for 15min, after which they were allowed to cool.

2.4 Confirmation of the Test Organisms

The test organisms were confirmed using morphological and microbiological staining techniques. The *candida albicans* were subjected to gram staining and germ tube tests. For the gram staining reaction, a microscope glass slide was placed on the bench, and a drop of distilled water was added to it. A moderate amount of the test organism's colony was collected on a sterile loop and mixed with the distilled water on the slide to make a smear and was allowed to air dry. The smear was heat-fixed using a Bunsen burner, flooded with crystal violet and left to sit for 1 minute before it was rinsed off the slide with water, then flooded with iodine solution and allowed to stand for 1 minute. It was rinsed again with water and then decolorized with ethyl alcohol for about 10 seconds and then rinsed with water. The slide was also flooded with safranin and allowed to stand for 1 minute and was rinsed again with water. It was gently dried, then examined under a microscope using oil immersion and was confirmed based on their spore morphology. A germ tube test was done by putting 1 ml of sheep serum into a small tube and a colony of yeast was touched with a sterile loop and gently emulsified in the serum. The tube was then incubated at 37°C for 4 hours. Microscopic examination of the loop-ful of the serum was done under high power objectives (Abiroo *et.al*, 2018).

The mold cultures (48 hours) growing on the PDA plates were identified macroscopically by observation of colony color, type texture (velvety, cottony, coarse), shape and growth pattern. Direct observation of culture under the light microscope (low power) by careful preparation of slides, stained with cotton blue-in-lactophenol was done. The diagnostic features was used in identification according to the manual and guides (Nelson *et al.* 1983, and Barnett and Hunter, 2000).

2.5 Inoculation of Organisms and Determination of Antifungal Effect of Ginger and Garlic

Two methods were compared in the determination of the antifungal effect of ginger and garlic. The methods employed were the Agar well diffusion method and the disk diffusion method. Inoculum of the test organisms (*Aspergillus flavus, Candida albicans and Penicillium* sp) were prepared in 3ml of sterile Normal saline (0.85 % NaCl) and standardized with 0.5 McFarland standard and labeled accordingly.

2.5.1 The Agar well diffusion

The Agar well diffusion method was carried out in a Petri dish containing Potato Dextrose Agar (PDA). Two wells that were evenly spaced were created using a sterile cork borer. Using a sterile swab stick, the test organism was introduced to the surface of the prepared agar plate, and labeled accordingly. With the use of a sterile syringe, both wells were filled with 1ml of a prepared extract solution and labeled

accordingly. This procedure was followed using all the concentrations of the plant extracts – 100mg/mL, 50mg/mL, and 25mg/mL. Control experiments with the fungal isolates inoculated without treatment were also set up and monitored for growth.

2.5.2 The disk diffusion

The disk diffusion test was carried out. In a Petri dish containing solidified potato dextrose agar (PDA), a sterile swab stick was used to introduce 0.5 ml of the known test fungal isolates to the surface of the prepared agar plate, and labeled accordingly. Two sterile disks were introduced into each concentration of the plant extract, and each disc was allowed to absorb the solution for one hour before it was placed in the Petri dishes containing the inoculated fungal isolates, and labeled accordingly. This procedure was followed using all the concentrations of the plant extracts – 100mg/mL, 50mg/mL and 25mg/mL. The control experiments were also set up, and all were incubated at 27 ^{0}C for 24 hours and then 48 hours to observe growth. The antifungal effects of ginger and garlic were observed for zones of inhibition. The zones of inhibition were measured in mm, using a plastic ruler.

2.6 Data Analysis

Descriptive statistics (mean and standard deviation) were calculated for replications. The mean difference for the two groups was ascertained using an independent t-test, while the mean difference for more than two groups was ascertained using one way ANOVA. Duncan was adopted as a post hoc test. All tests were conducted at a 5% level of significance.

3. Results

3.1 Morphological Characterization of the Test Organisms

The table below shows the morphological characterization of the fungal isolates of *Candida albicans*, *Aspergillus flavus* and *Penicillum* sp.

Table 1: Cultural and morphology of the known fungal isolates

Surface Colour	Reverse Colour	Zonation	Sporulation	Colonial topography	Phenotypic identity
Creamy	Yellowish brown	Conical	Light	Flat	*Candida albicans*
Granular	Tan	Less furrowed	Slightly heavy	Flat	*Aspergillus flavus*
Blue-green	Pale yellow	Radialley furrowed	Heavy	Flat	*Penicillium* sp

Table 2 shows the result for comparison of the effects of the concentrates of both extracts on *Candida albicans,* using the Disk Diffusion method. The effect was assessed by the zone of inhibition, measured in mm. The result indicated a significant difference in the mean values across the concentrations of both ginger and garlic extracts. However, for garlic extract, no significant difference was observed between the mean values of the inhibition zone at extract concentrations of 50mg/ml and 25mg/ml. Comparing the difference between the two extracts at each concentration, it was observed that there was no significant difference in mean values of the inhibition due to ginger and garlic at concentrations of 100mg/ml and 50mg/ml, but the mean value at 25mg/ml of garlic was significantly higher than that of ginger (t=13.13, p=0.006).

Table 2: Comparison of the concentrates' effects on *Candida albicans* (Disk Diffusion)

	Zone of inhibition for ginger in mm (Mean±SD)	Zone of inhibition garlic in mm (Mean±SD)	T	p-value
Concentration				
100mg/ml	22.5^{d}±0.7	21.0^{c}±1.4	1.34	0.408
50mg/ml	17.0^{c}±2.8	16.0^{b}±2.8	0.35	0.757
25mg/ml	5.5^{b}±0.7	12.0^{b}±0.0	13.13	0.006
Control	0.0^{a}±0.0	0.0^{a}±0.0	-	-
F-value	94.59	64.20		
p-value	<0.001	0.001		

Mean down the column with different alphabetical superscripts indicates significant differences, p<0.05 indicates significance.

Table 3 shows the result of the comparison of the effect of the concentrates on *candida albicans* using the Well Diffusion method. The result indicated a significant difference in the mean values of inhibition zones, on exposure to different concentrations of both ginger and garlic extracts. However, no significant difference was observed between the mean values at 100mg/ml and 50mg/ml concentrations of the garlic extract. Comparing the difference between the two extracts at each concentration, there was no significant difference in the mean values of inhibition zones.

Table 3: Comparison of the concentrates' effects on *Candida albicans* (Well Diffusion)

	Zone of inhibition for ginger in mm (Mean±SD)	Zone of inhibition garlic in mm (Mean±SD)	T	p-value
Concentration				
100mg/ml	20.5^{d}±0.7	20.0^{c}±0.0	1.010	0.419
50mg/ml	15.5^{c}±0.7	16.0^{c}±2.8	0.245	0.829
25mg/ml	9.0^{b}±1.4	5.0^{b}±1.4	2.857	0.104
Control	0.0^{a}±0.0	0.0^{a}±0.0	-	-
F-value	192.444	69.53		
p-value	<0.001	0.001		

Mean down the column with different alphabetical superscripts indicates significant differences, p<0.05 indicates significance.

Table 4 shows the result of the comparison of the effects of the concentrates on *Aspergillus flavus* using the Disk Diffusion method. The result indicated a significant difference in the mean values of inhibition zones across the concentrations of both ginger and garlic extracts. However, no significant difference was observed between the mean values of the control and both concentrates (ginger and garlic) at the concentration of 25mg/ml. Comparing the effects of the two extracts at each concentration, there was no significant difference in their mean values at all concentrations.

Table 4: Comparison of the concentrates' effects on *Aspergillus flavus* (Disk Diffusion)

	Zone of inhibition for ginger in mm (Mean±SD)	Zone of inhibition garlic in mm (Mean±SD)	T	p-value
Concentration				
100mg/ml	22.5^{b}±2.1	22.0^{c}±2.8	0.202	0.859
50mg/ml	15.0^{b}±4.2	15.0^{b}±0.0	-	-
25mg/ml	4.0^{a}±2.8	3.5^{a}±2.1	0.202	0.859
Control	0.0^{a}±0.0	0.0^{a}±0.0	-	-
F-value	27.689	66.387		
p-value	0.004	0.001		

Mean down the column with different alphabetical superscript indicates significant difference, $p<0.05$ indicates significance.

Table 5 shows the result of the comparison of the effects of the concentrates on *Aspergillus flavus* using the Well Diffusion method. The result indicated a significant difference in the mean values of zone inhibition across all the concentrations of both ginger and garlic extracts. However, no significant difference was observed between the mean value of inhibition due to the control, and due to garlic extract at concentration of 25mg/ml. Comparing the difference between the two extracts for each level of concentration, it was observed that there was no significant difference in the mean values of inhibition at all the concentrations

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Table 5: Comparison of the concentrates' effects on *Aspergillus flavus* (Well Diffusion)

	Zone of inhibition for ginger in mm (Mean±SD)	Zone of inhibition garlic in mm (Mean±SD)	T	p-value
Concentration				
100mg/ml	21.5^{d}±0.7	21.0^{c}±1.4	0.452	0.696
50mg/ml	14.5^{c}±0.7	13.0^{b}±0.0	3.031	0.094
25mg/ml	4.5^{b}±0.7	1.5^{a}±2.1	1.917	0.195
Control	0.0^{a}±0.0	0.0^{a}±0.0	-	-
F-value	502.556	121.923		
p-value	<0.001	<0.001		

Mean down the column with different alphabetical superscripts indicates significant differences, $p<0.05$ indicates significance.

Table 6 shows the result of the comparison of the effects of the concentrates on *Penicillum* sp, using the Disk Diffusion method. The result indicated a significant difference in the mean values across all the concentrations of both ginger and garlic extracts. However, no significant difference was observed between the mean values due to the control, and the values due to both extracts at concentration of 25mg/ml. Comparing the differences between the effects of the two extracts at each concentration, no significant difference in their mean values of inhibition was observed at all the concentrations.

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Table 6: Comparison of the concentrates' effects on *Penicillium sp* (Disk Diffusion)

	Zone of inhibition for ginger in mm (Mean±SD)	Zone of inhibition garlic in mm (Mean±SD)	T	p-value
Concentration				
100mg/ml	21.0^{c}±1.4	17.0^{c}±1.4	2.857	0.104
50mg/ml	13.5^{b}±2.1	11.5^{b}±0.7	1.278	0.330
25mg/ml	0.0^{a}±0.0	2.0^{a}±2.8	1.010	0.419
Control	0.0^{a}±0.0	0.0^{a}±0.0	-	-
F-value	133.615	48.937		
p-value	<0.001	<0.001		

Mean down the column with different alphabetical superscripts indicates significant differences, $p<0.05$ indicates significance.

Table 7 shows the result of the comparison of the effects of the concentrates on *Penicillium* sp using the Agar Well Diffusion method. It indicates a significant difference in the mean values of inhibition zones across all the concentrations of both ginger and garlic extracts. However, no significant difference was observed between the mean values due to the control, and the values due to both extracts at a concentration of 25mg/ml. Comparing the difference between the effects of the two extracts on the isolate, there was no significant difference in their mean values of inhibition at all the concentrations.

Table 7: Comparison of the concentrates effect on *Penicillum sp* (Well Diffusion)

	Zone of inhibition for ginger in mm (Mean±SD)	Zone of inhibition garlic in mm (Mean±SD)	T	p-value
Concentration				
100mg/ml	19.5^{c}±0.7	18.5^{c}±0.7	2.857	0.104
50mg/ml	12.0^{b}±0.0	11.5^{b}±2.1	1.278	0.330
25mg/ml	0.0^{a}±0.0	1.5^{a}±2.1	1.010	0.419
Control	0.0^{a}±0.0	0.0^{a}±0.0	-	-
F-value	1473.000	64.193		
p-value	<0.001	0.001		

Mean down the column with different alphabetical superscripts indicates significant differences, $p<0.05$ indicates significance.

4. Discussion

Growing the test fungi on media containing ginger and garlic showed obvious growth inhibitions of the pathogenic fungi. Ginger and garlic inhibited the growth of *Candida albicans, Aspergillus flavus* and *Penicillium* sp as shown in Table 2 to 7 which agrees with a study that investigated the effect of ginger extract and its active compounds on the growth of *Candida albicans* and *Aspergillus flavus*; The study found that ginger and garlic extract and its active compounds inhibited the growth of these fungi (Chang *et al*., 2013). In another study ginger and garlic extract was able to inhibit the growth of *Penicillium* sp and was attributed to the presence of several chemical compounds in them which can interfere with the fungal cell wall thereby leading to their death (Chuku *et al*., 2010). Another study found that ginger and garlic extract had a potent antifungal effect against *Aspergillus flavus* (Kumar *et al.*, 2015).

The results of this experiment also showed that both ginger and garlic extract inhibited the growth of the known test fungi in a dose-dependent manner which suggested that the extracts were more effective at greater concentrations, because, the greater the concentration, the higher the inhibition that occurred. This research supports the findings of Chuku *et al*. (2010) ,who observed that ginger and garlic inhibited the growth of pathogenic fungi in higher concentrations. This research showed a significant difference among all the concentrations tested on the three organisms, using both extracts, with 100mg/ml showing the highest zones of inhibition on all the fungal isolates using both extracts. The 50mg/ml concentrations showed mild or moderate inhibition on the three known fungal isolates, using both ginger and garlic isolates, the 25mg/ml concentrations showed little or no inhibition on the three fungi isolates, while the control showed no inhibition. The reason for this inhibition of the known pathogenic fungi might have been attributed to the active ingredients in ginger (Gingerol) and garlic (Allicin) (Sabitha *et al*., 2005). The minimal inhibitory concentration (MFC) of ginger and garlic was observed to be 100mg/ml and 50mg/ml respectively. This is in relation to a study that showed that ginger and garlic inhibited the growth andsporulation of *Aspergillus flavus* with the minimum inhibitory concentration ranging from 40-80mg/ml (Dhanasekar *et al*., 2015).

The result in the two different methods of disk diffusion and agar diffusion showed no significant difference. Also, there was no significant difference in the inhibition of the test organisms using the two extracts. Both extracts gave a dose-dependent inhibition with no significant difference between the two extracts. However, the mean value of inhibition at 25mg/ml of garlic against *Candida albicans*, using the disk diffusion method, was significantly higher than that of ginger with a t-value of 13.13 and a p-value of 0.006. This showed that garlic was more effective against *Candida albicans* at low concentrations than ginger, also that the disk diffusion method could be more sensitive for testing the antifungal properties of garlic. The result was in contrast with the findings of Hand (2013), who investigated the effectiveness of ginger and garlic on selected bacteria, using the disk diffusion method and agar well diffusion method. He revealed that the agar well diffusion method was more effective than the disk diffusion method. In addition to the direct antifungal activity of ginger and garlic, both ginger and garlic have been shown to enhance the immune system's response against fungal infections. This was thought to be due to their ability to increase the production of cytokines, signaling molecules that play a key role in the immune system's response to infections (Rappleye *et al.,* 2006).

Conclusion

The antifungal effects of ginger and garlic have been extensively studied and proven effective. The inhibitory dosage of ginger and garlic varied, depending on the fungal strain and the concentration of the active compounds. Overall, both ginger and garlic have high potency in inhibiting fungal growth, and their low toxicity makes them safe for use as alternative antifungal agents. Thus, incorporating ginger and garlic in our daily diet, or using them as complementary therapy could help prevent and treat fungal infections. Further research is needed to determine the optimal dosage and administration route for their antifungal effects.

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Authors Contribution

Concept development,Supervision of the work, Data analysis,Writing of the work *****Obaji, D.G; **Laboratory work** Nzerem, V.C . All authors read and approved the final manuscript.

Ethical approval

The present research work was permitted by the Ethical Committee of Alex Ekwueme Federal University, Ndufu-Alike..

Conflict of interest

Authors declare no conflict of interest.