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Antifungal Action of Garlic (*Allium sativum*) and Ginger (*Zingiber officinale*) on Some Pathogenic Fungi

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Authors' contributions

This work was carried out in collaboration among both authors. Author VAN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author CAA managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Background: The antifungal activities of fresh garlic (*Allium sativum*) and ginger (*Zingiber officinale*) on the growth of three known pathogenic fungi were investigated. The test organisms were *Aspergillus* spp *Penicillium* spp and *Candida albicans*.
Methodology: Two different concentrations of extracts were used. Concentration I which had crude extract of 100 g/ 100 ml of sterile distilled water and concentration II with extract of 100 g/50 m1 of distilled water. Ten mls of each extract was added to each 125 m1 of SAB (saboraud dextrose agar) media before and after sterilization.
Results: The extracts added to the media after its sterilization inhibited the growth of the pathogenic fungi samples more than the extracts added to the media before sterilization. This indicates that the active ingredients present in the extracts which have the antifungal effect observed is negatively affected and inactivated at 121ºC for 15 minutes. It can be inferred that garlic and ginger have different levels of therapeutic values on fungi.

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1. INTRODUCTION

Garlic (*Allium sativum*) is a biennial herb belonging to the family *Alliaceae* that consist of 30 genera and about 600 species [1,2]. It is an important vegetable crop, which is grown in Spain, Mexico, India, China, Italy, Korea, Thailand, Nigeria, Argentina and United states of America [3]. The crop is a widely consumed vegetable in Nigeria. It is a popular item in the diet of the Nepalese [4] who regarded it as a valuable spice food and lucrative commodity for income generation. The dependence of garlic is assumed to be due to its medicinal properties [1]. The crop is also reported to Strengthen and help protect the heart against circulatory diseases, is a powerful antibiotic and an agent that helps the body to inhibit the growth and development of dangerous microbes [5,6,7].

Garlic though very important has an offensive and pungent odour. Recent research has shown that fruits such as paw paw, tomatoes, banana and Oranges can be used to deodorize it when chewed. But amongst the fruits, tomatoes (*Lycopersicon escientum*) have the highest degree of effectiveness. It has also been shown that garlic just like ginger has different uses, which includes anti bacterial activity [8,9]. This property was attributed to diallyl suiphide which is an unstable sulphide in alkyl polysulphide [10].

Ginger (*Zingiber officinale*) on the other hand is from the Zingiberaceae that is usually cultivated in areas of much rainfall. Its pungency is as a result of an oleoresin, which may be extracted together with a volatile oil, by ether, alcohol or acetone [10]. It has a lot of uses which includes traditional, spices, pharmacological that comprises the anti inflammatory and anti microbial effects [11,12]. Both ginger and garlic have a common relationship as they help in regulating blood pressure, blood sugar, reduce cholesterol and prevent arteriosclerosis [12].

Though several investigators have worked on the antibacterial activities of these extracts, much attempt was not made to investigate the anti fungal activity of these important herbs on the growth and survival of pathogenic fungi.

2. MATERIALS AND METHODS

This study was carried out in the capital city of Rivers State popularly known as the Garden city. The city covers an area of about 369 kilometer squares. The study was done in the diagnostic Laboratory of Rivers state university Port Harcourt.

2.1 Sample/Pathogenic fungi used

Fresh garlic bulbs (*Allium sativum*) and ginger rhizomes (*Zingiber officinale*) were purchased from mile I and Mile III markets Port Harcourt, Rivers State of Nigeria and transported to the diagnostic Laboratory of Rivers state university Port Harcourt where they were sorted, washed and kept ready for use.

Fresh ginger and garlic samples were taken to the Food Science and Technology Laboratory in Rivers State University of Science and Technology for proximate analysis. The parameters analysed were done according to the Association of Official Analytical Chemists methods in 1990 [13].

The pathogenic fungi used were *Penicillium spp, Aspergillus spp* and *Candida albicans* all gotten from the culture collections of the University of Port Harcourt Teaching Hospital (U.P.T.H), Port Harcourt. Wet mounts of the fungi on cotton blue placed in Lactophenol were examined microscopically and the fungi confirmed based on their spore morphology, nature of the mycelia [14,15].

2.2 Preparation of Extracts

Two concentrations of extracts were prepared. Concentration I is a lesser concentration than concentration II which was done to achieve a greater concentration of the antifungal ingredients in the extract.

2.3 Preparation of Garlic Extract

Concentration I:

With the aid of a weighing balance, 100 g of garlic cloves were measured. It was peeled with the aid of a sterile lancet washed properly with sterilized water and pounded with the aid of a sterile mortar and pestle, 100 ml of distilled water was added to it and the extract was gotten with the aid of a sterile muslin cloth. 20 mls of the extract was obtained and kept in two different test tubes, 10 mls in each.

Concentration II:

With the aid of a weighing balance, 100 g of garlic cloves was also measured, peeled with the aid of a sterile lancet and washed with sterilized water. A sterile mortar and pestle was used in pounding it and 50 mls of distilled water was added to it. The extract was then gotten with the aid of a sterile muslin cloth; 20 mls of the extract was also obtained and kept in two [2] different test tubes. 10 mls in each test tube.

2.4 Preparation of Saboraud Dextrose Agar Garlic Incorporated Extracts Media

Normally, 65g of SDA (saboraud dextrose agar) is supposed to be dissolved in 1000 ml of distilled water but for 500 mls of distilled water, 32.5 g of SDA was used. The 500mls of distilled water and 34.5 g of SDA mixture was now divided into four [4] equal parts of 125m1s each. Into two parts, test tubes G_{al} and G_{all}, 10mls of concentration I and concentration II was added. All including the other two test tubes G_{al} and G_{all} containing the agar without the extracts were also sterilized at 121ºC for l5minutes. They were allowed to cool for 45ºC after sterilization, then 10 mls of concentration I and 10 mls of concentration II were added to test tubes G_{al} and G_{all} the plates were then poured and a Bunsen flame was used to remove air bubbles and also for sterilization. The plates were labeled and stalked.

2.5 Preparation of Ginger Extract

Concentration I:

With the aid of a weighing balance, 100 g of ginger rhizome were measured. They were peeled using a sterile lancet washed properly in sterilized water and pounded with the aid of a sterile mortar and pestle. It was dissolved in 100mls of distilled water and the extract was obtained sieving through a sterile muslin cloth. 20 mls of the extract was obtained and kept in two different test tubes 100 mls in each.

Concentration II:

With the aid of a weighing balance, 100 g of ginger rhizomes was measured, peeled, washed, pounded and extracted as in concentration 1 above. Using only 50 mls of distilled water for the extraction. 20 mls of the extract was also obtained and kept in two different test tubes 10 mls in each.

2.6 Preparation of SDA Ginger Extract Incorporated Media

With the aid of a weighing balance, 32.5 g of SDA (saboraud dextrose agar) weighed and dissolved in 500 mls of distilled water and the mixture was shared into four [4] equal parts J_{al} J_{all} J_{bl} and J_{bll}. Into J_{al} and J_{all}, l0mls of the extracts of concentration I and concentration II were added correspondingly, sterilized at 121ºC for 15 minutes. The other two parts without the extracts (J_{bi} and J_{bll}) were also sterilized at same temperature and time. They were allowed to cool to 45ºC and then the l0ml extracts of concentration I and concentration II were added to the agar without the extracts before sterilization (J_{bl} and J_{bll}) and poured into agar plates. A Bunsen flame was used for sterilization and removal of air bubbles. The plates were then labeled and stalked.

2.7 Inoculation of Pathogenic Fungi

The various pathogenic fungi namely *Penicilium spp, Aspergillus spp* and *Candida albicans* were inoculated aseptically onto the garlic-agar and ginger —agar media. This was done with the aid of a sterile wire loop that was flamed at intervals for proper sterilization. The plates were incubated at ambient temperature (28-35ºC) for 3.5 days; plates that showed growth of the pathogenic fungi were examined and recorded. Those that inhibited the growth of the pathogenic fungi were also observed and recorded.

3. RESULTS

The anti fungal effects of the sample extracts on the known pathogenic fungi species are shown in the tables below. Table 1 show the inhibition of fungal growth by sterilized concentration II (100g/50ml) of garlic and ginger. *Candida albicans* (75/75%) growth was inhibited in the two media more than *Peniicillium spp* (50/75%) which showed lesser inhibition on sterilized garlic medium while the growth of *Aspergillus spp* (50/50%) was fairly mild in both media. Table 2 on the other hand shows the inhibition of fungal growth by unsterilized concentration II (100 g/50 ml) of garlic and ginger. There was complete inhibition (100%) in the three [3] pathogenic fungi species; *Candida albicans*, *Penicillium spp* and *Aspergillus spp*.

Table 3 shows the inhibition of pathogenic *Fungi spp* by sterilized concentration I (100g/ 100 ml) of

Table 1. The inhibition of pathogenic *Fungi spp* by sterilized concentration II (100 g/50 ml) of garlic and ginger extracts (G_{all} and J_{all})

Types of fungi	Media type	
	Sterilizer garlic (100 g/50 ml)	Sterilized ginger (100 g/50 ml)
Aspergillus spp	50%	50%
Penicillium spp	50%	75%
Candida aibicans	75%	75%

Table 2. The inhibition of pathogenic *Fungi spp* by unsterilised concentration II (100 g/50 ml) of garlic and ginger extracts (G_{bll} and J_{bll})

Types of fungi	Media type	
	Sterilizer garlic (100 g/50 ml)	Sterilized ginger (100 g/50 ml)
Aspergillus spp	100%	100%
Penicillium spp	100%	100%
Candida aibicans	100%	100%

Table 3. The inhibition of pathogenic *Fungi spp* by sterilized concentration I (100 g/100 ml) of garlic and ginger extracts (G_{al} and J_{al})

Types of fungi	Media type	
	Sterilizer garlic (100 g/100 ml)	Sterilized ginger (100 g/100 ml)
Aspergillus spp	25%	25%
Penicillium spp	50%	75%
Candida aibicans	75%	75%

Table 4. The inhibition of fungi spp by unsterilised concentration I (100 g/100 ml) of garlic and ginger extracts (G_{bl} and J_{bl})

Types of fungi	Media type	
	Sterilizer garlic (100 g/100 ml)	Sterilized ginger (100 g/100 ml)
Aspergillus spp	50%	50%
Penicillium spp	50%	25%
Candida aibicans	25%	25%

garlic and ginger extracts. *Candida albicans* (75/75%) growth was inhibited in the two media more than *Penicillium* spp (75/50%), which showed lesser inhibition on sterilized garlic medium while the growth of *Aspergillus* spp (25/25%) was fairly mild in both media. In Table 4, the inhibition of fungal growth by unsterilized concentration I (100 g/ 100 ml) of garlic and ginger extracts was shown. There was more inhibition of *Candida albicans* (75/75%) as compared to *Aspergillus* spp (25/50%) and *Penicillium* spp (50/25%) where there was mild growth using both unsterilised garlic and ginger incorporated media.

The antifungal action of garlic and ginger on the three [3] known pathogenic Fungi spp are shown in tables 1-4. Under the conditions of test from the tables, the more concentrated extracts (100g/50ml) inhibited the Fungi spp more than the less concentrated (100 g/ 100 ml) extracts where moderate inhibition was observed. The actions of these extracts in these organisms were more on garlic. Between the two extracts used, the best in terms of inhibition is garlic followed by ginger.

4. DISCUSSION

Our interest in researching on garlic and ginger is as a result of their several therapeutic purposes. Studies have shown that they can be used as a natural treatment of diabetes [16], hypertension [17], cancer [18], inflammation, thrombosis [19], cardiovascular and Alzheimer's diseases [20, 21]. This research has shown that garlic and ginger have antifungal effects based on the concentration of extracts used and the application of heat to the extracts. During sterilization or whether they are used raw

(unsterilized). The unsterilized garlic media in concentration II (100 g/50m1) (G_{bII}) inhibited the growth of all the three [3] known pathogenic fungi in this work which are *Aspergillus spp*, *Penicilhium spp* and *Candida albicans.* The reason for the inability of these fungi to grow could be attributed mainly to the active ingredients, allicin in garlic and ginger oleoresin in ginger that inhibited the growth of some pathogenic bacteria [7,8] (Table 2). Whereas the unsterilized garlic and ginger incorporated media for the same concentration II did not inhibit the growth of the pathogenic *fungi spp* since limited growth was observed. This is an indication that heat probably had negative effect on the extracts by denaturing the active ingredients allicin and oleoresin. Areas where growth did not occur remained the same even after two weeks of incubation of inoculated agar plates. This indicates that garlic and ginger have some antifungal effects and their active ingredients are likely to be heat labile hence raw extracts (unsterilzed) of concentration II showed a complete inhibition on the test fungi while the heated (sterilized) concentration II did not inhibit their growth as shown on table 1 and table 2.

On the other hand, in the sterilized concentration I (100 g/100 ml) of garlic and ginger extract media, *Candida albicans* growth was inhibited more in the media than *Penicillium spp* which showed lesser inhibition on sterilized garlic media while the growth of *Aspergillus spp* was lowest. Whereas, the inhibition of fungal growth by unsterilized concentration I (100 g/100 ml) of garlic and ginger extract incorporated media shows little inhibition of the three pathogenic fungi using unsterilized ginger as compared to unsterilised garlic where there was mild inhibition of *Candida albicans* as compared to *Aspergillus spp* and *Penicillium spp* where there was mild growth. Other studies have shown ginger and garlic having antifungal activities on some other test fungi [21,22,23,24].

5. CONCLUSION

In this study using the following test *fungi spp; Candida albicans, Aspergillus spp* and *Penicillium spp* and growing them on garlic and ginger incorporated media, there was inhibition of the growth of the pathogenic fungi. The application of heat to extract reduces its antifungal activity. Therefore, the extracts are at their best when used as crude unheated natural extracts. The concentration is also important because the greater the concentration, the better the inhibition. Conclusively, garlic and inhibited the growth and survival of pathogenic *Fungi spp Candida albicans, Aspergillus spp* and *Penicillium spp*.

Based on this study, garlic and ginger could be used for therapeutic purposes and useful in the pharmaceutical industries. They can also be used in storage of materials to prevent fungi activities in a developing country like Nigeria because it is cheap, accessible and an available natural material.

COMPETING INTERESTS

Authors have declared that no competing interest exists.

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