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Research Article

ANTIFUNGAL ACTIVITY OF GARLIC EXTRACT ON FUNGI ASSOCIATED WITH URINARY TRACT INFECTIONS AMONG FEMALE STUDENTS OF JOSEPH SARWUAN TARKA UNIVERSITY

Agada Emmanuel Oloche, Benson Terer Targema*, Mary Chinenye Ugwuanyi

Joseph Sarwuan Tarkaa University, Biological Sciences, Microbiology, Makurdi, Nigeria

**Corresponding Author:*
E-mail: bensontargema@mail.com

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ABSTRACT. Researchers from all around the world are becoming more interested in the medicinal properties of plant extracts. Since modern medicine has its own benefits and drawbacks, plant-based solutions are becoming more and more popular since they are risk-free, readily available, and reasonably priced. The main purpose of this study was to determine the antifungal activity of garlic (*Allium sativum*) extract on fungi associated with urinary tract infections among female students of Federal University of Agriculture Makurdi. Garlic cloves were purchased from North Bank market and were transported to Microbiology Laboratory. They were blended into paste and were extracted using maceration method. Urine samples collected were analyzed using standard microbiological methods. Extract concentrations from 500mg/mL-100mg/mL were prepared using a double standard dilution method. About 50 urine samples were collected and the fungi present in the urine were isolated using standard microbiological methods. Susceptibility analysis of the fungal isolates was done using the aqueous and ethanolic garlic extract, and the zones of inhibitions obtained at the various concentrations were compared with a positive control (fluconazole). Analysis of data obtained was done using a Statistical Package for Social Science (SPSS). The result showed a mean zone of inhibition of 17.6×10± 1.00×10 mm at a concentration of 500mg/mL, 13.6×10 ± 1.52×10 mm at a concentration of 250mg/mL and 22.0×10 ± 1.00x10mm for the positive control (fluconazole) on *Candida albicans* using the aqueous garlic extract while for the ethanolic garlic extract showed a mean zone of inhibition of 18.3x10±1.52×10mm at a concentration of 500mg/mL 13.0×10 ± 2.00×10 mm at 250mg/mL. The mean zones of inhibition for 200mg/mL, 125mg/mL, and 100mg/mL were 9.67x10±5.77×10 mm, 7.67×10 ±5.77×10 mm and 3.67×10± 5.77×10mm respectively. Garlic extract has potential antifungal activity on the isolated urinary tract organisms and as such can be used as a natural antifungal agent in the control of fungal urinary tract infections.

Keywords: *Antifungal activity, garlic extract, isolates, urinary tract infections.*

INTRODUCTION

Researchers from all around the world are becoming more interested in the medicinal properties of plant extracts. Since modern medicine has its own benefits and drawbacks, plant-based solutions are becoming more and more popular since they are risk-free, readily available, and reasonably priced [1]. Several substances show antifungal action [2]. One of the most significant and ancient herbs used for medicine is garlic. Since it is a key food spice plant, garlic has been used all round the world. It is crucial for disease prevention and control, and many illnesses can be treated with it. It has long been

employed against human diseases. However, there are few research on the effectiveness of garlic in treating plant diseases. The effects of garlic on infections have been covered in earlier works [3]. Since the dawn of time, people have used garlic as a folk remedy for a number of diseases which includes hemorrhoids, snake bites, rheumatism, stomachaches, and parasitic infections. Today, in many nations, garlic is used as a remedy for various illnesses [4]. Many academics demonstrated garlic's insecticidal, antibacterial, antiprotozoal, and antifungal properties. With regard to pathogenic yeasts, particularly *Candida albicans*, Kabelic showed that garlic extract was more efficient [5]. Sulfur-containing chemicals make up garlic. These sulfur-based chemicals, the most significant of which are allicin and the breakdown products diallyl sulphide (DAS) and diallyl disulphide (DADS), are thought to be responsible for the therapeutic properties of garlic [6]. Because they interact with numerous nearby proteins, including the allinase enzyme, the allicin molecules produced have an extremely short half-life [7]. This enzyme therefore becomes almost suicidal. The ability of allicin, which is derived from fresh garlic, to enter cell membranes, act as an antioxidant, and attach sulfhydryl (SH) groups to enzymes, and block sulfhydryl enzymes are all factors in its therapeutic action [8].

MATERIALS AND METHODS

Sample collection

Garlic cloves were purchased in North bank market Makurdi metropolis, they were transported to Microbiology research laboratory, Joseph Sarwuan Tarka University, Makurdi. The fresh garlic cloves were peeled and blended with a blender to obtain the paste from which the extract was made. About 50 urine samples were randomly collected from female students in hostels of Joseph Sarwuan Tarka University Makurdi, they were transported to the Research Laboratory and were analysed using standard microbiological procedures under aseptic conditions.

Preparation of garlic extract

Aqueous garlic extract preparation

After swirling the mixture intermittently for a while to achieve homogeneity, it was combined with 500 mL of double standard distilled water. The combination was then filtered and centrifuged once more for 20 minutes at 10,000 rpm. In order to get rid of any impurities, the supernatant was filtered through a Wattman filter paper grade 1 with 0.2 mm pore size [9].

Ethanolic garlic extract preparation

500 ml of ethyl alcohol and 250 g of crushed garlic paste were combined in a glass container and heated to 350°C for several minutes to create a uniform mixture. After filtering, the mixture underwent a second, 20-minute centrifugation at 10,000 rpm. To get rid of any contaminants, the supernatant was filtered through Wattman filter paper grade 1 with 0.2 mm pore size. As a result, the alcoholic extract was heated to evaporate the ethyl alcohol and yield the crude extract [9].

Preparation of garlic extracts concentrations

The crude garlic extracts were obtained in five different concentrations, ranging from 500 mg/mL to 250 mg/mL, 200 mg/mL to 125 mg/mL, and 100 mg/mL, using the double standard dilution method. In order to do this, the extracts were diluted in the right solvents at the relevant milligram concentrations [10].

Media Preparation and Inoculation of Samples

The preparation of Potato Dextrose Agar (PDA) utilized followed the guidelines provided by the manufacturers. It was sterilized for 15 minutes at a pressure of 15 pounds and 121 degrees Celsius in an autoclave. After chilling to a temperature of around 42°C, it was put into plates, and the swap samples were infected using the pour plating method after a serial dilution [10].

Fungal Isolation and ıdentification

The morphological traits, such as shape and pigmentation, as well as microscopic analysis employing lactophenol cotton blue stain, were used to identify the fungal isolates.

Microscopic examination of the ısolates

For a microscopical examination for spores and vegetative bodies, isolates of the fungi were mounted in lactophenol cotton blue stain solution on slides with cover slips. With the use of the agar well diffusion technique, the thus-obtained isolates were exposed to aqueous and ethanolic garlic extracts at various concentrations.

Inoculums' preparation using macfarland standard

A straight nichrome wire that was sterilized was used to move the colonies from the plates to the broth medium. Using a freshly made 0.5 MacFarland unit turbidity standard made by mixing barium chloride and sulfuric acid, the cloudiness was visually corrected using the broth medium to match that standard [11].

Inoculation of agar plate

To get rid of extra inoculum, a sterile cotton swab was rotated against the tube wall above the liquid after being dipped into the inoculum. A potato dextrose agar plate was streaked three times, covering the entire surface. To achieve uniform dispersion, the plates were rotated by about 60 degrees in between streaking. The inoculated plates were let to stand for at least 3 minutes, but no more than 15 minutes, before the wells in the agar plate were punched. Heat was applied to a hollow tube with a 5 mm diameter. In order to create a well in the inoculated agar plate, it was pressed onto the plate and removed right away. Six wells were also drilled into each plate [12]. Following the addition of the chemical, the plates were incubated at 37°C for 18–24 hours at different AGE and EGE concentrations of 500 mg/mL, 250 mg/mL, 200 mg/mL, 125 mg/mL, and 100 mg/mL, 0.1 mL (100µl) each in the corresponding wells on each plate. The growth on confluent or nearly confluent plates was read. To the nearest whole millimeter, the inhibition zone's diameters were measured [13].

RESULTS AND DISCUSSION

In this research, the antifungal activity of ethanolic and aqueous garlic extract on urinary tract isolates (*Candida albicans* and *Aspergillus niger*) showed a significant antifungal activity at each concentration from 500mg/mL to100mg/mL. The effect observed in concentrate shows that the antifungal activity of garlic extract *on Candida albicans* and *Aspergillus niger* was dependent on concentration as the inhibitory activity of the aqueous garlic extract recorded at a concentration of 500mg/mL was higher ($17.6\times10\pm 2.51\times10$ by mm) than the rest of the concentrations. This therefore shows that the antifungal activity of aqueous garlic extract is dependent on concentration since the highest concentrations had a higher zone of inhibition than the lower concentrations. The result of this study is in agreement with the work of Lemar et al. [14], who also recorded a positive antifungal activity of garlic at varying concentrations.

The result of the ethanolic garlic extract on *Candida albicans* showed a significant antifungal activity also as that of the aqueous garlic extract. However the antifungal activity of the ethanolic garlic extract showed a higher effect on *Candida albicans* than the aqueous garlic extracts, and also in the ethanolic garlic extract, the antifungal activity recorded was dependent on concentration as the antifungal activity observed on the isolated fungus was in ascending order. However the effect of the positive control was higher ($22.6\times10\pm 2.51\times10$mm) than the all the prepared garlic concentrations. This result also agrees with the work of Lemar *et al.* [14], who also recorded a higher antifungal activity of ethanolic garlic extract on *Candida albicans*.

The result of the aqueous garlic extract shows a significant antifungal activity on *Aspergilus niger*. The antifungal activity of aqueous garlic extract shows that the extract was less effective as compared to the positive control (Fluconazole) used on *Aspergilus niger*. However the antifungal activity of garlic extract observed was also dependent on concentration as the antifungal activity of aqueous garlic extract observed was higher at higher concentrations than lower concentrations. This is in agreement with the work of Shams-Ghahfarokhi et al. [8].

The ethanolic garlic extract also showed a reasonable antifungal activity on *Aspergillus niger*. In a similar way, the antifungal activity of the ethanolic garlic extract was dependent on the extract concentration as 500mg/mL has a higher ($20.0\times10 \pm 2.00\times10$mm) antifungal activity as compared to the other concentrations. The antifungal activity of this extract was however lesser than the positive control (fluconazole) which shows that garlic is less effective as an antifungal agent than fluconazole as the antifungal activity of fluconazole that was in each case higher than both the aqueous and the ethanolic garlic extract.

Mean zones of ınhibition of aqueous garlic extract on Candida albicans

The mean zones of inhibition of aqueous garlic extract on *Candida albicans* were presented in the Table 1 as shown below. The effect of the extract was highest ($17.6\text{x}10 \pm 2.51\text{x}10$) at a concentration of 500mg/mL and least ($2.00\text{x}10 \pm 1.00\text{x}10$) at a concentration of 100mg/mL. The positive control used exhibited highest effect ($22.0\text{x}10 \pm 1.00\text{x}10$) as can be seen in Table 1.

Table 1. *Mean Zones of Inhibition of Aqueous Garlic Extract on Candida albicans*

Treatment	Mean Inhibition (mm)
Fluconazole	22.0x10 ± 1.00x10
500mg/mL	17.6x10 ± 2.51x10
250mg/mL	13.6x10 ± 1.52x10
200mg/mL	10.3x10 ± 1.52x10
125mg/mL	7.00x10 ± 1.73x10
100mg/mL	2.00x10 ± 1.00x10
	df = 5

Results are expressed as mean of different concentration plus or minus the standard deviation.

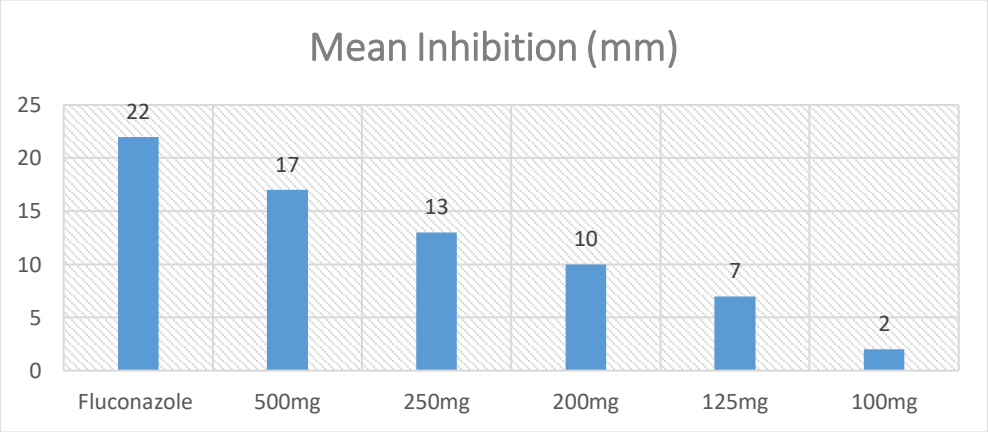

Fig 1: Mean Zones of Inhibition of Aqueous Garlic Extract on Candida albicans

Mean zone of ınhibition of ethanolic extract on Candida albicans

Table 2 presented the mean zones of inhibition of ethanolic garlic extract on *Candida albicans*. The effect of the extract was highest (18.3x10 ± 1.52x10) at a concentration of 500mg/mL and least (3.67x10 ± 5.77x10) at a concentration of 100mg/mL. The positive control used however exhibited highest effect (22.0x10 ± 1.00x10) as can be seen in Table 2.

Table 2. *Mean Zone of Inhibition of Ethanolic Extract on Candida albicans*

Treatment	Mean zone of inhibition
Fluconazole	22.6x10 ± 2.51x10
500mg/mL	18.3x10 ± 1.52x10
250mg/mL	13.0x10 ± 2.00x10
200mg/mL	9.67x10 ± 5.77x10
125mg/mL	7.67x10 ± 5.77x10
100mg/mL	3.67x10 ± 5.77x10
	df = 5

Results are expressed as mean of different concentration plus or minus the standard deviation.

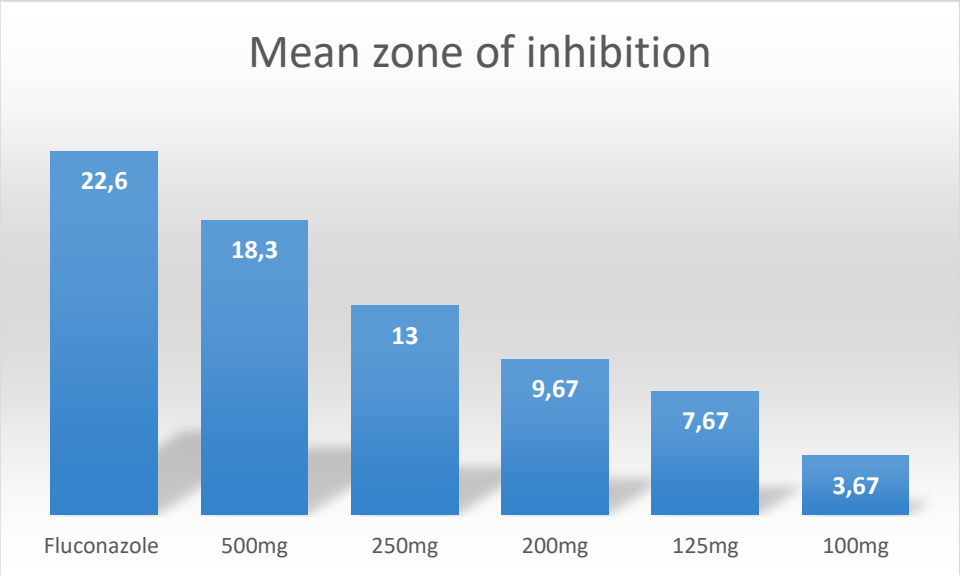

Fig 2. *Mean Zone of Inhibition of Ethanolic Extract on Candida albicans*

Mean zones of ınhibition of ethanolic garlic extract on Aspergillus niger

In Table 3, the mean zone of inhibition of ethanolic garlic extract were presented on *Aspergillus niger*. The effect as observed was highest (20.0x10 ± 2.00x10) at a concentration of 500mg/mL and least (3.67x10 ± 1.52x10) at a concentration of 100mg/mL. The positive control used however exhibited highest effect (22.3x10 ± 1.52x10) as can be seen in Table 3.

Table 3. *Mean Zones of Inhibition of Ethanolic Garlic Extract on Aspergillus niger*

Treatment	Mean zone of Inhibition (mm)
Fluconazole	22.3x10 ± 1.52x10
500mg/mL	20.0x10 ± 2.00x10
250mg/mL	16.6x10 ± 1.52x10
200mg/mL	11.6x10 ± 1.52x10
125mg/mL	7.00x10 ± 1.00x10
100mg/mL	3.67x10 ± 1.52x10
	df = 5

Results are expressed as mean of different concentration plus or minus the standard deviation.

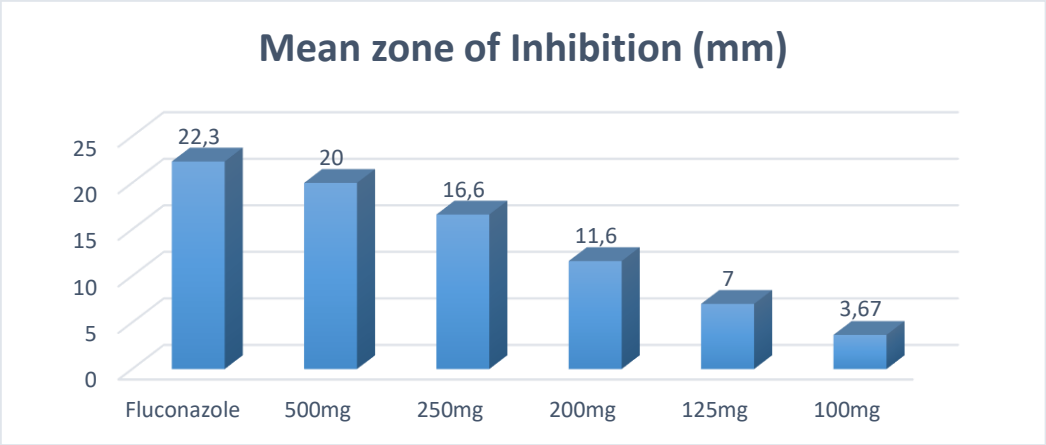

Fig 3. *Mean Zones of Inhibition of Ethanolic Garlic Extract on Aspergillus niger*

Mean zones of ınhibition of aqueous garlic extract on Aspergillus niger

Table 4 is a representation of the antifungal activity of aqueous garlic extract on *Aspergillus niger*. The effect was highest (21.3x10 ± 1.52x10) at a concentration of 500mg/mL and lowest (5.00x10 ± 1.00x10) at 100 mg/mL. However the fluconazole used as positive control exhibited a higher activity (24.3x10 ± 5.70x10) on the test isolates.

Table 4. *Mean Zones of Inhibition of Aqueous Garlic Extract on Aspergillus niger*

Treatment	Mean zone of inhibition (mm)
Fluconazole	24.3x10 ± 5.70x10
500mg/mL	21.3x10 ± 1.52x10
250mg/mL	18.3x10 ± 1.52x10
200mg/mL	13.0x10 ± 2.64x10
125mgmL	8.33x10 ± 5.77x10
100mg/mL	5.00x10 ± 1.00x10
	df = 5

Results are expressed as mean of different concentration plus or minus the standard deviation.

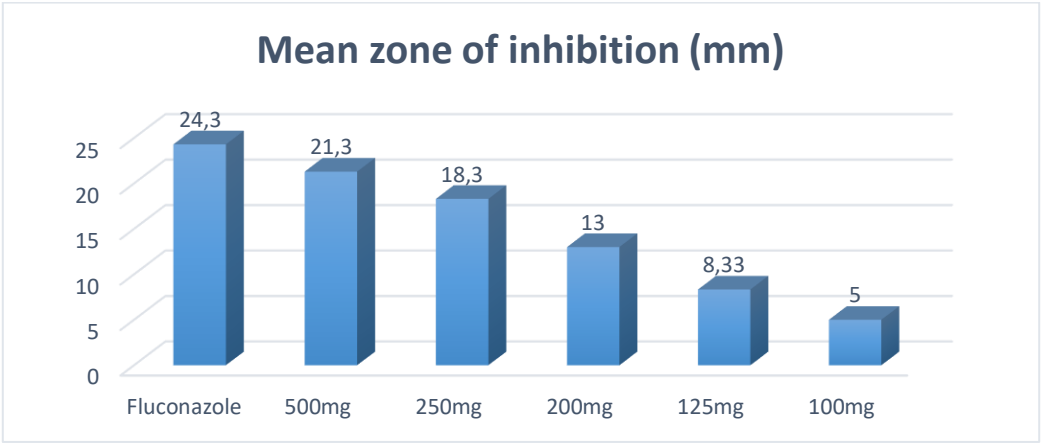

Fig 4. *Mean Zones of Inhibition of Aqueous Garlic Extract on Aspergillus niger*

CONCLUSION

Garlic extract has potential antifungal activity on the isolated urinary tract organisms including *Aspergillus niger* and *Candida albicans* at varying concentrations. Therefore, the use of products incorporated with garlic can be effective in the management of fungal urinary tract infections caused by *Aspergilus niger* and *Candida albicans*.

Conflict of Interest. The authors declared that there is no conflict of interest.

Authorship Contributions. Concept: A.O., Design: A.O., Data Collection or Processing: M.U., Analysis or Interpretation: B.T., Literature Search: A.O., M.U., B.T., Writing: B.T.

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