Journal of Biotechnology Research
ISSN(e): 2413-3256, ISSN(p): 2413-8878
Vol. 3, No. 11, pp: 106-109, 2017
URL: http://arpgweb.com/?ic=journal&journal=16&info=aims

Potential Antifungal Activity of Fresh Garlic Cloves (*Allium sativum* L.) from Sudan

Emad Mohamed Abdallah Department of Laboratory Sciences, College of Sciences and Arts, Qassim University, Al-Rass, Saudi Arabia

Abstract: The incidences of fungal infections are increasing due to the growing phenomena of antifungal resistance and a limited number of antifungal drugs. As some fungi adversely affect on human health such as *Candida albicans*, or on its economic plants such as *Aspergillus niger*. In the current study, minced cloves of garlic from the Sudanese variety was tested against two referenced fungi, namely *Aspergillus niger* ATCC®6275™ and *Candida albicans* ATCC®10231™. The results showed that garlic has a noticeable and high antifungal activity against both fungal strains, the inhibition zones of garlic were 41.0±4.0 mm and 28.0±1.0 mm, respectively, compared with clotrimazole which was 22.5±1.5 mm and 27.5±0.5 mm, respectively. This antifungal activity was statistically significant. Though, the fresh minced cloves of garlic was a competitor to that antifungal drug. With the available data, it can be concluded that garlic bulb is a potent antifungal agent.

Keywords: Antifungal; Garlic; *Allium sativumm*; Medicinal plant; Sudan.

1. Introduction

Natural products remain the main source for remedies since antiquity. Garlic is one of these famous natural products which was used since thousands of years ago as a vegetable, condiment and as a remedy, it was prescribed in many ancient civilizations such as Egyptian, Indian and Chinese civilizations [1]. Garlic (*Allium sativum* L.) is a bulb-shaped plant belonging to family Amaryllidaceae, there are about 300 varieties of garlic cultivated in many countries all over the world [2]. The production of garlic in Sudan is humble compared to its enormous agricultural potential, it is the 22nd in the world, the top 10 countries in production of garlic are China, India, South Korea, Bangladesh, Egypt, Russia, Burma, Ukraine, Spain and United States [3]. Regarding the chemical constituents of garlic, garlic bulbs are rich in phytochemical constituents, such as phenolic compounds, flavonoids, alkaloids, terpenoids and fatty acids [4]. It is believed that the antimicrobial activity of garlic is attributed to a sulfoxide compound isolated from fresh ground garlic pulp known as Allicin [5]. However, some researchers disagree and believed that Allicin itself is very unstable and decomposes rapidly and claim that some smaller metabolic breakdown products also exert strong antimicrobial effects [6]. The flavor attributes which have unpleasant smell after consumption is related to its sulfur compounds which present in garlic in high quantity, recent scientific studies showed that these sulfur compounds are responsible for many medical benefits such as antimicrobial, anti-inflammatory, immunomodulatory, cardioprotective, antidiabetic, antioxidant, and anticancer activity [7]. Regarding the tested microorganisms, *Aspergillus niger* is a filamentous fungus, usually grows in nature on any organic matter and decay it, it is generally considered as non-pathogenic microorganisms. However, its dust may cause allergy or lung problem and it can occasionally attack the human body as an opportunistic invader in immune-compromised or AIDS patients [8]. Its mycotoxins may cause some ailments to human and it is also associated with many plant diseases leading to heavy economic loss [9]. *Candida albicans* is an opportunistic fungus compose some life-threaten infections, particularly for the immunocompromised, cancer, organ transplant or HIV infected patients [10]. Candida is difficult to treat microorganism, it can easily switch between yeast shape to hyphae shape and able to forming biofilms that give it the ability to become resistant to antifungal therapy [11]. This paper attempts to evaluate the antifungal potential of the Sudanese variety of garlic, which is preferred between households in Sudan for its strong odor compared to that imported from South Asian region, although the latter is bigger in size and easy peeling.

2. Materials and Methods

2.1. Plant Material and Preparation

Garlic bulbs were purchased from local markets in Khartoum, Sudan. Fresh bulbs of the garlic were divided into cloves and peeled, and then it was minced and squeezed until obtained some garlic solution or juice, without adding

any additives or water. Immediately, garlic juice was filtered using muslin cloth, the filtrate was considered as 100% aqueous garlic juice, which was used in the same day in the antifungal testing.

2.2. Preparation of Discs

Paper discs, size 6 mm in diameter were cut from Whatman No.1 filter paper, placed in a well-tighten bottle and autoclaved. Then, sterile dry discs were divided into two groups. The first group was saturated with Clotrimazole solution (10mg/ml) and served as a standard antifungal (positive control). The second group were discs saturated with the fresh garlic juice (100%) which served as a test group. The pre-experimental examination revealed that the blank disc absorbs approximately 15 µl.

2.3. Preparation of Fungal Strains

The referenced fungal strains used in this investigation were *Candida albicans* ATCC®10231™ and *Aspergilllus niger* ATCC®6275™, which was available in the Department of Laboratory Sciences, College of Sciences and Arts. Qassim University. Strains were cultured from the stock in Sabouraud dextrose agar for 48-72 hours. Microorganisms were examined microscopically and sub-cultured in Sabouraud dextrose broth for 48 hours at 25 ^{o}C. Then, broth cultures were kept in the refrigerator until used.

2.4. Antifungal Sensitivity Testing

The antifungal activity of fresh garlic juice was determined using Kirby-Bauer disc diffusion method as reported in Abdallah, *et al.* [12] with minor modification. Bottles containing 20 ml of Sabouraud dextrose agar were autoclaved and poured hot on sterile Petri-dishes (90 mm in diameter) and left at room temperature until solidified. Then, then 100 µl from each fungal strain from the broth cultures (previously prepared) was decanted over the agar plates and spread using sterile cotton swap. Discs saturated with garlic juice (about 15 µl/disc) and also discs saturated with 15 µl of Clotrimazole (10mg/ml) as antifungal positive control was placed on the seeded agar plates, then incubated at 25°C for 48-72 hours. After incubation, the diameter of the clear zone of inhibition around the discs was measured in millimetre (mm). The test was repeated twice and the mean was calculated. The antifungal activity was expressed as a zone of inhibition (mm) ± standard error of the mean.

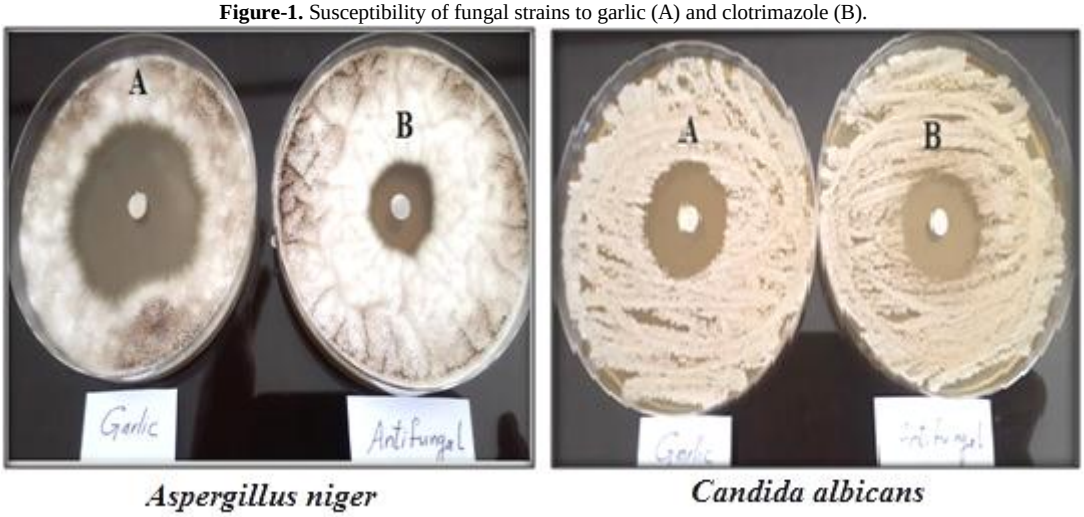
2.5 Statistical analysis

SPSS software (Version 15.0) was used in the statistical analysis of data and graphs. Paired-Sample T-Test was used to compare the activity of garlic juice and the antifungal drug.

3. Results and Discussion

Interestingly, *Aspergillus niger* and *Candida albicans* were highly susceptible to garlic juice in comparison with the antifungal drug (Figure 1). The values of antifungal activity of tested fungi are tabulated in Table (1) and expressed in bar-graph as shown in Figure (2). The mean zone of inhibition of *Aspergillus niger* was 41.0±4.0 mm with garlic juice and 22.5±1.5 mm with clotrimazole. This exciting result leads to conclude that garlic juice was highly effective against *Aspergillus niger* and much better than the commercial antifungal drug (Clotrimazole). As well, The mean zone of inhibition of *Candida albicans* was 28.0±1.0 mm with garlic juice and 27.5±0.5 mm with clotrimazole. This result indicated that that garlic juice has an equal or a better effect against *Candida albicans* compared with a commercial antifungal drug (Clotrimazole). The statistical analysis showed that when to compare the antifungal efficacy of garlic and clotrimazole, garlic as antifungal was statistically significant ($P<0.05$). When putting in consideration that garlic is a natural product with minimal side effects, available and low in cost compared with clotrimazole, though garlic is a better choice as promising natural antifungal drug.

Figure-1. Susceptibility of fungal strains to garlic (A) and clotrimazole (B).



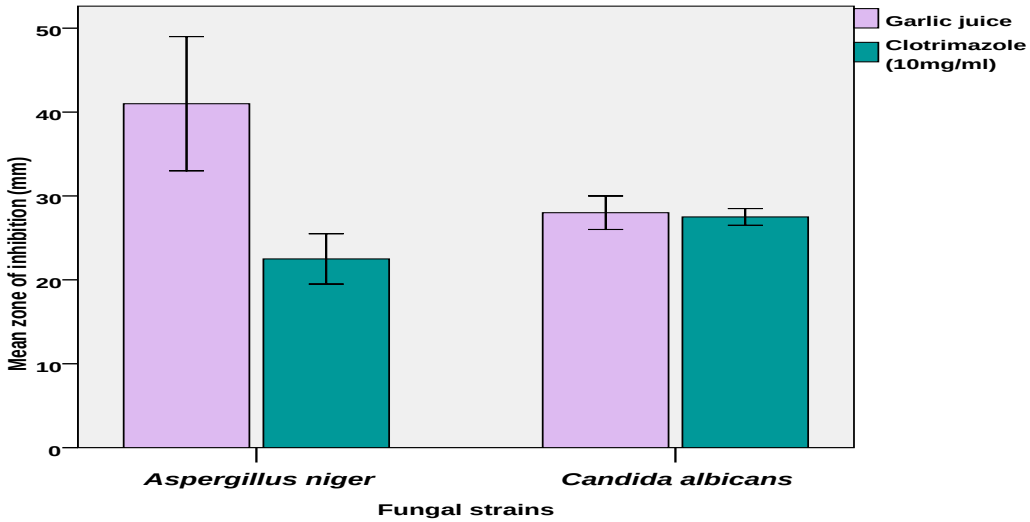
Many previous studies support the current findings, Rees, *et al.* [13] cited that all referenced fungal strains of *Aspergillus fumigates*, *Fusarium laceratum*, *Geotrichum candidum* and *Trichoderma hamatum* were susceptible to garlic. Allicin in its pure form extracted from freshly crushed garlic was found to exhibit *Candida albicans* Ankri and Mirelman [14]. The ethanol extract of garlic has efficiently inhibited the growth of three fungal isolates of food-grain associated moulds, which were *Aspergillus niger*, *Aspergillus ustus* and *Penicillium sp.* [15]. The aqueous, methanolic,propanolic and benzyl alcohol extracts of garlic gave good and varied values of antifungal activity against *Aspergillus niger*, *Candida albicans* and *Trichophyton rubrum*, only benzyl alcohol extract showed less or no inhibition, while other extracts were good antifungal agents [16]. The antifungal potential of garlic was also evaluated against some post-harvest fungal pathogens, namely *Penicillium expansum*, *Botrytis cinerea* and *Neofabraea alba*; The aqueous and ethanol extracts of garlic inhibited the growth of all tested fungi, particularly *Neofabraea alba* which cause bull's eye rot in apples [17]. In an interesting study, Burian, *et al.* [18] cited that, a fungus known as *Sporothrix schenckii*, which is a causative agent of subcutaneous mycosis in Latin America was treated with garlic *in vivo* using mice, the results revealed that garlic has effective antifungal against *Sporothrix schenckii* and the oral administration of garlic influenced the immune system of mice to respond more effectively in order to fight the infection by influences the release of cytokines of macrophages.

Table-1. Antifungal activity of garlic juice compared with clotrimazole.

Tested compound	Mean zone of inhibition (mm)	
	Aspergillus niger	***Candida albicans***
Garlic juice (15µg/disc)	41.0±4.0	28.0±1.0
Clotrimazole* (15µg/disc)	22.5±1.5	27.5±0.5

*Clotrimazole 10mg/ml.

Figure-2. Mean zone of inhibition of garlic juice and clotrimazole



4. Conclusion

The garlic bulb (Sudanese variety) revealed a considerable antifungal efficacy against two importation opportunistic yeast and fungi (*Candida albicans* and *Aspergilllus niger*), the antifungal activity was a competitor to

the commercial antifungal drug. Accordingly, introduction garlic in food behavior and eating habits of people lived in low-income countries, and poor hygienic situation such as refugees will lead to decrease the probability of acquiring fungal infections. On the other side, future research should extract and purify these antifungal compounds, tested *in vivo* and supported with other essential pharmacological studies in order to introduce it as an effective antifungal drug.

References

[1] Jangam, G. B. and Badole, S. L., 2014. *Garlic (Allium sativum): Role in Metabolic Disorder. In: Polyphenolics in Human Health and Disese, Watson RR and Victor R (Eds). Chapter 46* vol. 1, pp. 611-614.

[2] FAO, 2007. "Garlic: Post-harvest operation. J. De La Cruz Medina and H.S. García (Eds), Food and Agriculture Organization of the United Nations." *Instituto Tecnologico de Veracruz,* Available: http://www.itver.edu.mx

[3] Factfish, 2017. "Sudan: Garlic, production quantity (tons)." Available: http://www.factfish.com/statistic-country/sudan/garlic,+production+quantity

[4] Saha, M. and Bandyopadhyay, P. K., 2017. "Phytochemical screening for identification of bioactive compound and antiprotozoan activity of fresh garlic bulb over trichodinid ciliates affecting ornamental goldfish." *Aquaculture,* vol. 473, pp. 181–190.

[5] Cavallito, C. J., Bailey, J. H., and . 1944. "Allicin, the antibacterial principle of Allium sativum. isolation, physical properties and antibacterial acrion." *J. Am, Chem, Soc,,* vol. 66, pp. 1950-1951.

[6] Harris, J. C., Cottrell, S. L., Plumer, S., and Lloyd, D., 2001. "Antimicrobial properties of Allium sativum (garlic)." *Appl Microbiol Biotechnol,* vol. 57, pp. 282-286.

[7] Martins, N., Petropoulos, S., and Ferreira, I. C. F. R., 2016. "Chemical composition and bioactive compounds of garlic (Allium sativum L.) as affected by pre- and post-harvest conditions: A review." *Food Chemistry,* vol. 211, pp. 41-50.

[8] Schuster, E., Dunn-Coleman, N., and Frisvad, J. C. D., P. W. M., 2002. "On the safety of Aspergillus niger – a review." *Appl. Microbiol. Biotechnol.,* vol. 59, pp. 426-435.

[9] Sharma, R., 2012. "Pathogenicity of Aspergillus niger in plant." *Cibtech Journal of Microbiology,* vol. 1, pp. 47-50.

[10] Yang, C. R., Zhang, Y., Jacob, M. R., Khan, S. I., Zhang, Y. J., and Li, X. C., 2006. "Antifungal activity of C-27 steroidal saponins." *Antimicrobial Agents and Chemotherapy,* vol. 50, pp. 1710-1714.

[11] Soliman, S., Alnajdy, D., El-Keblawy, A. A., Mosa, K. A., Khoder, G., and Noreddin, A. M., 2017. "Plants' natural products as alternative promising anti-candida drugs. Pharmacognacy review." vol. 11, pp. 104–122.

[12] Abdallah, E. M., Kamal Ahmad Qureshi, K. A., and Musa, K. H., 2017. "Antimicrobial, antioxidant and phytochemical screening of Lupin seeds (Lupinus Termis Forrsk.) from Sudan." *Cibtech Journal of Microbiology,* vol. 6, pp. 1-8.

[13] Rees, L. P., Minney, S. F., Plummer, N. T., Slater, J. H., and Skyrme, D. A., 1993. "A quantitative assessment of the aanti-microbial activity of garlic (Allum sativum)." *World Journal of Microbiology and Biotechnolog,* vol. 9, pp. 303-307.

[14] Ankri, S. and Mirelman, D., 1999. "Antimicrobial properties of allicin from garlic." *Microbes and Infection,* vol. 2, pp. 125-129.

[15] Akinmusire, O. O., Omomowo, I. O., and Usman, I. M., 2014. "Evaluation of the phytochemical properties and antifungal activities of ethanol extract of Allium sativum." *International Journal of Current Microbiology and Applied Sciences,* vol. 3, pp. 142-149.

[16] Ikegbunam, M., Ukamaka, M., and Emmanuel, O., 2016. "Evaluation of the antifungal activity of aqueous and alcoholic extracts of six spices." *American Journal of Plant Sciences,* vol. 7, pp. 118-125.

[17] Daniel, C. K., Lennox, C. L., and Vries, F. A., 2015. " In-vitro effects of garlic extracts on pathogenic fungi Botrytis cinerea, Penicillium expansum and Neofabraea alba." *S. Afr. J. Sci.,* vol. 111, pp. 1-8.

[18] Burian, J. P., Sacramento, L. V. S., and Carlos, I. Z., 2017. "Fungal infection control by garlic extracts (Allium sativum L.) and modulation of peritoneal macrophages activity in murine model of sporotrichosis." *Braz. J. Biol.,* vol. 77, pp. 848-855.