

Antimycotic Effect of *Allium Sativum* on Selected Fungi

GABRIEL C. DISEGHA & NEDIE P. AKANI
Rivers State University of Science and Technology, Nigeria

ABSTRACT This study was aimed at evaluating the antifungal activities of extracts derived from *Allium sativum* (garlic) on *Aspergillus nidulans*, *Penicillium niger*, *Candida albicans* and *Scupularis* species using the agar well diffusion method. Extracts included crude, ethanol and aqueous (cold and hot) extracts. A conventional antifungal drug Fluconazole (10mg) was used as a positive control. Results obtained by measuring zones of inhibition of fungal growth after 24hours of incubation showed high but varied levels of antifungal efficacy. Crude extract was more effective ($P \geq 0.05$) than ethanol and aqueous (cold and hot) extracts. There was an increase ($P \geq 0.05$) in the zone of inhibition as concentration of extract increased. Crude extract had the highest (16.0 ± 0.00 mm at 40g/l) inhibitory effect on *Aspergillus nidulans* while fresh cold extract was least (0.00 ± 0.00 mm at 10g/l) on *Candida albicans*. Generally, Fluconazole showed greatest activity ranging between 26.33 ± 0.57 and 9.67 ± 0.58 mm. The minimum inhibitory concentration (MIC) of the fresh cold and hot aqueous and ethanol extracts of *Allium sativum* on *Aspergillus nidulans*, *Penicillium niger* was 10g/ml while that of *Candida albicans* was 20g/ml. The present study reveals that garlic extracts possesses antifungal properties at its crude and varied concentrations and may be used as crude or aqueous mixture for antifungal remedies in cases implicating the test organisms. The use of crude extracts of *Allium sativum* as an antifungal agent by herbalists in the treatment of the fungal diseases is advocated. It is pertinent, therefore, that more clinical studies be carried out in order to assess the spectrum of activity of the extracts which could serve as possible therapeutic solution to other human and plant pathogenic fungal infections.

Keywords: antifungal, *Allium sativum*, sensitivity, fungi, minimal inhibitory concentration.

Introduction

Allium sativum (Garlic) is a hardly liliaceous sterile plant, the strong-scented, pungent bulb of which purposes. Garlic is considered to have originated from central Asia and was domesticated in ancient times in Mediterranean areas (Batchuaron, 1993; Larsen, 2011)). It is now cultivated in various countries with increasing production. The garlic bulb contains 4 to 20 cloves which serve as garlic seed for the next generation. Medicinal plants are good reservoirs of chemotherapeutants with considerable potential (Damodaran *et al.*, 1994, Farooqui *et al.*, 1998; Disegha, 2014). Human beings are susceptible to microbial infections caused by bacteria, fungi, viruses, protozoan *etc.* (Prescott *et al.*, 1993). However the infective pathogenic fungi are known to develop resistance to synthetic antifungal drugs (Groll *et al.*, 1998; Graybill, 1996). Since the fungal pathogens are eukaryotes, the treatment may also affect the infected patients (Klepser *et al.*, 1997). *Allium sativum* is a bulbous plant that grows up to 0.6m (2ft) in height. It produces hermaphrodite flowers and pollination occurs by insects and bees. Its bulb is normally divided into numerous fleshy sections called cloves. Garlic has a characteristic pungent, spicy flavor that mellows and sweetens considerably with cooking. The garlic plants bulb is the most commonly used part of the plant and has long been a staple in the Mediterranean region as well as a frequent seasoning in Asia, Africa and Europe. It was known to Ancient Egyptians and has been used for both culinary and medicinal purposes (Ensminger, 1994).

When crushed, *Allium Sativum* yields allicin an antibiotic and antifungal compound (phytoncide) discovered by Chester J Cavallito and colleagues in 1994. Fresh or crushed garlic also affords the sulfur containing compounds allicin, ajoene, diallyl polysulfides, vinylthiins, s- allylcysteine and enzymes, B vitamins, proteins, mineral, saponins, flavonoids and maillard reaction products which are not sulfur containing compound (Garba *et al.*, 2013). Furthermore a phytoalexin (alliixin) was found a non-sulfur compound with a Y – pyrone skeleton structure with antioxidant effects, antimicrobial effect, antitumor promoting effect, inhibition of aflatoxin B2 DNA binding and neurotrophic effects (Garba *et al.*, 2013). Modern science has shown that garlic is a powerful natural antibiotic, can have a powerful antioxidant effect. Initial reports of antimicrobial activity of garlic showed that allicin (allyl 2-propene thiosulfinate); a notable flavonoid in garlic is formed when garlic cloves are crushed (Garba *et al.*, 2013). Garlic may help improve your iron metabolism. Help our blood vessels expand and keep our blood pressure in check. . Modern medicine has found that garlic contains allicin which scavenges hydroxyl radicals (OH). This in turn is thought to prevent LDLs from being oxidized (Cholesterol) Meredith (2015). The composition of the bulbs is approximately 84.09%, water, 13.38% Organic matter and 1.53% inorgan-

ic matter while the leaves are 87.14% water, 11.27% organic matter and 1.59% in organic matter (Block, 2010).

Although allicin and the volatile oil fraction are clearly the most potent active anticandida component (Lemar *et al.*, 2002, Kim *et al.*, 2004), aqueous garlic extract have been shown *in vivo* to be effective, even at a dilution of 1: 100 against the common tinea corporis, capitis and cruris fungal skin infection (Venugopal *et al.*, 1995; Larsen, 2011). In one study at the major Chinese hospital, garlic therapy alone was used effectively in the treatment of cryptococcal meningitis, one of the most serious fungal infections imaginable. Fungi are eukaryotic organisms that are spore bearing, have absorptive nutrition, lack chlorophyll and reproduce both sexually and asexually. They degrade complex organic materials in the environment to simple organic compound and inorganic molecules. In this way, carbon, nitrogen, phosphorus and other critical constituents of dead organisms are released and made available for living organisms. They are important in the commercial production of many organic acids, certain drugs and antibiotics. Fungi can occur as yeast, molds or as a combination of both forms. Some fungi are capable of causing superficial, cutaneous, subcutaneous, systemic or allergic diseases in plants animals or humans (Michael *et al.*, 1996; Disegha, 2014).

Threat of fungal infections to life; increase in the cost of synthetic antifungal drugs, and allergic reactions caused by such drugs; emergence and prevalence of resistant strains of fungi due to use and abuse of synthetic drugs, and existence of fake, non-drug products, and proliferation of adulterated drugs in the markets today, and to appreciate the gift of nature and better utilize the resources available in the tropical environment and necessitated this study. The study attempts to investigate the *in-vitro* activity of fresh cold aqueous, fresh hot aqueous and ethanol extracts of *Allium sativum* on *Candida albicans*, *Aspergillus nidulans*, *Penicillium niger* and *Scupularis* species and to determine the minimum inhibitory concentration of *Allium sativum* extracts on the test organisms.

Materials and Methods

Collection of Sample

The garlic bulbs were purchased from local market in Port Harcourt, Nigeria.

Plate 1: Garlic gloves (*Allium sativum*)

Source: Mile 3 Market, Port Harcourt, Nigeria.

Test Organisms

The test organisms, *Aspergillus nidulans*, *Penicillium niger*, *Candida albicans* and *Scupularis* species were isolated from soup thickeners such as *Bra-*

chystegia eurycoma (achi), *Detarium microcarpum* (ofor), and *Mucuna flagelipes* (ukpo) collected from local markets in Port Harcourt, Nigeria.



Plate 2: pure culture of *Aspergillus niger*



Plate 3: Pure culture of *Penicillium nidulans*



Plate 4: Pure culture of *Candida albicans*



Plate 5: Pure culture of *Scupularis* species

Preparation of Aqueous Garlic Extract

Fresh garlic was sorted for uniformity and absence of defect. The sorted ones were washed with clean water, peeled and grounded using a blender. After which 10g, 20g, 30g and 40g of the ground garlic were weighed and put separately into sterile container and 90ml, 80ml, 70ml and 60ml of ethanol, hot water and cold distilled water respectively was added, stirred with a stirrer to and allowed for 24 hours in an air tight container (Uzama, 2009). This was done in triplicates.

Sensitivity Testing

Agar well diffusion method was used as an antifungal method. Sterile Sabouraud Dextrose Agar (SDA) was poured into the Petri plates (9cm diameter). Then the agar was allowed to solidify at 42⁰C. 0.2ml of each culture of test organism was inoculated on the different plates and a sterile bent glass rod spreader was used to lawn the test organism. The SDA plates were left to dry and divided into four (4) sections with a marker pen. A sterile standard borer of 7mm diameter was used to bore five (5) wells on each of the sections and the centre of the plate.



Plate 6: Showing inhibition on Aspergillus nidulans



Plate 7: Showing inhibition on Candida albicans



Plate 8: Showing inhibition on Scupularis species.

Determination of Minimal Inhibitory Concentration (MIC)

Thirteen (13) test tubes were labeled and set up, then 1ml of broth was pipette into tubes 2 – 10, 11 and 13 after which 2ml broth was pipette into tube 12. N/B Tube 11 is the inoculums control, tube 12 is the broth control and tube 13 is the dry control. 1ml of working drug (pure garlic) was pipetted into tubes 1, 2 and 13. A doubling dilution was prepared from tube 2 up to 10 using 1 ml of the solution and 1 ml of standardized working inoculum (organism) was introduced into tubes 1 to 11. Tubes were incubated at 37⁰ C for 24 hours (Ochei *et al.*, 2000). Observations were made for visible growth of fungi. The highest dilution (lowest concentration) showing no visible growth was regarded as minimal inhibitory concentration (MIC). Cells from the tubes showing no growth were sub - cultured on SDA plates and incubated at 37⁰C for 24 hours to determine if the inhibition was irreversible or permanent.

Data analysis

All obtained data were analyzed statistically using Analysis of variance techniques and employing Turkey mean separation option.

Results and Discussion

Evaluation of antifungal activities of crude extracts of *Allium sativum* (garlic) and conventional antifungal agent (Fluconazole 10mg) on *Aspergillus nidulans*, *Penicillium niger*, *Candida albicans* and *Scupularis* species using the agar well diffusion method yielded varying results as presented in the following tables.

Table 1 shows the antifungal activity results of crude, cold, hot aqueous extracts and standard antifungal drug (Fluconazole 10mg) at 10% concentration on the test organisms. All extracts of *A. sativum* demonstrated antifungal activity on all test organisms except fresh cold aqueous extracts which did not show any activity on *C. albicans*. It had the highest activity on *Scupularis* species followed by *Aspergillus nidulans*, *Penicillium niger* and *Candida albicans* in decreasing order. There was no significant difference between the activities of the extract on *A. nidulans* (11.33 ± 1.53^b), *P. niger* (8.33 ± 0.58^b) and *Scupularis* species (14.00 ± 1.73^b) at $p \geq 0.05$. But the antifungal activity on them was significantly different from the activities on *C. albicans* (2.00 ± 1.73^a) at $p \geq 0.05$. With respect to the extract types, crude extract of *A. sativum* demonstrated highest activity on *A. nidulans* and its effect was significantly different from the effects of fresh cold, fresh hot and fresh ethanol extract at $p \geq 0.05$. The effect of the extracts on *C. albicans* was generally low. Fresh ethanolic extract only showed slight activity on the test organisms. Aqueous extracts of *A. sativum* are may be preferred as treatment for pathologic conditions implicating the test organisms. The activity of Fluconazole was higher in all respects than the activity of *A. sativum* extracts. This implies low activity indices using a relative measure of zones of inhibition produced by extract by zones of inhibition produced by Fluconazole (Borgio *et al.*, 2008). The effect of *A. sativum* extract on *A. nidulans* supports the work of Pai and Platt (1995). They reported that aqueous garlic extract AGE and especially concentrated garlic oil were found to have antifungal activity.

Table 2 shows the antifungal activity values of the treatments at 20% concentration on the test organisms. All extracts of *A. sativum* demonstrated antifungal activity on all test organisms except fresh cold aqueous extracts which showed slight any activity on *C. albicans* (1.00 ± 1.73^a). Crude extract had the highest activity on *Scupularis* species (14.33 ± 0.58) followed by *Aspergillus nidulans* (12.67 ± 0.58), *Penicillium niger* (12.33 ± 1.53) and *Candida albicans* (3.33 ± 2.89) in decreasing order. Column wise, there was significant difference between the activities of the crude extract and those of fresh cold, fresh ethanol, and fresh hot aqueous extracts on *A. nidulans* at $p \geq 0.05$. The activities of extracts on *P. niger* differ significantly from one an-

other with crude extract being the highest (12.33 ± 1.53) and fresh hot aqueous extract the lowest (4.67 ± 0.58). On *C. albicans*, there was no significant difference between the activities of the extracts at $p \geq 0.05$. The activity of crude extract on *Scopularis* species was higher than the activities of all other treatments including that of Fluconazole and this value was significantly different at $p \geq 0.05$. Alam *et al.*, 2002 reported high levels of inhibition of spore/ conidia germination of some fungal species using extracts of rice, wheat, straws and tobacco leaf. The above result clearly confirms the fact that soluble extract of garlic have antifungal properties and are able to inhibit the growth of the fungi; *Aspergillus nidulans*, *Pencillium niger*, *Candida albicans* and *Scopularis species* albeit to different extents. This confirms *in vitro* activity of some plant extract including garlic, ginger, and onion on seed -borne fungi of wheat such as the *Aspergillus species* (Hasan *et al.*, 2005).

Table 1: Antifungal activity of crude extracts of *A. sativum*, Fluconazole (10 mg), and ethanol, cold and hot aqueous extracts of *A. sativum* at 10% concentration.

Treatment	Zone of inhibition (mm)			
	<i>Aspergillus nidulans</i>	<i>Penicillium niger</i>	<i>Candida albicans</i>	<i>Scopularis species</i>
Crude extract	11.33 ± 1.53^b	8.33 ± 0.58^b	2.00 ± 1.73^a	14.00 ± 0.73^b
Fresh cold extract	4.00 ± 1.00^a	2.67 ± 0.58^a	0.00 ± 0.00^a	5.33 ± 0.58^a
Fresh ethanol extract	4.33 ± 0.58^a	7.33 ± 1.16^a	0.67 ± 1.16^a	4.67 ± 0.58^a
Fresh hot extract	3.00 ± 0.00^a	4.33 ± 0.58^b	0.67 ± 1.16^a	2.33 ± 0.08^a
Fluconazole (10 mg/ml)	18.00 ± 0.00^c	22.67 ± 0.58^c	17.67 ± 0.58^c	15.00 ± 0.00^c
R ²	0.985	0.993	0.983	0.957

^{a,b,c} Means with same superscript at columns are not significantly different ($p \geq 0.05$)

Table 2: Antifungal activity of crude extracts of *A. sativum*, Fluconazole (10 mg), and ethanol, cold and hot aqueous extracts of *A. sativum* at 20% concentration.

T	Treatment	Zone of inhibition (mm)			
		<i>Aspergillus nidulans</i>	<i>Penicillium niger</i>	<i>Candida albicans</i>	<i>Scopularia species</i>
Crude extract	12.67±0.58 ^b	12.33±1.53 ^c	3.33±2.89 ^a	14.33±0.58 ^c	
Fresh cold extract	7.67±2.08 ^a	10.00±1.00 ^{ab}	1.00±1.73 ^a	9.67±2.52 ^{ab}	
Fresh ethanol extract	5.67±0.58 ^a	8.00±1.00 ^b	4.33±2.31 ^a	6.67±0.58 ^{ab}	
Fresh hot extract	5.00±1.00 ^a	4.67±0.58 ^a	5.00±1.00 ^a	4.67±1.16 ^a	
Fluconazole (10mg)	17.00±0.00 ^c	18.33±0.58 ^d	22.00±1.00 ^b	9.67±0.59 ^b	
R ²	0.963	0.969	0.958	0.903	

^{a,b,c} Means with same superscript at columns are not significantly different ($p \geq 0.05$)

Table 3: Antifungal activity of crude extracts of *A. sativum*, Fluconazole (10 mg), and ethanol, cold and hot aqueous extracts of *A. sativum* at 30% concentration.

Treatment	Zone of inhibition (mm)			
	<i>Aspergillus nidulans</i>	<i>Penicillium nigricans</i>	<i>Candida albicans</i>	<i>Scopularia species</i>
Crude extract	14.67±0.58 ^b	13.67±0.58 ^b	12.00±1.73 ^a	14.33±1.16 ^{ba}
Fresh cold extract	8.00±2.65 ^a	11.00±0.00 ^a	6.00±5.20 ^a	10.33±1.53 ^{ab}
Fresh ethanol extract	7.33±0.58 ^a	8.33±2.08 ^a	4.67±4.04 ^a	8.33±0.58 ^{ab}
Fresh hot extract	7.67±0.58 ^a	8.67±1.16 ^a	5.33±1.16 ^a	5.00±4.48 ^a
Fluconazole (10mg)	17.00±0.00	22.00±1.00	26.33±0.58	17.33±0.58
R ²	0.940	0.964	0.912	0.849

^{a,b,c} Means with same superscript at columns are not significantly different (p≥ 0.05)

Table 4: Antifungal activity of crude extracts of *A. sativum*, Fluconazole (10 mg), and ethanol, cold and hot aqueous extracts of *A. sativum* at 40% concentration.

Treatment	<i>Aspergillus nidulans</i>	<i>Penicillium niger</i>	<i>Candida albicans</i>	<i>Scupularis</i> species
Crude extract	16.00±0.00 ^b	14.67±1.53 ^a	14.67±1.53 ^b	15.67±1.16 ^b
Fresh cold extract	9.67±1.53 ^a	11.33 ^a	12.00 ^a	12.00 ^a
Fresh ethanol extract	8.67±1.16 ^a	10.00±2.65 ^a	15.33±0.58 ^b	9.67±0.58 ^a
Fresh hot extract	8.00±0.00 ^a	11.33±0.58 ^a	14.33±0.58 ^{ab}	9.67±1.16 ^a
Fluconazole (10 mg/ml)	15.00±0.00 ^b	20.33±3.22 ^b	22.00±1.00 ^c	18.00±0.00 ^b
R²	0.958	0.837	0.955	0.933

^{a,b,c} Means with same superscript at columns are not significantly different ($p \geq 0.05$)

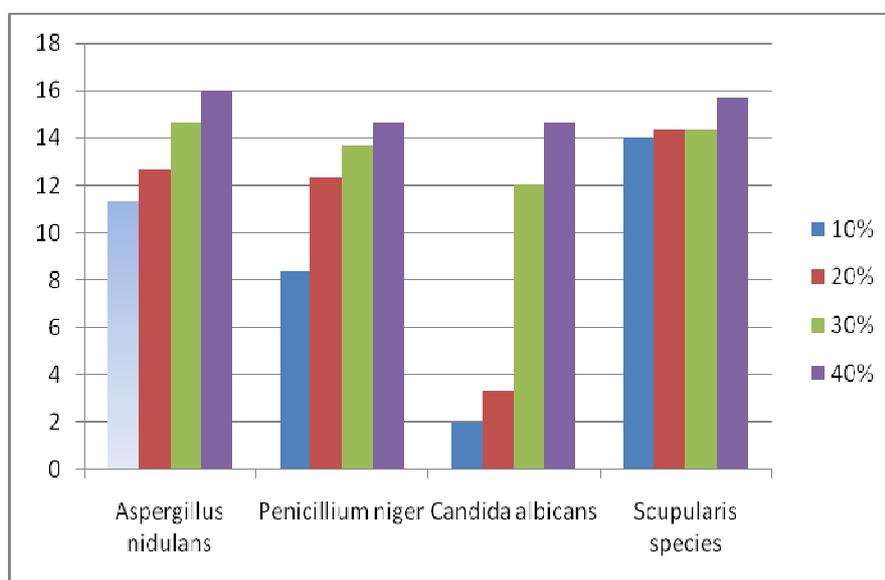


Figure 1: Concentration-related antifungal activities of crude extract of A. sativum on test organisms

Table 3 and 4 are similarly presented, showing the mean activity values of extracts on the test organisms. Column wise, crude extract showed highest activity on all test organisms and the values are significantly different from the activities of all other extracts at $p \geq 0.05$. According to the report of Pai and Platt (1995), concentrated extracts of garlic showed more antifungal efficacy against *Aspergillus* species.

Generally all extracts of *A. sativum* demonstrated antifungal activity on all test organisms, except fresh cold aqueous extract on *C. albicans* (0.00 ± 0.00). This differs or seemingly contradicts the report of Venugopal *et al.*, (1995) that from a clinical perspective, inhibition of *Candida albicans* was the most significant, because both *in vivo* and *in vitro* studies showed garlic to be more potent than nystatin, gentian violet and six other reputed antifungal agents. However, in the present study, crude extract showed the highest efficacy at all concentrations, with a gradual increase in zones of inhibition as concentration increases (Figure 1): 10% (11.33 ± 1.53), 20% (12.67 ± 0.58), 30% (14.67 ± 0.58^b) and 40% (16.00 ± 0.00). Akinmusire, Omonowo, and Usman (2014) reported that efficacy of garlic extracts was concentration-dependent, as percentage mycelia growth increased with increase in concentration.

Conclusion and Recommendations

There is interest in herbal therapy in recent times. It is established from the present study that garlic extract demonstrated antifungal activities against the test organisms at varying concentrations. As crude extracts yielded the highest mean zone sizes, the use of ethanol as extraction solvent is discouraged, since its use will imply additional cost in preparation without adding to the efficacy of the extract. The findings of the study suggest that garlic extracts may be used solely as crude extract or as aqueous mixture for herbal antifungal remedies, especially in cases implicating *A. nidulans*, *P. niger*, *C. albicans* and *Scupularis* species. The present study reveals that garlic extracts possess antifungal properties and may be used as crude or aqueous mixture for antifungal remedies in cases implicating the test organisms. The use of crude extracts of *Allium sativum* as an antifungal agent by herbalists in the treatment of the fungal diseases is advocated. It is pertinent, therefore, that more clinical studies be carried out in order to assess the spectrum of activity of the extracts which could serve as possible therapeutic solution to

other human and plant pathogenic fungal infections, as well as anti-contaminant on food thickeners. It is also recommended that toxicity studies on *A. sativum* be carried out and its toxicity and therapeutic indices established so that administration of garlic-based drugs would be regulated to avoid contraindication and side-effects. The study was however limited by the researchers' deficiencies to purify the extracts using modern separation techniques, hence could not ascertain activity patterns of pure extracts on the test organisms if employed as experimental treatments. Also the number of test organisms was rather too few, thus restricting predictive statements to include related microbes.

Policy Implications

- Current awareness programmes with respect to danger of ethanol to health is not communicated enough by the appropriate authorities and it is not reaching the masses as effectively as it is supposed to.
- More efforts are needed to educate tradomedical practitioners and the general public to use crude or water-based preparations of garlic or other alternatives to ethanol solvents in the preparation of herbal drugs consisting of garlic to avoid the impending health consequences posed by ethanol.

Correspondence

Gabriel Charles Disegha
Department of Applied and Environmental Biology
Rivers State University of Science and Technology
Port Harcourt, Nigeria.
Tel:+ 2348034739654
Email: gabrieldisegha2@gmail.com

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