



Original Research Article

Evaluation of the phytochemical properties and antifungal activities of ethanol extract of *Allium sativum*

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ABSTRACT

The antifungal activity of ethanolic crude extract of *Allium sativum* bulb (Garlic) was investigated on three food-grain associated moulds, which included *Aspergillus niger*, *Aspergillus ustus* and *Penicillium* species. The preliminary phytochemical screening indicated the presence of alkaloids, flavonoids, glycosides, saponins, and tannins. . In this investigation, the poisoned food technique methodology was used. The ethanolic garlic extract inhibited the growth of the food associated fungi *Aspergillus niger*, *A. ustus* and *Penicillium species*. This inhibition was concentration-dependent. Mycelia growth inhibition in *A. ustus* was 23.53% at a concentration of 50mg/ml. At a concentration of 100mg/ml, the mycelia growth inhibition was 43.12% while 100% inhibition was recorded at a concentration of 200mg/ml. All the three different concentrations (50mg/ml, 100mg/ml and 200mg/ml) of the crude extract used inhibited all the fungi. At a concentration of 100mg/ml, the growth of *A. niger* was retarded the most (72.12%). This was followed by *Penicillium* species (61.39%) and then *A. ustus* (43.12%). The concentration of 200mg/ml showed the highest level of inhibition compared to the other two concentrations. *A. ustus* was most inhibited (100%) followed by *A.niger* (93.03%) and then *Penicillium* species (92.97%). The three food-grain associated fungi differed with regard to their susceptibility to crude garlic extracts. The antifungal activity of the crude garlic extract is therefore discussed.

Keywords

Allium sativum, phytochemical properties, antifungal activities

Introduction

Herbs have been used as a source of medicine to treat infectious diseases in nearly all cultures from ancient times to the present day. In African countries there is prevalence ethno-pharmacological use of plants in the treatment of malaria, diarrhoea, stomach disorders and other infectious diseases since these plants are easily

available and cheaper than the conventional drugs. Some of these plants which are used in their natural state as herbal medicines in West Africa include garlic, ginger, aloe vera, and lime fruit (Onyeagba *et. al.*, 2004; Oyagade *et. al.*, 1999; Lino and Deogracious, 2006).

Garlic (*Allium sativum L*) is a common spice that has been traditionally popular with strong folkloric awareness. It is the edible bulb of the lily family liliaceae which is widely used as a spice in flavouring food and is used by the traditional medicine practitioners in the treatment of bacterial related diseases such as pile, cough and rheumatism. It contains aromatic sulphur based compounds which contributes to its odour and taste. Allicin is the key component to which the antimicrobial activity of garlic is attributed; it is a volatile molecule that gives it its characteristic odour. Allicin has also been found to be effective as an anti fungal, antibacterial, antiviral and anti parasitic agent. (Lawal et. al., 2010; Jabar and Al-Mossawi, 2007; Reuter et. al., 1996)

Much effort has been devoted to the search for new antimicrobial agents for the management of infectious diseases and for food preservation. This search has focused on natural sources such as plants and microbes. (Nielsen and Rios, 2000). Bioactive compounds in the plants which confer protection on them against bacteria, fungi and viruses have been linked to the antimicrobial activities of the extracts of such plants. (El-Mahmood and Amey, 2007).

Mycotoxins are known to be potent hepatocarcinogens in animals and humans and *Aspergillus* species are the most common fungal species with the ability to produce these toxins in foods and feedstuffs (Soliman and Badeaa, 2002).

Aflatoxin B1 produced by the *Aspergillus* species is the most toxic form of mycotoxins for mammals. It has been found to have hepatotoxic, teratogenic and mutagenic properties, and it causes damage such as toxic hepatitis, haemorrhage, edema, immunosuppression and hepatic carcinoma (Speijers and Speijers, 2004). The

consumption of aflatoxin contaminated food and feed is responsible for several reported disease outbreaks of aflatoxicosis in humans and animals (Reddy and Raghavender, 2007).

The main theme of this study is to assess the potential of inhibiting the growth of mycotoxin producing moulds in foods and feedstuffs using natural products that are known to be nontoxic thereby reducing the health risks associated with their consumption.

Materials and Methods

Garlic bulbs used in the production of the extract were purchased from Maiduguri's Monday Market, Nigeria, and were identified at the Department of Biological Sciences, University of Maiduguri, Nigeria.

Preparation of *Allium sativum* (Garlic) extract

The epicarps of the bulbs were peeled off and separated from the stalks for easy assessment. The peeled bulbs were thoroughly washed and dried under the sun for 3 days after which the bulbs were grinded into a fine powder (Lin and Lineback, 1990).

95% ethanol was used for the extraction of bioactives from the garlic clove according to the method of (Barreto et al., 2002). 150g of grinded garlic powder was dissolved in 300ml of ethanol. The mixture was kept at room temperature for 24 hours in a sterile flask covered with aluminium foil to avoid evaporation, it was then filtered through a glass wool filter, and the extract evaporated in a rotary evaporator until about 25ml of extract was left in the container. This was further allowed to air dry in a vacuum desiccator until all the ethanol had evaporated. The solidified extracts were

diluted by ethanol to make different concentrations and stored at 4°C (Bokhari, 2009). Three different concentration of the extract; 50mg/ml, 100mg/ml and 200mg/ml, were used in this research.

Phytochemical screening of Garlic extract

Phytochemical screening was done in order to detect the presence of bioactive constituents such as alkaloids, tannins, saponins, phenols, glycosides, phlobatannins, flavonoids and glycosides using the methods described by Sofowora (1978), Trease and Evans (1989).

Test for saponins

Two milliliter of the aqueous and ethanolic extracts in a test tube was shaken for two minutes. Frothing which persisted on shaking was taken as evidence for the presence of saponins.

Test for alkaloids

Three milliliter of the ethanolic extracts was stirred with 5 ml of 1% HCL on a steam bath for twenty minutes. The solution obtained was cooled and filtered and few drops of Mayer's reagent/picric acid were added to the filtrate. A cream precipitate indicated the presence of alkaloid.

Test for phenolics

Two drops of 5% ferric chloride were added to 5 ml of the crude ethanolic extract in a test tube. A greenish precipitate was taken as indication of phenolics.

Test for tannins

A volume of 1 ml of freshly prepared 10% potassium hydroxide was added to a volume of 1 ml of the ethanolic extracts. The

presence of a dirty white precipitate was taken as indication of tannins.

Test for steroids

To a volume of 1 ml of the extracts, five drops of concentrate tetra-oxo-iosulphate VI acid was added. Red coloration indicated the presence of steroids.

Test for flavonoids

To a volume of three milliliter of the ethanolic extract, a volume of 1 ml of 10% sodium hydroxide was added. A yellow coloration indicated the presence of flavonoids.

Test for glycosides

To a volume of 3 ml of the ethanolic extract, 2 ml of chloroform was added. Tetraoxiosulphate VI acid was carefully added to form a lower layer. A reddish brown color at interface indicated the presence of a steroidal ring.

Isolation and Maintenance of test organisms

The fungi used were isolated from stored grains and identified following the method of (Domsch *et al.*, 1980). They were stored on Potato Dextrose Agar slant in the refrigerator at 4°C prior to use.

Antifungal Activity Assay

The antifungal activity of garlic extract was evaluated against the food-associated fungi by using poisoned food technique (Pundir *et al.*, 2012). All the three food-associated test fungi were separately inoculated on Potato Dextrose Agar (PDA) plates that contains chlorophenicol supplement to suppress bacterial growth and incubated for 3 days at

room temperature ($28 \pm 2^{\circ}\text{C}$), to obtain young, actively growing colonies of molds. 1ml of garlic extract was mixed with 15ml of cooled (45°C) molten PDA medium and allowed to solidify at room temperature for thirty minutes. A mycelial disc 6mm diameter, cut out from periphery of 3 day old cultures, was aseptically inoculated onto the agar plate containing the garlic extract, PDA plates containing 1ml of griseofulvin were used as positive control. PDA plates with 1ml of distilled water were used as negative control (Georgii and Korting, 1991; McCutcheon et al., 1994). The inoculated plates were done in triplicates and incubated at room temperature ($28 \pm 2^{\circ}\text{C}$).

Colony diameter was measured and recorded after 3 days and the mean of three readings is recorded (Pundir et al., 2012). Percent mycelia growth inhibition was calculated as given below.

Percent mycelia
growth inhibition % = $\frac{D_a - D_b}{D_a} \times 100$

Where D_a represents control colony diameter and D_b represents treated colony diameter. The colony diameter is in millimetres.

Results and Discussion

The result of the phytochemical screening of garlic extract indicated the presence of phenolics, alkaloids, flavonoids, steroids, glycosides, saponins and tannins (Table 1). Dutta (1993) also reported some compounds as an indication of the potential medicinal value of the plants in which they appear.

The results obtained from the antifungal activity assay using the crude ethanolic garlic extract, showed that growth of all the fungi was inhibited by the garlic extract (Table 2). The ethanolic extract of garlic showed the highest activity against *Aspergillus ustus*. The extract inhibited the

growth of *Aspergillus ustus* by 100% at a concentration of 200mg/ml. This was followed by *Aspergillus niger*. The growth of *A. niger* was inhibited by 93.03% at a concentration of 200mg/ml. The lowest activity was recorded for *A. ustus* at a concentration of 50mg/ml (23.53%). *Penicillium* species showed moderate inhibition of 50.87% and 61.39% at a concentration of 50mg/ml and 100mg/ml respectively. All the three different concentrations of the extract used inhibited all the fungi. At a concentration of 50mg/ml, highest activity was recorded for *Penicillium* species (50.87%), followed by *A.niger* (49.60%), and the least was recorded for *A. ustus* (23.53%). At a concentration of 100mg/ml, the growth of *A.niger* was retarded the most (72.12%). This was followed by *Penicillium* species (61.39%) and then *A.ustus* (43.12%). The concentration of 200mg/ml showed the highest level of inhibition compared to the other two concentrations. *A.ustus* was most inhibited (100%) followed by *A.niger* (93.03%) and then *Penicillium* species (92.97%).

The three fungi differed with regard to their susceptibility to garlic extracts. At a concentration of 50mg/ml, *Penicillium* species was the most susceptible followed by *A.niger* and then *A. ustus*. At a concentration of 200mg/ml, *A.ustus* was the most susceptible and the least in susceptibility was *Penicillium* species (Table 3).

The results of the phytochemical screening indicated the presence of phenolics, alkaloids, flavonoids, steroids, glycosides, saponins, and tannins. The result is in agreement with the work of Lawal *et al.*, (2010) who reported that aqueous garlic extract revealed the presence of alkaloids, saponins, tannins, flavonoids, glycosides, cardiac glycosides, volatile oils and steroids.

Table.1 Qualitative determination of phytochemical groups of ethanolic extract of *Allium sativum* clove

Phytochemicals	Results
Alkaloids	+
Flavonoids	+
Glycosides	+
Saponins	+
Tannins	+
Phenolics	+
Steroids	+

+ = Present, - = Absent / not detected

Table.2 Antifungal activity of ethanolic garlic extract (diameter of the zone of mycelial extension, mm) against food-associated fungi

Fungi	Concentration of garlic extract(mg/ml)			Negative control	Positive control
	50.00	100.00	200.00		
<i>Aspergillus niger</i>	19.33±0.47	10.67±0.47	2.67±0.47	38.33±1.25	0.00
<i>Aspergillus ustus</i>	13.00±0.82	9.67±0.47	0.00	17.00±0.47	0.00
<i>Penicillium species</i>	18.67±1.27	14.67±0.94	2.67±0.47	38.00±0.82	0.00

Data represent average mean of three replicate ± S.D

Table.3 The percentage (%) mycelial growth inhibition of garlic extract against food-associated fungi by poisoned food technique

Fungi	Concentration of garlic extract(mg/ml)			Negative control	Positive control
	50.00	100.00	200.00		
<i>Aspergillus niger</i>	49.60	72.16	93.03	0.00	100.00
<i>Aspergillus ustus</i>	23.53	43.12	100.00	0.00	100.00
<i>Penicillium species</i>	50.87	61.39	92.97	0.00	100.00

The present investigation revealed that ethanolic extract of garlic inhibits the growth of *Aspergillus ustus*; *Aspergillus niger* and *Penicillium* species. The growth of *Aspergillus ustus* was the most retarded. These results confirm the earlier observations of Tagoe *et al.*, (2011) who had reported that ethanol extract of garlic is active against *Aspergillus flavus*, *Aspergillus niger* and *Cladosporium herbarum*. The results of this work also supported the work of Virmani *et. al.*, (2008) who reported that the growth of *Aspergillus fumigatus* was inhibited by alcoholic extracts of garlic bulb.

Yin and Cheng (1998) reported that water soluble extracts of garlic bulb had an inhibitory effect against *Aspergillus flavus* and *Aspergillus niger*.

In this study, the ethanol extract of garlic was effective in terms of percent mycelia growth inhibition (23.53%-100%) (Table 2). The extract showed excellent antifungal activity against *Aspergillus ustus* with complete mycelia growth inhibition (100%). Several workers (Pundir *et. al.*, 2012; Wu *et. al.*, 2009 and Hasan *et. al.*, 2005) have earlier reported that ethanol garlic extract

showed antimycotic activity against fungal genera such as *Aspergillus*, *Rhizopus*, *Fusarium* and *Cladosporium*, which is in harmony with the present study. Pundir *et.al.* (2012) showed that garlic extract inhibited *Rhizopus stolonifer* (50%), *Mucor* species (40%), *Aspergillus luchuensis* (30%), *A. flavus* (30%) and *Scopulariopsis* species (20%) but lacked an inhibitory activity against *Penicillium oxalicum*. In this investigation, ethanol garlic extract inhibited the growth of the food associated fungi *Aspergillus niger*, *A. ustus* and *Penicillium species*. This inhibition was concentration-dependent. Mycelia growth inhibition in *A. ustus* was 23.53% at a concentration of 50mg/ml. At a concentration of 100mg/ml, the mycelia growth inhibition was 43.12% while 100% inhibition was recorded at a concentration of 200mg/ml. A similar survey by Alhussaen *et al.*, (2011) indicated that garlic extract had a concentration-dependent activity against *Pythium ultimum* isolated from tomato seedlings. Undiluted garlic extract showed a high control activity with no growth as compared to the biotic control without the extract whereas diluted garlic extracts 10% and 5% reduced the fungal growth to 15.5% and 41% respectively (Alhussaen *et al.*, 2011). The ability of garlic to inhibit the growth of fungi could be attributed to the fact that garlic contains many antimicrobial compounds (Jabar and Al- Mossawi, 2007). The mechanism of action of phytochemicals against microorganism vary and depend on these phytochemicals (Aly and Bafiel, 2008).

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