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Evaluation of the antifungal activities of extracts of garlic (*Allium sativum*) on fungi isolated from guinea corn

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Abstract
The study investigated the effects of extracts of chloroform, methanol and ethanol of Allium sativum on the growth of species of *Aspergillus fumigatus, Penicillium and Rhizopus stolonifer* isolated from guinea corn. Between the minimum inhibitory concentration ranges of 20 mg / mL to 40 mg / mL the fungi were susceptible. The chloroform extract showed more inhibitory effect compared to ethanol and methanol extracts. An assay of the antifungal properties of these extracts indicated that the most resistant of all the test fungi appeared to be *Aspergillus fumigatus while the most susceptible species were Penicillium.* The investigation on the antifungal effect of *Allium sativum* on the growth of fungi isolated from Guinea corn demonstrated that *Allium sativum* has proven to be an effective antifungal agent against species *of Aspergillus fumigatus Rhizopus stolonifer*, and *Penicillium*. The results therefore suggests that extracts of *Allium sativum* may be an important preservative for Guinea corn. Phytochemical screening of extracts of *Allium sativum* also showed that the extracts contained saponins, tannins flavonoids, and anthraquinones.

Keywords: *Allium sativum, Aspergillus fumigatus, Penicillium*, Anti-fungal agents

Introduction

Guinea corn (*Sorghum bicolor*) belongs the family *Poaceae* of flowering plants which is a native precisely in North Africa. Its grains are used for the production of ethanol and it also serves as a source of food for human beings and animals because of its rich nutrients (Tashikalma *et al*., 2010) [20]. According to Abdullah *et al.*, 2014 the Nigerian sorghum production rose from 11.5 tons in 2010 and forecast was 11.7 tons in 2011. The crop yield increased greatly because the farmers accepted improved varieties that has been developed by local research institutes. There are numerous methods for the traditional preparations of guinea corn meals. Boiling guinea corn is one of the simplest and the most desirable. The whole grains are either decorticated before grinding or ground directly to floor to produced flour or fine particles and is then used in various traditional meals (Dada, 2013) [12].

Guinea corn is a valuable crop to the food and beverage industry especially for the production of non-alcoholic drinks as well as confectionery industry in Nigeria (Baiyengunhi and Fraser, 2009) [3]. The fifth most important cereal crop in the world is Sorghum after rice, wheat, maize and barley. It is an annual crop which grows in clumps reaching over 4m high. Its grains are small in size, with a diameter range of 3 - 4 mm (Kingston, 2008). Sorghum thrives in a wide range of high altitudes, temperatures, varying soil types which may be toxic and it recovers quickly after drought. It can be said to be able to survive unfavourable environmental conditions. A sample of good quality and mature guinea corn contains atleast; starch (74.63 %), dietary fibre (6.30 %), fat (3.30 %), protein (11.30 %) and 4.47 % although other micro- nutrients indicates that it could be a potential source of starch. Sorghum is also known as millet and guinea corn in West Africa, the South Africa call it corn, it is known as dura in Sudan, the East Africans call it matma, it is called jowar in India and kaolinang in China. In United States it is simply referred to as milo or millo-maize (Brandt, 2013) [9]. *Sorghum bicolor* belongs to *Andropogonae* grass family. The numerous health benefits of this crop includes prevention of cancer, improves digestion, control of diabetes, circulation and development of red blood cells, it is used to treat celiac disease, and it is a great source of energy (Brem and Lips, 2008) [10]. Sorghum also has high phenolic and tannin contents (Samson *et al*., 2001). Annual losses from in Africa and Asia as a result of grain mould contamination is over 130 million US dollars (Chandrashekar *et al*., 2000) [11]. In Nigeria and most parts of the world the mycotoxins and mycoflora of these mould has been documented. Niger state in Nigeria is the major producer of Sorghum (Dada, 2013) [12]. Fungi together with its toxins causes significant damage leading to a gross reduction in crop production, livestock production and diseases in humans more studies need to be carried out (Hawksworth and Livrinho, 2017) [14]. The Sorghum leaves are green in colour, flat but not as broad as the leaves of maize. Its leaves are smaller than that of maize and it is long, narrow and pointed (Bradley, 2008) [8]. As the leaves mature the are upright, and the blades bend and point downwards. (Brem and lupa, 2008) [10]. Depening on the environmental conditions the leaves number between 8-22 per plant (Abarca *et al*., 2014) [1]. On sandy soils Sorghum grows poorly unless where heavy textured subsoil is found (Hibbett *et al.*, 2007) [15]. Sorghum should be stored as whole grain at 12 % to 13 % moisture or less (James *et al*., 2002) [16]. The objective of storage is to try as much as possible to preserve the value of the grain for future use. This means to either retain a proportion of the viable seeds for next planting season so as to harvest bountifully (Lemar *et al.*, 2007) [18]. A number of factors may lead to the loss of viability and nutrients of the grains; however, depredations of pests (insects, birds and rodents) are the main causes of loss globally. Mould damages are also not left out

because guinea corn is highly susceptible to contamination by micro organisims especially fungi. Thus leading to huge economic losses and serious health challenges. In developing countries sorghum is stored in small quantities in traditional containers, most often on the farm (Tsao and Yin, 2001) [21]. The aim of this study therefore was to determine the effect of *Allium sativum* on the growth of fungi isolated from guinea corn

Materials and Methods

Plant Materials

The *Allium sativum* and Guinea corn were used in this study. *Allium sativum* were purchased at modern market Bida, Niger state Nigeria. While Guinea corn used was obtained from Etsu Musa Market opposite Etsu Usman Zaki Palace Bida Niger state, Nigeria.

Test Microoganisms

The test microorganisms used in this study were *Rhizopus stolonifer*, *Aspergillus fumigatus* and *Penicillium* species. These organisms were isolated from guinea corn and were identified after staining with lactophenol cotton blue. The organisms were cultured on potato dextrose agar slant and stored in the refrigerator until required.

Preparation of Plant Extracts

The One hundred gram (100 g) of the dried powder of *Allium sativum* (garlic) was weighed separately into 400 mL of ethanol, methanol, and chloroform in different conical flasks. These were stirred intermittently over a period of 72 hours. The extracts were then filtered into different conical flasks. The extracts obtained were evaporated to dryness using a water bath at (Barrinyton C 25) 80 ºC. The extracts were then assayed immediately after preparation (Banso, 2016) [5].

Determination of antifungal properties of extract

The antifungal properties of the extracts were determined using agar dilution method of (Banso and Mann, 2006) [6]. Different volumes of the extracts were introduced into McCartney bottles containing appropriate volumes of sterile potato dextrose agar. The mixtures were poured into different petri dishes and allowed to solidify. The plates were inoculated with 5 mm diameter of the fungal culture. Control experiment was performed without the extracts. The plates were incubated at 28 ± 2 ºC for 48 hours. Antifungal activity was expressed in terms of diameter of growth (mm).

Determination of minimum inhibitory concentration (MIC)

Various medium concentrations (10 mg / mL, 20 mg / mL, 30 mg / mL and 40 mg / mL) of the extracts were prepared. Four of these was added to 15 mL of potato dextrose broth in test tubes and inoculated with 0.1 mL spores of the spore suspended of *Rhizopus stolonifer*, *Penicillium* species and *Aspergillus fumigatus* diluted in order to give a final spore suspension of 10^6 spores / mL. The mixtures were then incubated for 48 hours at 28 ± 2ºC. The least concentration of the plant extracts that does not permit the growth of inoculated test organism was regarded as the minimum inhibitory concentration control experiment were performed without the extracts (Banso, 2016) [5].

Determination of Minimum Fungicidal Concentration

The content of the tubes which showed no visible fungal growth in the minimum inhibitory concentration, (MIC) experiment was cultured into freshly prepared potatoes dextrose agar plates to assay of fungicidal effects of the extracts. The plates containing the test fungi was incubated at 28 ± 2ºC for 72 hours. The lowest concentration of the extracts that did not yield any visible fungal growth on the solid medium was regarded as the minimum fungicidal Concentration (Banso *et al*., 2010) [7].

Phytochemical Screening of Extracts

Extracts of the Spice used in this study were screened for active ingredients using the method described by (Banso and Fawole, 2010) [7].

Test for sterols

Two millitres of distilled water was added to 0.3g of the extract to form a Solution. To 1 mL of the solution a few drops of 10 % ferric chloride was added and observed. The absence of a green precipitate indicated the presence of sterols.

Test for flavonoids

Five millitres of distilled water was added to 0.3 g of the extract and 10 % lead acetate solution was also added. A precipitate indicated the presence of flavonoids.

Test for Tannins

Two millitres of distilled water was added to 0.3g of the extract to form a solution. To 1 mL of the solution a few drops of 10 ferric chloride was added and observed for the presence of tannins. The presence of green precipitate indicates the presence of tannins.

Test for phenols

Three millitres of distilled water was added to 0.3 g of the extract one millitres of ferric chloride and 1 mL of 1 % potassium ferric cyanide were added and observed for the presence of phenols. Development of a blue green color indicates the presence of phenols.

Test for anthraquinones

Three milllitres of distilled water was added to 0.3g of the extract. To 1ml of the solution 0.2 mL of dilute sulpheric acid (H_2SO_4) and 9 mL of benzene were added upon separation of benzene layer into another test tube, three drops of dilute ammonium solution was added. Presence of pink colour in the ammonium solution phase indicates negative result.

Test for saponins

Two millitres of distilled water was added to 0.3 g of the extract and shaken. Development of frothing that persisted on warming was primary identification of saponins.

Results

Antifungal properties of extracts of *Allium sativum*

The results of the antifungal properties of *Allium sativum* are shown in Table 1. The mean deviation of growth of *Rhizopus stolonifer* when ethanol extract of *Allium sativum* was assayed against *Rhizopus stolonifer* was 10.0 ± 0.2mm. Methanol was 11.0 ± 0.1 mm while a value of 9.0 ± 0.1 mm was recorded against chloroform.

The value of 10.5 ± 0.1 mm was recorded against *Aspergillus fumigatus* when ethanol extract of *Allium sativum* was assayed against test fungi. The value recorded against methanol extract was 12.0 ± 0.2 mm while a value of 9.5 ± 0.1 mm was recorded against chloroform extract.
A value of 12.5±0.1mm was recorded against *Penicillium* species when methanol extract of *Allium sativum* was assayed against the test fungi. The value recorded against ethanol extract was 9.5.0 ± 0.1 mm while a value of 8.5 ± 0.2 mm was recorded against chloroform.

Table 1: Antifungal properties of extracts of *Allium sativum*

Diameter of growth (mm) ± SD				
Organism	**Control**	**Ethanol extract**	**Methanol Extract**	**Chloroform Extract**
		n = 3	n = 3	n = 3
R. stolonifer	13.0 ± 0.2	10.0 ± 0.2	11.0 ± 0.1	9.0 ± 0.1
A. Fumigatus	13.5 ± 0.3	10.5 ± 0.1	12.0 ± 0.2	9.5 ± 0.1
Penicillium spp.	11.0 ± 0.1	9.5 ± 0.1	12.5 ± 0.1	8.5 ± 0.2

SD = Standard deviation n= Number of samples

The Minimum inhibitory concentration of extract of *Allium sativum* Against The Test Organisms
The results minimum inhibitory concentration of extracts of *Allium sativum* against *Rhizopus stolonifer, Aspergillus fumigatus* and *Penicillium* species are shown in Table 2. Minimum inhibitory concentration values recorded against *Rhizopus stolonifer*, *Aspergillus fumigatus* and *Penicillium* species when ethanol extract of *Allium sativum* was assayed against the fungi were 30 mg / mL, 20 mg / mL and 30 mg / mL respectively. Minimum inhibitory concentration values of 30 mg / mL, 30 mg / mL and 20 mg / mL were recorded against *Rhizopus stolonifer*, *Aspergillus fumigatus* and *Penicillium* species respectively when methanol extract of *Allium sativum* was assayed against the fungi. Minimum inhibitory concentration values recorded against *Rhizopus stolonifer, Aspergillus fumigatus* and *Penicillium* species when chloroform extract of *Allium sativum* was assayed against the fungi were 40 mg / mL, 30 mg / mL and 40 mg / mL respectively. The results of minimum inhibitory concentration of the extracts against the test organism correlate with the report that microorganisms varied in the degree of their susceptibility to antifungal agents.

Table 2: Minimum Inhibitory Concentration of Extracts of *Allium sativum*

Minimum inhibitory concentration (mg / mL)			
Organism	**Ethanol extract**	**Methanol Extract**	**Chloroform Extract**
R. stolonifer	30	30	40
A. Fumigatus	20	30	30
*Penicillium*spp	30	20	40

Minimum Fungicidal Concentration of extracts of *Allium sativum* Against The Test Organisms
The results (Table 3) show that the minimum fungicidal concentration of *Allium sativum* against *Rhizopus stolonifer, Aspergillus fumigatus* and *Penicillium* species ranged between 30 mg / mL and 40 mg / mL. A minimum fungicidal concentration value of 40mg/ml was recorded against *Rhizopus stolonifer*, *Aspergillus fumigatus* and *Penicillium* species value of 30 mg / mLwas recorded against all the test fungi when ethanol extract of *Allium sativum* was assayed against the fungi. Minimum fungicidal concentration values of 40 mg / mL, 40 mg / mL and 30 mg / mL were recorded against *Rhizopus stolonifer*, *Aspergillus fumigatus* and *Penicillium* species respectively when methanol extract of *Allium sativum* was assayed against the fungi.

Table 3: Minimum Fungicidal Concentration of Extracts of *Allium sativum*

Minimum inhibitory concentration (mg/ml)			
Organism	**Ethanol extract**	**Methanol Extract**	**Chloroform Extract**
R. stolonifer	40	40	40
A. Fumigatus	30	40	40
Penicillium spp	40	30	40

Phytochemical Constituents of *Allium sativum*
Table 4 shows the results of the phytochemical screening of ethanol extract of *Alliium sativum*. Ethanol extract of *Allium sativum* contains flavonoids, tannins, anthraquinones and saponins. Phenol and steroids were absent in the extract. These constitutes may be responsible for antifungal activity of the extract used in this study. Biologically active substances have been found to be responsible for antifungal activities of certain plants (Banso, 2016) [5].

Table 4: Phytochemical constituents of ethanolof extracts of *Alliumsativum*

Constituent	Ethanol extract of *Allium sativum*
Steriods	_
Flavonoids	+
Tannins	+
Phenols	_
Anthraquinones	+
Saponins	+

- = Absent
+ = Present

Discussion
Antifungal test of extracts of *Allium sativum* illustrated that the extracts exhibited antifungal activities against the test fungi (Table1). This could be as a result of the presence of active ingredients in the plant materials. Plants generally are known to produce many secondary metabolites which constitutes an important source of microbiocides, pesticides and pharmaceutical drugs (Banso *et al*., 2010) [7]. These spices contain phenolic essential oils which are strong inhibitory agents to microorganisms (Banso, 2016) [5]. The results of these study justifies the traditional use of *Allium sativum* as food preservative. Previous studies have demonstrated that *Allium sativum* has antimicrobial activities against some selected microorganisms (Banso *et al*., 2010) [7]. The minimum inhibitory concentration results of the extracts on the test fungi coincides with the study that microorganisms varied in their degree of susceptibility to most antifungal agents (Banso *et al*., 2010; Banso and Ayodele, 2005) [7, 4]. Flavonoids, tannins, phenols, Anthraquinones and saponins are contained in *Allium sativum* which are inhibitory to microorganisms. Banso and Ayodele in 2005 [5] also reported the antifungal effect of flavonoids and anthraquinones.

Conclusion

The plant (A*llium sativum)* extracts used in this study demonstrated inhibitory effects against *Rhizopus stolonifer*, *Aspergillus fumigatus* and *Penicillium* species isolated from deteriorating Guinea corn. Extracts of *Allium sativum* may be an important source of preservative for Guinea corn.

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