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Studies on the Antimicrobial Properties and Phytochemical Screening of Garlic (*Allium sativum*) Extracts

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Abstract

This study was carried out to evaluate the antimicrobial properties of garlic (*Allium sativum)* extracts on three fungi namely *Aspergillus flavus*, *Curvularia lunata* and *Fusarium moniliforme* using the pour plate method. A phytochemical screening of the extracts was also carried out to determine the constituents in garlic. Water, ethanol and acetone were the extractants used. Results showed that radial growth in all the three test organisms was impaired by the addition of the extracts in the culture medium used. The test organisms differed in their reaction to the different extracts but on the whole, growth inhibition increased with the concentration of each extract. Phytochemical screening of the different extracts showed that garlic contains important compounds such as carbohydrates, reducing sugars, lipids, flavonoids, ketones, alkaloids, steroids and triterpenes. Tannins were, however, not detected in any of the extracts under the conditions of this study. The significance of these results is discussed.

Key words: Garlic, extracts, constituents, antimicrobial, inhibition.

Introduction

Garlic (*Allium sativum*) qualifies as an important vegetable because not only is it an indispensable cookery ingredient, it can well and delightfully be eaten as and for itself (Anon, 2009). The *Allium* genus belongs to the Liliaceae family comprising onions, leeks, shallots, asparagus etc. Modern garlics are of only two species; *Allium sativum*, the soft necks and *A. ophioscorodon*, the hard necks. Taste, storage ability and suitability in growing are the critical factors in selecting garlic classes of interest. The hard necks have more intense flavours but less storage capabilities while the soft necks are excellent keepers but often milder. Hard necks are generally grown in cooler climates while the soft necks grow closer to the equator (Al-Zahim *et al.,* 1997). Garlic requires a sunny spot and the soil should be rich but not too rich or the tops will overdevelop.

The medicinal value of plants has assumed a more important dimension in the past few decades. This is due largely to the discovery that extracts from plants contain not only minerals and primary metabolites but also a diverse array of secondary metabolites with antioxidant potentials (Sofowora, 1993, Okigbo *et al*. 2009a). The use of plant extracts in traditional medicine is a worldwide practice. Medicinal plants form the basis of primary health care for majority of the people living in the rural and remote areas in Nigeria and other third world countries (Awosika, 1993). A number of medicinal plants have been found and put into use in ethnomedicine by traditional healers in the management of many diseases.

According to Sofowora (1993), African medicinal plants rank highest among plants used in the investigations of antimicrobial properties. This could be due to their high traditional medicinal use and also the ease of carrying out such tests. Among medicinal plants of African origin are *Psidium guajava* (guava), *Azadirachta indica* (neem), *Vernonia amygdalina* (bitter leaf), *Anacardium occidentales* (cashew), *Allium cepa* (onion) and *A. sativum* (garlic). The organs or parts used in these plants vary from one plant to the other and these include the leaves, bark, roots, stem, flowers, fruits and even the seeds (Farombi, 2003, Nguelefack *et al*., 2005). While garlic

is primarily used as a herb to enhance many food dishes in various cultures (Table 1), it contains many substances which studies have shown act together to prevent disease and age-related conditions (Anon, 2009). According to Johnson *et al*. (2008), biodiversity provides mankind enormous direct benefits and indirect essential services through natural ecosystem function and stability. 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 (Cavallito *et al*., 1945; Ross *et al*., 2000). Allicin formation follows the action of an enzyme, allinase of the bundle sheet cells upon the alliin of the mesophyl cells. When crushed, *A. sativum* yields allicin, a powerful antibiotic and antifungal compound (phytoncide). However, due to poor bioavailability, it is of limited use for oral consumption. Garlic also contains some sulphur-containing compounds such as alliin, ajoene, diallylsulphide, dithin, S-allylcysteine and enzymes as well as some non sulphur-containing compounds including vitamin B, proteins, minerals, saponins and flavonoids. Yukihiro *et al.,* 2002 have reported a phytoalexin called allixin in garlic.

The objective of this study is to evaluate the potential of garlic (*A. sativum* L.) extracts in inhibiting the growth of three phytopathogenic fungi, *A. flavus, C. lunata* and *F. moniliforme in vitro*. This study will also determine the phytochemical constituents of garlic and the efficiency of their extraction using different solvents.

Materials and Methods

Materials used and Extraction Procedure

Cured bulbs of *A. sativum* were obtained from Idi-Ape Market in Ilorin, Kwara State of Nigeria. The test organisms used (*Aspergillus flavus, Curvularia lunata* and *Fusarium moniliforme*) were sourced in-house from the stock cultures in the Plant Pathology Laboratory, Department of Plant Biology, University of Ilorin. Fresh cultures were prepared from the stock and used for all the experiments carried out in this study. Potato Dextrose Agar (PDA) was the only culture medium used and it was prepared routinely.

The aqueous, ethanol and acetone extracts of garlic were prepared following one of the various acceptable procedures. After removing the papery skin of *A. sativum* cloves, 25g were weighed into a beaker, surface-sterilized with sodium hypochlorite for 2 minutes, rinsed twice with sterile distilled water and then crushed using sterile mortar and pestle. The resultant paste was soaked in 100ml of any of the chosen extractants for 24 hours. The extract was then filtered through two layers of sterile Whatman No. 1 filter papers into conical flasks and used as stock. The extract was stored in the refrigerator at 3^{o}C for subsequent use.

$$\text{Concentration of extract} = \text{Weight of garlic cloves (g)} \ / \ \text{Volume of extractant (ml)}$$

$$= 25\text{g} / 100\text{ml} = 0.25 \text{ g/ml}$$

Effect of Extracts on Growth of Test Organisms

Four concentrations of garlic extracts were tested against three pathogenic fungi namely *A. flavus, C. lunata* and *F. moniliforme*. One hundred and twenty (120) millilitres of sterile molten agar was amended separately with 5, 10, 20 and 40ml of the stock extract. The amended agar medium was dispensed into sterile Petri dishes and allowed to solidify. Each concentration was replicated three times. The control experiment comprised three agar plates amended with sterile distilled water. Two diagonal lines were drawn with a marker to cross each other at the centre on the reverse side of each Petri dish. Each plate was inoculated at the centre with a 2mm-diameter mycelial plug cut from the edge of a growing culture using a sterile cork borer. Growth was measured daily along the diagonal lines at the back of the plates.

Phytochemical Screening

The aqueous, ethanol and acetone extracts of *A. sativum* were screened for the presence of secondary metabolites using the procedure of Sofowora (1993). Two (2) milliliters of each extract was measured into a test tube for each of the tests and concentrated by evaporating the extractant in a water bath. Tests were carried

out for carbohydrates, reducing sugars, tannins, polyphenols, lipids, flavonoids, ketones, alkaloids, steroids and triterpenes. This aspect of the research was carried out in the Department of Chemistry of the University of Ilorin, Ilorin, Nigeria.

Results and Discussion

Growth Inhibition

Radial growth in all the organisms tested in this study was inhibited by the three extracts investigated. The effectiveness of the extracts in hindering growth of the test fungi differed with the extracts, the organisms and also between concentrations. At the highest concentration (40ml extract: 120ml molten agar) tested in this study, all the extracts inhibited growth completely in all the test fungi. At 20ml extract: 120ml molten agar concentration, the acetone extract was most effective against *A. flavus* inhibiting growth completely while permitting slight growth in both *C. lunata* and *F. moniliforme*. At the same 20:120 concentration of the aqueous and ethanol extracts, both *A. flavus* and *C. lunata* recorded slight radial growth. Only *F. moniliforme* was completely inhibited at this concentration.

At both the 5:120 and 10:120 concentrations, growth was recorded in all the test fungi with more growth occurring at the 5:120 concentration than at 10:120 of all the extracts. At the lowest concentration (5ml extract: 120ml molten agar), the aqueous extract was more inhibitory to the growth of *F. moniliforme* than any of the other two extracts. However, there was more growth in all the test fungi in the control plates than in any of the extract concentrations tested under the conditions of this study. Observations from the control plates also showed that *C. lunata* was the fastest growing organism among the three organisms used in this study while *F. moniliforme* was the slowest.

Phytochemical Screening

Screening tests showed that the constituents of *A. sativum* extracts differed with respect to the extractant employed. The aqueous and ethanol extracts of *A. sativum* showed similar reactions in all the tests carried out while the acetone extract showed slightly different reactions with the same reagents (Table 2). Both the aqueous and ethanol extracts were found to contain carbohydrates, reducing sugars, lipids, flavonoids, ketones, alkaloids, steroids and triterpenes. In the acetone extract alkaloids, steroids and triterpenes were absent ((Table 3). Tannins and polyphenols were not detected in any of the *A. sativum* extracts under the conditions of this study.

Growth inhibition was observed in the present study when *A. sativum* extracts were incorporated into potato dextrose agar (PDA) medium. All the extracts irrespective of the extractant used were effective against the three fungi tested though the level of antimicrobial activity differed slightly. At higher concentrations the extracts completely inhibited the growth of the fungi tested in this study. The acetone extract was inhibitorier to the radial growth of all the test fungi. This may be due to the additional effect of the extractant. Antifungal activities have been detected in crude medicinal plant extracts of 20 plant species against *Fusarium oxysporum* causing wilt disease in *Solanum melongena* L. It has been reported that the potency of any plant extract depends on its concentration and the method of extraction. The systemic screening of antimicrobial plant extracts represents a continuous effort to find new compounds with potential to act against multi-resistant pathogenic bacteria and fungi (Shariff *et al*., 2006). Investigations into the chemical and biological activities of plants during the past two centuries have yielded compounds for the development of modern synthetic organic chemistry and the emergence of medicinal chemistry as a major route for the discovery of novel and more effective therapeutic agents (Roja and Rao, 2000).

The result of phytochemical screening showed that water and ethanol extracted more components from crude garlic extract than acetone under the conditions of this study. While carbohydrates, reducing sugars, lipids, flavonoids, ketones, alkaloids, steroids and triterpenes were extracted both in water and ethanol, acetone could not extract alkaloids, steroids and triterpenes. Farombi (2003) and Okigbo *et al*. (2009a) had reported some

of these components in many other plants. In most traditions, decoctions or infusions of herbs are usually made with either alcohol or water as the solvent. This may be related to their efficiency in extracting most of the active principles in plants. At times, marked differences exist between the phytochemical profile of alcoholic and aqueous extracts of plants. For *A. sativum*, the aqueous extract is recommended because no vital phytochemical constituent seemed to be left out and also because of probable unwanted effects that alcohol which is another drug on its own may produce.

The inhibitory activity of *A. sativum* and its healing power has been reported (Cavallito, 1945). This may be due to the presence of active components like flavonoids, alkaloids, steroids and triterpenes. Flavonoids are potent water-soluble antioxidants and free radical scavengers, which prevent oxidation, cell damage and have strong anticancer activity. They also lower the risk of heart diseases (Sofowora, 1993). Alkaloids have been documented to possess analgesic, antispasmodic and bactericidal effects (Okigbo et al., 2009b). Garlic powder preparations have been reported to be of some clinical use in subjects with mild hypertension.

The fungi used for this study are economically important for different reasons. *A. flavus* produces a violent toxin called aflatoxin. It is a highly toxic substance which has some carcinogenic effects and may cause cancer of liver in humans and animals (Sharma, 2005). *Curvularia lunata* is a member of the family Dematiaceae of the order Moniliales. It occurs on rice and many other crops, causing leaf spots, blights, grain deformation, grain discolouration and even root rot. *Fusarium moniliforme* belongs to the family Tubercul ariaceae and occurs on many crop plants such as rice (*Oryza sativa* L.), maize (*Zea mays* L.) and sorghum (*Sorghum vulgare* L.). It causes serious wilting in the host plants as well as diseases like foot rot, seedling blight, stalk rot and top rot (Pandey, 2008). More research activities may be required to elucidate the exact structures of the active principles in garlic responsible for the observed antimicrobial properties.

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Table 1. Nutritional value per 100g (3.5oz) of raw garlic.

Mineral	**Weight**
Carbohydrates	33.06g
Sugars	1.00g
Dietary fiber	2.1g
Fat	0.5g
Protein	6.39g
-B-carotene	5Ng
Thiamin (vit. B1)	0.2mg
Riboflavin (vit. B2)	0.11mg
Niacin (vit. B3)	0.7mg
Pantothenic acid (B5)	0.596mg
Vitamin B6	1.235g
Folate (vit. B9)	3Ng
Vitamin C	31.2mg
Calcium	181mg
Iron	1.7mg
Magnesium	25mg
Potassium	401mg
Sodium	17mg
Zinc	1.16mg
Manganese	1.672mg
Selenium	14.2mg

Table 2. Phytochemical Observation of *A. sativum* Extracts.

Constituent	Observation	
	Water and Ethanol Extracts	**Acetone Extract**
Carbohydrates	Two layers of ethanol above and acid below were formed with a reddish-brown ring at the junction of the two layers.	Two layers of ethanol above and acid below were formed with a reddish-brown ring at the junction of the two layers.
Reducing sugars	Brick-red colouration	Brick-red colouration
Tannins/ Polyphenols	Golden precipitate instead of a bluish-black colouration	Golden precipitate instead of a bluish-black colouration
Lipids	Reddish precipitate	Reddish precipitate
Flavonoids	Orange colouration	Orange colouration
Ketones	Yellowish precipitate	Yellowish precipitate
Alkaloids	Orange and yellowish-white precipitate for Mayer's and Dragendorff's reagent respectively.	Golden and creamy colouration for Dragendorff's and Mayer's reagent respectively.
Steroids and triterpenes	Greenish-blue and violet precipitates for steroids and triiterpenes respectively.	Two layers of reagents above and acid below were formed with a reddish-brown ring at the junction of the two layers.

Table 3. Phytochemical Constituents of *A. sativum* Extracts.

Constituent	Extract		
	Water	**Ethanol**	**Acetone**
Reducing sugars	+	+	+
Tannins/ Polyphenols	–	–	–
Lipids	+	+	+
Flavonoids	+	+	+
Ketones	+	+	+
Alkaloids	+	+	+
Steroids and Triterpenes	+	+	+

+ Present - Absent