

Full Length Research Paper

Phytochemical screening and antimicrobial studies on the methanolic bulb extract of *Allium sativum* L.

Ameh, G. I.^{1*}, Eze, S. C.² and Omeje, F. U.¹

¹Department of Applied Biology and Biotechnology, Enugu State University of Science and Technology, Enugu, Enugu State, Nigeria.

²Department of Crop Science, University of Nigeria, Nsukka, Enugu State, Nigeria.

Accepted 5 July, 2012

Methanolic extract of the bulb of *Allium sativum* was screened for phytochemical and antimicrobial properties. The crude extract of *A. sativum* bulb showed positive results for carbohydrates, glycosides and proteins in high concentrations; alkaloids, saponins, reducing sugars, oils and steroids in medium concentrations, while flavonoids and acidic compounds were present in low amounts. Tannins, resins and terpenoids were however absent in the extract. All the six tested microorganisms exhibited varied susceptibility to the plant extract. The minimum inhibitory concentration (MIC) of the organisms were, 50 mg/ml for *Salmonella paratyphi*, 25 mg/ml for *Bacillus subtilis*, 100 mg/ml for *Klebsiella pneumoniae*, 12.5 mg/ml for *Candida albicans*, 3.12 mg/ml for *Candida parolopsis* and 6.25 mg/ml for *Candida tropicalis*. *A. sativum* showed both antifungal and antibacterial properties.

Key words: Phytochemical screening, antimicrobial activity, *Allium sativum*, bulb extract.

INTRODUCTION

Plants have long been serving mankind as sources of useful drugs, food, additives, flavouring agents, colourants, binders and lubricants (Falodun et al., 2006). Medicinal plants are sources of important drugs used in the treatment of diseases either alone or in combination with other plants (Awonubi, 1988). Chemical substances found in plants include alkaloids, glycosides, essential oil, saponins, tannins, steroids, terpenoids, resins, flavonoids, proteins and others. These substances are potent bioactive compounds found in medicinal plant parts that can be used for therapeutic purposes (Soforowa, 1993; Nwadiaro and Nwachukwu, 2007). These inherent bioactive principles differ from plant to plant as a result of their biodiversity and they produce a definite physiological effect on human body. Several authors have screened different medicinal plants for the presence of these active principles. The knowledge of medicinal plants are important in pharmaceutical industry. Banson and Olutimayin (2001) have shown that Plants are composed of a wide variety of active principles.

These established a good support to the use of these plants in herbal medicine and as base for the development of new drugs and phytomedicine. It has been demonstrated by several workers that some of these bioactive principles of plant origin possess antimicrobial properties. The increased reliance on the use of medicinal plants in industrialized countries had been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used rural herbal medicine (UNESCO, 1998).

Allium sativum L., commonly called garlic is a widely cultivated herbal plant, belonging to the family Alliaceae. Garlic is a plant with long, flat grass like leaves and a papery hood around the flowers (Milner, 2005). The greenish white or pink flowers are grouped together at the end of a long stalk. The stalk rises directly from the flower bulb, which is the part of the plant used as food and medicine (Awonubi, 1988). The most active components of fresh garlic are alliin and an enzyme called allinase. When garlic is chewed, chopped, bruised or cut, these compounds mix to form allicin, which is responsible for garlic's strong smell. It's medicinal claims have included cures for toothaches, cold, coughs and

*Corresponding author. E-mail: g_ameh@yahoo.com.

Table 1. Phytochemical analysis of garlic bulb extract.

Active Ingredient	Extract
Carbohydrates	+++
Reducing sugars	++
Alkaloids	++
Glycosides	+++
Saponins	++
Tannins	–
Flavonoids	+
Resins	–
Proteins	+++
Oils	++
Steroids	++
Terpenoids	–
Acidic compounds	+

–, Absent; +, low concentration; ++, medium concentration; +++ high concentration.

other viral infections, open wounds and evil demons (Fluck, 1973). The aim of this study was to evaluate the antimicrobial activities of active principles from *A. sativum* bulb extract.

MATERIALS AND METHODS

Collection and identification of plant material

Fresh bulbs of garlic were collected from Nsukka Central Market in Enugu State and authenticated by Mr. A. Ozioko, a Taxonomist of Bioresource Development and Conservation Programme (BDGP), Nsukka.

Processing of plant samples

The scales around the fresh bulbs of garlic were removed while the bulbs were washed and rinsed properly in tap and sterile distilled water, respectively. The bulbs were macerated, dried in an electric oven and milled mechanically before being stored in a plastic container.

Extraction of plant material

The methanol extract of the garlic bulb was prepared using the procedure described by Harbone (1994). Twenty five gram (25 g) of the powdered sample was soaked in a mixture of methanol and distilled water in the ratio of 3:2 for four days and later filtered to obtain the methanol extracts. The mixture was first concentrated by evaporation using water bath at 100°C for 1 h.

Test microorganisms

Clinical isolates of *Salmonella paratyphi*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Candida albicans*, *Candida parolopsis* and *Candida tropicalis* used in this study were obtained from Pharmaceutical Microbiology Laboratory of the University of Nigeria, Nsukka.

Sensitivity tests

The antimicrobial test was carried out using the agar diffusion method described by Opara and Anasa (1993). Nutrient agar and Sabouraud dextrose agar (SDA) were prepared for the sensitivity tests. The test organisms were inoculated on nutrient agar plates for bacteria and SDA for fungi, and spread uniformly using a sterile glass spreader. Cavities of 1 cm diameter were made on the nutrient agar using a sterile cork borer. The cut agar disks were removed with forceps sterilized by flaming. To each of the cavities was introduced 0.1 ml of the plant extract. One of the cavities was filled with 0.1 ml of absolute ethanol to serve as control. The plates were allowed to stand for 1 h on the bench for diffusion to occur before the growth of the organisms commenced and incubated at 37°C for 24 to 48 h. The zones of inhibition were then observed and recorded.

Determination of minimum inhibitory concentration (MIC) of the bulb extract on the test organisms

The minimum inhibitory concentration of the extract was determined according to the technique of Baron and Finegold (1990). Standardized suspensions of the test organisms were inoculated into a series of sterile Petri dishes of nutrient broth containing dilutions of the bulb extract (100, 50, 25, 12.5, 6.25, 3.125 and 1.563 mg/ml) and incubated at 37°C for 24 h. The MICs were read as the least concentration that inhibited the growth of the test organisms.

Phytochemical screening

The phytochemical screening of the garlic bulb extract was carried out according to the procedures described by Trease and Evans (1989).

RESULTS

The crude extract of *A. sativum* bulb showed positive results for alkaloids, glycosides, saponins, flavonoids, steroids, proteins, carbohydrates, oils, reducing sugars and acidic compounds (Table 1). The extract of the plant material was, however devoid of tannins, resins and terpenoids. Carbohydrates, glycosides and proteins occurred in high concentrations. Alkaloids, saponins, steroids, reducing sugars and oils were present in medium concentrations, while flavonoids and acidic compounds had low concentrations (Table 1).

The results of the sensitivity tests showed that all the six micro-organisms exposed to the methanolic extract of garlic bulb were sensitive to the plant extract. The micro-organisms studied had obvious differences in their susceptibility to garlic bulb extract. *C. tropicalis* showed the highest inhibition zone diameter (30 mm), followed by *C. albicans* (29 mm), while *S. paratyphi* showed the lowest (15 mm) inhibition zone diameter (Table 2).

Table 3 shows the MIC of the garlic bulb extract on the test isolates. The MIC results for the test organisms were *S. paratyphi* (50 mg/ml), *B. subtilis* (25 mg/ml), *K. pneumoniae* (100 mg/ml), *C. albicans* (12.5 mg/ml), *C.*

Table 2. Inhibition zone diameter produced by garlic bulb extract against the test organisms.

Test organism	Inhibition zone diameter (IZD) (mm)
<i>Salmonella paratyphi</i>	15
<i>Bacillus subtilis</i>	16
<i>Klebsilla pneumoniae</i>	25
<i>Candida albicans</i>	29
<i>Candida parolopsis</i>	21.8
<i>Candida tropicalis</i>	30

Table 3. Minimum inhibitory concentration (MIC) exhibited by the bulb extract against the test isolates.

Test organism	Zone of inhibition (mm)						
	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	3.125 mg/ml	1.563 mg/ml
<i>S. paratyphi</i>	+	10	+	+	+	+	+
<i>B. subtilis</i>	+	13.5	10	+	+	+	+
<i>K. pneumoniae</i>	25	+	+	+	+	+	+
<i>C. albicans</i>	+	19	16	14	+	+	+
<i>C. parolopsis</i>	+	25	23	21	13	11	+
<i>C. tropicalis</i>	+	20	16	13	11	+	+

+ = No inhibition.

parolopsis (3.125 mg/ml) and *C. tropicalis* (6.25 mg/ml).

DISCUSSION

Phytochemical screening of the garlic bulb extract revealed the presence of alkaloids, glycosides, saponins, flavonoids, steroids, proteins, carbohydrates, oils, reducing sugars and acidic compounds. These bioactive principles are believed to be responsible for the observed antimicrobial effect of the plant extract. Several workers have attributed the antimicrobial effect of plant extract to the presence of these secondary plant metabolites (Nweze et al., 2004; Gandhiraja et al., 2009). Phytochemical analysis of leaf extracts of *Ocimum gratissimum* revealed the presence of alkaloids, cardiac glycosides, flavonoids, glycosides, resins, steroidal terpenes and tannins (Mbata and Saika, 2008). The presence of active principles in the bulb extract of *A. sativum* could be used to establish a good support for the use of the plant in herbal medicine.

In this study, the six microorganisms studied were sensitive to the plant extract. The plant extract showed varying degrees of antimicrobial activity on the micro organisms. This is in agreement with the work of Rojas et al. (2006) on ten medicinal plants. The inhibitory activities of the plant extract agree with the report of Leven et al. (1979). Nwadiaro and Nwachukwu (2007) linked the antimicrobial activities of plants to the presence of tannins, alkaloids, flavonoids and saponins. It has been reported that various plants that are rich in alkaloids,

tannins and glycosides possess antimicrobial activity against a number of micro organisms (Adebajo et al., 1983). This suggests that the plant extract used in this study has a general antimicrobial activity. The antifungal potential of *A. sativum* bulb extract is more pronounced than its antibacterial properties. This is in consonance with the report of Nantitanon et al. (2007) on *Hyptis suaveolens*.

The various sizes of inhibition zones obtained in this study is an indication of the potency of the bioactive principles contained in the plant extract. The varied MICs of the plant extract on the test organisms correlates with the report indicating that micro organisms exhibits varied level of susceptibility to plant extracts. The MIC result in this study agrees with Banso and Adeyemo (2007) showing that antimicrobial agent with low activity against an organism has a high MIC, while a highly active antimicrobial agent gives a low MIC. The present study therefore shows that *A. sativum* bulb extract has useful antimicrobial properties. Further work is needed to isolate the active principles from the plant in order to test the specific antimicrobial activity of the respective phytochemical components.

REFERENCES

- Adebajo, AO Adewumi, CO and Essien, EE (1983). Antiinfective agent of higher plants. International Symposium of Medium Plants. 5th ed. University of Ife, Nigeria. Pp. 152-158.
- Awonubi MO (1988). Use of Local Herbs for Curing Diseases. Pharma. Herbal Med. 4(2):26 -27.
- Banso A, Adeyemo SO (2007). Phytochemical and Antimicrobial

- Evaluation of Ethanolic extract of *Dracaena mannii* Bark. Nig. J. Biotech. 18(1-2):27-32.
- Banso A, Olutimayin T (2001). Phytochemical and Antimicrobial Evaluation of Aqueous extracts of *Daniella oliveri* and *Nauclea latifolia*. Nig. J. Biotechnol. 12(1):114-118.
- Baron JE, Finegold SM (1990). Method for Testing Antimicrobial Effectiveness. In: Bailey Scotts Diagnostic Microbiol. Mosby, C.V. (ed.), Missouri, pp. 171-194.
- Falodun A, Okunrobo LO, Uzoamaka N (2006). Phytochemical Screening and Anti-Inflammatory Evaluation of Methanolic and Aqueous extracts of *Euphorbia heterophylla* Lim. Afr. J. Biotechnol. 5(6):259-531.
- Fluck H (1973). Medicinal Plants and their uses. W. feulsham and Coy. Ltd. New York, pp. 7-15.
- Gandhiraja N, Sriram S, Meena V, Srilakshini JK, Sasikumar C, Rajeswari R (2009). Phytochemical screening and antimicrobial activity of the plant extracts of *Mimosa pudica* L., against selected microbes. Ethnobot. Leaflets 13:618-624.
- Harbone NV (1994). Phytochemical methods. A guide to Modern Techniques of Plant analysis. 2nd Ed. Chapman and Hall, London, p. 425.
- Leven M, Vanden-Berghe DA, Marten I, Vilientnick A, Lomweas EC (1979). Screening of higher plants for biological activity. Planta Medica 36:311-312.
- Mbata TI, Saika A (2008). Antimicrobial activity and Phytochemical Screening of Crude ethanolic extract of leaves of *Ocimum gratissimum* L. on *Listeria monocytogenes*. Internet J. Microbiol. 4(2):1-13.
- Milner JA (2005). Garlic (*Allium sativum*). In: Encyclopedia of Dietary Supplements. Black, M. and Cragg, G. (Eds.). Marcel Dekker, New York, pp. 229-240.
- Nantitanon W, Chowwanapoonpohn S, Okonogi S (2007). Antioxidant and Antimicrobial activities of *Hyptis suaveolens* essential oil. Sci. Pharma. 75:35-46.
- Nwadiaro PO, Nwachukwu I (2007). Inhibition of Pathogenic Fungi by ethnobotanical extracts of *Cymbopogon citrates*, *Ceiba pentandra* and *Loranthus bengwelensis*. Nig. J. Biotechnol. 18(1-2):61-65.
- Nweze EI, Okafor JI, Njoku O (2004). Antimicrobial Activities of Methanolic Extracts of *Trema guineensis* and *Morinda lucida* used in Nigeria Herbal Medicinal Practice. J. Biol. Res. Biotechnol. 2(1):39-46.
- Opara AA, Ansa MA (1993). The Antimicrobial activity of tea and Coffee on selected Organisms. J. Med. Lab. Sci. 3:45-48.
- Rojas JJ, Ochoa VJ, Ocampo SA, Munoz JF (2006). Screening for Antimicrobial activity of ten Medicinal Plants in Colombian folkloric Medicines: A possible alternative in the treatment of non-nosocomial infections. Comp. Altern. Med. 6:2-12.
- Soforowa A (1993). Medicinal Plants and Traditional Medicine in Africa. Chichester John, Willey and Sons, New York, p. 256.
- Trease GE, Evans WC (1989). Pharmacognosy. 13th Edition. Bailliere Tindall Ltd., London, pp. 350-389.
- UNESCO (1998). FIT/504 – RAF - 48. Terminal Report: Promotion of ethnobotany and the sustainable use of Plant Resources in Africa. Paris, p. 60.