MERIT RESEARCH JOURNALS
www.meritresearchjournals.org

Merit Research Journal of Biochemistry and Bioinformatics Vol. 2(2) pp. 034-037, December, 2014
Available online http://www.meritresearchjournals.org/bb/index.htm

Original Research Article

Antimicrobial and phytotoxic effects of *Allium sativum* methanolic extract

Odutuga A. A., *Dairo J. O., Ukpanukpong R. U. and Uyabeme R. N.

Department of Biochemistry, College of Natural Sciences, Joseph Ayo Babalola University, Ikeji-Arakeji, Osun State, Nigeria.

***Corresponding Author's E-mail: olugbenga005@yahoo.com**

Abstract

The phytochemical screening of *Allium sativum* was investigated showing large quantities of phenolics, glycosides and flavonoids. Six pathogenic microorganisms, namely; *Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli, Staphylococcus aureus, Aspergilleus niger* and *Rhizopus arrhizus* were used for antimicrobial test. At concentration of 10% *Allium sativum* methanolic extract, microbial growth rates were inhibited except *Rhizopus arrhizus.* Nevertheless, *Escherichia coli* demonstrated the highest level of inhibition at 10% concentration of the extract with 45% inhibition rate. Methanolic extract of *Allium sativum* has enhanced the growth of bean and maize seedlings with shoot growth rate of 34.49 ± 0.35% and 21.35 ± 0.25% taller than the control at 2% concentration respectively. The methanolic extract of *Allium sativum* served as growth enhancer and exhibited good antimicrobial properties.

Keywords: *Allium sativum*, phytochemicals, pathogenic microorganism

INTRODUCTION

Allium sativum commonly known as garlic is a species in the onion family Alliaceae. .Its close relatives include the onion, shallot, leek and chive (Foster, 1996). Garlic has been used throughout history for both culinary and medicinal purposes. The garlic plant bulb is the most commonly used part of the plant .with the exception of the single clove types. The bulb is divided into numerous fleshy sections called cloves.

The cloves are used for cloning, consumption when raw or cooked and have a characteristic pungent spicy flavor that mellows and sweetens considerably with cooking (Gernot, 2007). The papery protective layers of the skin that covers various parts of the plant are generally discarded during preparation for most culinary uses, although in Korea immature whole heads are sometimes prepared with the tender skins intact (Amanda, 2010).The composition of the bulbs is approximately 84.09% water, 13.38% organic matter and 1.53% inorganic matter, while the leaves are 87.14% water, 11.27% organic matter and 1.59% inorganic matter (Chalson and McFerren, 2007). Garlic contains 0.1-0.36% of a volatile oil which are generally considered to be responsible for most of the pharmacological properties of garlic. Garlic contains at least 33 sulfur compounds like Allicin and Ajoene (Daniel and Maria, 2000).

The sulfur compounds are responsible both for garlic's pungent odor and many of its medicinal effects. The odor is formed by the action of the enzyme allinase on the sulfur compound allicin. The enzyme is inactivated by heat, which accounts for the drastic reduction in odour intensity found in cooked garlic with similar physiological effects (Koch and Lawson, 1996).

The s-allylcysteine and s-allylmercaptor-l-cysteine are the two major compounds in aged garlic with highest radical scavenging activity. In addition, some organosulfur compounds derived from garlic including s-allylcysteine have been found to retard the growth of chemically induced and transplantable tumor (Chan, 2007). This study therefore investigated the antimicrobial

and phytotoxic effects of *Allium sativum* methanolic extract.

MATERIALS AND METHODS

Local species of *Allium sativum* was purchased from Ogbomosho market, Oyo state Nigeria and identified by a Botanist at the department of Biological Sciences, Joseph Ayo Babalola University. Cloves of fresh garlic bulb were peeled and sliced into smaller pieces and oven-dried at 70ºC until a constant weight was obtained. The dehydrated garlic was homogenized with electrical blender into a powdery form and stocked for subsequent use.

Preparation of aqueous extract of *Allium sativum*

200g of the powdered garlic sample was soaked in 500ml n-Methanol, stirred at intervals of four hours and kept at room temperature for forty eight hours. The solvent was filtered by a suction pump, evaporated by a rotary evaporator and the methanolic extract stored in the refrigerator until required for use.

Phytochemical screening and Phytotoxicity assessment of *Allium sativum*

The chemical classes of constituents in the freshly prepared extracts were detected using standard phytochemical reagents and procedures by Odebiyi and Sofowora (1978), while phytotoxicity assessment was experimented in an aseptic environment.

Preparation of test solution

Test solution was prepared by serial dilution of the methanolic extract of *Allium sativum* in distilled water. 0.2g of the extracts was mixed with 25ml water to get a 2% test solution. This test solution was diluted to get 2%, 1%, 0.5%, 0.2% and 0.1% solution in distilled water.

Preparation of maize and beans culture

Maize and bean seeds were cultured in petri-dishes as follows: Cotton wool was placed at the bottom of the petri-dishes. 10ml of each of the serially diluted solution was pipette into separate dishes so that the cotton wool was completely wet. Four healthy seeds were well spaced in a circle. About 10ml of distilled water was added to each culture everyday from the second day of culturing so as to replace water loss through evaporation. The experiments were done in triplicates and at room temperature. The culture was observed for nine days for seed germination and growth patterns. At the end of nine days, the root and shoot lengths were measured. The growth profiles of the test samples were compared with those of controls to get an index of inhibition of seed germination and seeding growth as described by Morebise and Fafunso (1998).

Administration of *Allium sativum* methanolic extract on test organisms

20 ml of molten nutrient agar was introduced into different petri dishes and seeded with 0.2ml of the pure culture of test organisms; *Pseudomonas aeruginosa, Klebisiella spp, Escherichia coli and Staphylococcus aureus*. The petri-dishes were swirled slowly to enhance even distribution of microorganism and allowed to solidify following the procedure described by Ebi and Ofoefula (1997). The petri-dishes were labeled A, B, C, D and control, sterile cork borer were used to make 5 different holes on the nutrient agar medium and different concentrations of *Allium sativum* methanolic extract were inoculated into the holes using a sterile Pasteur pipette to establish a sensitivity test. The dishes were allowed to stand for 30 minutes at room temperature to allow proper diffusion of the extract on the test organisms. The petri-dishes were incubated at 37ºC for 24hours and mean diameters of inhibition were measured, recorded and inhibitory concentration was determined.

Statistical analysis

All data were statistically analyzed using ANOVA while values expressed in mean ± SEM, at $P < 0.05$ values were considered significant (Duncan *et al*., 1977).

RESULTS AND DISCUSSIONS

The phytochemical analysis of methanolic extract of *Allium sativum* showed little presence of tannin, alkaloids, glycosides but with higher quantities of phenolics and flavonoids.

Result of the antimicrobial screening revealed that *Allium sativum* methanolic extract was effective against all the microbial strains used except *Rhizopus arrhizus*. The investigation showed that methanolic extract of *Allium sativum* has anti-bacterial potency against gram positive and gram negative organisms. *Staphylococcus aeureus* is a non-sporing organism that causes boils, carbuncle, acute food poisoning and *Aspergillus nige*r with endless pathogenic manifestations were also inhibited by *Allium sativum* methanolic extract. The extract confers immense pharmaceutical potential and may be implored in medical formulations (Igile, 1995).

Allium sativum methanolic extract showed inhibitory phytotoxicity effects on shoots and roots growth of a maize seedlings. The extract however, has an enhancing

Table 1. Phytochemical analysis of *Allium sativum* extract

Chemical composition	Results
Phenolics	++
Tannins	+
Alkaloids	+
Steroids	-
Saponins	-
Flavonoids	++
Glycosides	+

Key
+ Detected
++ Strongly detected
- Not detected

Table 2. Antimicrobial screening of *Allium sativum* methanolic extract

	M./ORG. / CONC.	P. aeruginosa	K. pneumonia	E. Coli	S. Aureus	A. niger	R. spp
ZONE OF INHIBITION (mm)	CONTROL	0	0	0	0	0	0
	A (25µg/ml)	15	15	25	0	25	0
	B (50µg/ml)	17	20	30	10	30	0
	C (100µg/ml)	20	20	30	15	35	0
	D (200µg/ml)	25	35	45	20	30	0
	MIC.	25µg/ml	25µg/ml	25µg/ml	50µg/ml	25µg/ml	0µg/ml

- MIC = Minimum Inhibitory Concentration

Table 3. Growth profile of seedling under methanolic extract of *Allium sativum*

Concentration of methanolic extract (%)	Maize		Bean	
	Shoot	Root	Shoot	Root
2.0	27.37±0.32	33.25 ± 0.52	33.25 ± 0.23	10.05 ± 0.57
1.0	25.05 ± 0.37	31.75 ± 0.34	31.45 ± 0.65	9.70 ± 0.43
0.5	24.45 ± 0.44	29.40 ± 0.37	30.10 ± 0.74	8.75 ± 0.61
0.2	23.30± 0.34	28.20 ± 0.46	29.30 ± 0.35	8.20 ± 0.27
0.1	22.36± 0.25	27.05 ± 0.38	28.20 ± 0.43	7.85 ± 0.45
Control	20.35± 0.53	26.30 ± 0.73	27.40 ± 0.28	7.45 ± 0.83

Values: Mean ± SEM of 3 analyses

Table 4. Percentage growth of seedlings enhanced by *Allium sativum* methanolic extracts

Concentration of methanolic extract (%)	Maize		Bean	
	Shoot (%)	Root (%)	Shoot (%)	Root (%)
2.0	34.49 ± 0.35	26.43 ± 0.51	21.35 ± 0.25	34.90 ± 0.75
1.0	23.09 ± 0.24	20.72 ± 0.31	14.78 ± 0.64	30.20 ± 0.44
0.5	20.14 ± 0.31	11.79 ± 0.23	9.85 ± 0.72	17.45 ± 0.61
0.2	14.49 ± 0.42	7.22 ± 0.45	6.93 ± 0.55	10.06 ± 0.47
0.1	9.87 ± 0.37	2.85 ± 0.33	2.92 ± 0.32	5.36 ± 0.83

Values: Mean ± SEM of 3 analyses

rather than inhibiting effect on the growth of bean shoot and root seeding.

The bean seeds were more sensitive to *Allium sativum* methanolic extract enhancing effect, especially the root when compared with the shoot. The root of maize seedling was equally sensitive to the extract, especially the roots compared with the shoot. This difference may be that beans is a legume while maize is a cereal and this finding is agreement with the reported of Morebise and Fafunso (1998). The result showed that the enhancing potentials of the beans seed growth and the inhibition effect of the maize seed growth were concentration dependent and this phenomenon might be structure dependent as well (Waller and Yamasaki,1996). The phytotoxicity effect were thought to occur at hormonal and enzymatic levels involving inhibitory or enhancing effects on plant growth hormones like auxins and the gibberellins (Waller, 1989). *Allium sativum* methanolic extract exhibit enhancing properties to maize and bean seedling at the test concentrations of 0.1% to 2.0% used. It showed that even at lower concentrations it may exhibit the same effects.

CONCLUSION

The investigation showed that Allium sativum extract has anti-bacterial potency against gram positive and gram negative organisms respectively.

REFERENCES

Amanda G (2010). “Glossary of Food and Food Term in Korea”. http:/www.food-links.com/countries/korea/food-term-korea.php.

Chalson M, McFerren M (2007). Garlic: What We Know and What We Don’t Know Arch. Intern. Med. 167 (4): 325 – 326

Chan KC, Yin MC, Chao WJ (2007). Effects of Diallyl Trisulfide-Rich Garlic Oil On Blood Coagulation and Plasma Activity of Anticoagulation Factors in Rats. Food Chem. Toxicol 45 (3):502 507

Daniel Z, Maria H (2000).Domestication of plant in the old World, third edition p.197, Oxford University Press

Duncan RC Knapp R.C, Miller M.C (1977). Test of Hypothesis in population means. introduction biostatistics for the Health Sciences. John Wiley and Sons Inc. N.Y. Pp 71-96.

Ebi GC, Ofoefule SI (1997). Investigation into the folkloric anti-microbial activities of Landolphia owerriance. Phytother. Res. 11: 147 – 151.

Foster S (1996). Garlic –Allium. Botanical Series. American Botanical Council. Austin, Texas: 2nd. ed.No.311.

Gernot K (2007). ‘‘Spice pages: Garlic (*Allium sativum*, garlick)”.http://www.uni-graz.at/-Katzer/engl/Alli_sat.html ‘‘Small Farm News Archive’’.

Igile, G.O (1995). Phytochemical and Biological Studies on Constituents of Vernonia amygdalina (Compositae) Leaves PhD Thesis, Department of Biochemistry, University of Ibadan, Nigeria.

Koch, H.P, Lawson L.D (1996). Garlic-The Science and Therapeutic Application of Allium sativum L. and Related Species Williams, Wilkins, 2nd ed. Baltimore.

Morebise O, Fafunso, MA (1998). Anti-Microbial and Phytotoxic Activities of Saponins Extracts from Two Nigeria Edible Medicinal Plants. Biokemistri 8: (2) 69 -77

Odebiyi A, Sofowora, AE (1978). Phytochemical Screening of Nigeria Medicinal Plants (Part III). Lloydia 41: 234-236.

Oleszek, (1993). Allelopathic Potential of Alfafa Saponins, J. Chem. Ecol. 19, 60, 1063

Waller, GR, Yamasaki, K (1996). Saponins Used in Traditional and Modern Medicine. Vol. 404 of the Series Adv. Exp. Biol. Plenum Press, New York