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# Allelophatic and Anti-Microbial Activities of Aqueous Extract of *Allium Sativum*

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***Abstract:***
*The phytochemical screening of Allium sativum was carried out and the presence of phenolics, glycosides and flavonoids were revealed in large quantities. Allium sativum aqueous extract has an enhancing capability on the growth of both bean and maize seedling with shoot growth rate of 34.49 ± 0.35%and 21.35 ± 0.25% taller than the control at 2% concentration respectively. Six pathogenic microorganisms namely Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli, Staphylococcus aureus, Aspergilleus niger and Rhizopus spp. were used for anti-microbial test. At concentration of 10% of Allium sativum aqueous extract, the growth of all bacterial and fungi used were inhibited except Rhizopus spp.. E.coli was the most inhibited with 45% inhibition rate at 10% concentration of the extract. The aqueous extract of Allium sativum serves as a growth enhancer and display good anti-microbial properties.*

***Keywords:*** *Allium sativum, allelophatic, anti-microbial, phenolics, flavonoids.*

## 1. Introduction

*Allium sativum,* a species in the family of Alliaceae, is commonly known as garlic. The cloves are used for cloning, consumption (raw or cooked) and for medicinal purposes and have a characteristic pungent spicy flavor that mellows and sweetens considerably with cooking (Gernot, 2007). 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 but with the exception of the single clove types. The peppery protective layers of the skin that covers various parts of the plant are generally discarded during preparation for most culinary uses; though 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 (Charlson and McFerren, 2007). Garlic contains 0.1-0.36% of a volatile oil. These volatile compounds 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 two major compounds in aged garlic, s-allylcysteine and s-allylmercaptor-l-cysteine, had the 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 et al., 2007). The sulfur compounds are responsible both for garlic's pungent odor and many of its medicinal activities. The odor is formed by the action of the enzyme Allinase on the sulfur compound alliin. The enzyme is inactivated by heat, which accounts for the fact that cooked garlic produced neither as strong an odour as raw garlic nor nearly as powerful physiological activities (Koch and Lawson, 1996).

## 2. Materials and methods.

Local cultivar of *Allium sativum* species were purchased from Bodija market, Oyo state, South-western part of Nigeria and identified by a botanist at the department of Biological Sciences, Joseph Ayo Babalola University.The cloves of the fresh garlic bulb were peeled and sliced into smaller pieces and were oven-dried at 70ºC to constant weight. The dried garlic was then grinded into a powdery form with electrical blender and kept for subsequent use.

*2.1. Preparation of aqueous extract of Allium sativum*

200g of the powdered garlic sample was soaked in 500ml distilled water. It was stirred at interval of 4 hours and kept at room temperature for 48 hours. The solvent was filtered by a suction pump and then evaporated.The *Allium sativum* aqueous extract was also stored in the refrigerator until required for use.

*2.2. Phytochemical screening and preparation of plant extract test solution*

The chemical classes of constituents in the freshly prepared extracts were detected using standard photochemical reagents and procedures by Odebiyi and Sofowora (1978).Test solution was prepared by serial dilution of the aqueous 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%, 0.1% solution in distilled water.

*2.3. Preparation of maize and bean cultures*

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 roots and shoot lengths were measured. The growth profiles of the test samples were compared with those of controls to get an index of inhibition/stimulation of seed germination and of seeding growth as described by Morebise and Fafunso (1998).

*2.4. Anti-microbial activities of Allium sativum aqueous extract*

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 aureginsa, Klebisiella sp, E coli and Staphylococcus aureus*. The petri-dishes were rotated slowly to enhance even distribution of microorganism following the procedure described by Ebi and Ofoefula, (1997) and were allowed to solidify. Sterile cork borer was used to make 5 different holes in the agar and different concentrations of the *Allium sativum* aqueous extract were inoculated into the holes using a sterile Pasteur pipette. The dishes were allowed to stand for 30 mins. at room temperature to allow proper diffusion of the extract to take place. The petri-dishes were labeled A, B, C, D and control. The petri-dishes were then incubated at 37°C for 24hrs. per each extract concentration. The mean diameter of inhibition were measured and recorded. The inhibitory concentration was also determined.

**Statistical analysis**

Data were statistically analysed using ANOVA, values obtained were expressed in mean ± SEM. The values of $p < 0.05$ were considered as significant.

## 3. Results

| Chemical Composition | Results |
|---|---|
| Phenolics | ++ |
| Tannins | + |
| Alkaloids | + |
| Steroids | − |
| Saponins | − |
| Flavonoids | ++ |
| Glycosides | + |

*Table 1: Phytochemical analysis of Allium sativum extract*
*+ Detected, ++ Strongly detected, - Not detected*

| | 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 |

*Table 2: Anti-microbial screening of allium sativum aqueous extract*

- MIC = Minimum Inhibitory Concentration

| Concentration of aqueous 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 |

*Table 3: Growth profile of seedling under aqueous extract of Allium sativum*

Values are mean ± of 3 determinations. Values with different alphabet superscripts are significantly different at Pp< 0.05

| Concentration of aqueous 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 |

*Table 4: percentage of growth enhancement of seedlings by Allium sativum aqueous extracts.*

**4. Discussion**

The phytochemical analysis of aqueous extract of *Allium sativum* showed the presence of phenolics and glycosides in high quantities. Result of the anti-microbial screening revealed that *Allium sativum* aqueous extract was effective against all the microbial used except *Rhizopus spp.,* which is a fungus. The results showed that aqueous extract of *Allium sativum* has an anti- bacteria potency especially against both gram +ve and gram negative bacteria. The results are interesting as the gram negative bacterial have been shown to be generally more resistant to antibiotics. *Staphylococcus aeureus* is a non-sporing organism that causes skin lesions such as boils and carbuncle. it can also affect the bone tissue as in *Staphylococcal osteomyellties*. It produces a toxin which if ingested with food can give rise to intense and acute food poisoning, a common manifestation of its infection is the production of pus (Oleszek, 1993).The fact that aqueous extract of *Allium sativum* were highly effective against both gram+ve and gram negative and against *Aspergillums niger* confer on them immense potential in pharmaceutical and medical formulations. *Allium sativum* aqueous extract showed varied allelophatic effect. Both maize shoot and roots growth were inhibited while the extract has an enhancing rather than inhibiting effect on the growth of bean shoot and root seeding.

The beans seeds were more sensitive to *Allium sativum* aqueous extract enhancing effect, especially the root when compare with the shoot.The root of maize-seedling were equally sensitive to the extract, especially the roots compare with the shoot.This difference might be due to the fact that the bean is a legume while maize is a cereal. This finding agreed with what was reported by Morebise and Fafunso (1998). The result showed that the enhancing capability of the bean seed growth and the inhibition effect of the maize seed growth ware concentration–dependent and this phenomenon might be structure dependent as well (Oleszek,1993). The allelophaticity effect were thought to occur at hormonal and enzymic levels involving inhibitory or enhancing activities on plant growth hormones like auxins and the gibberellins (Waller and Yamasaki,1996).

It has been reported that retarded seed germination might be by inhibition of endogenous gibberellic acid (GAB) and indole-3-acetic acid (IAA), both responsible for seedling elongation (Igile,1995).Since *Allium sativum* aqueous 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 concentration it would exhibit the same activities.

**5. Conclusion**

The findings portrayed that aqueous extract of *Allium sativum* has an anti-bacteria potency against gram positive and gram negative bacteria respectively. And that allelophaticity effects may have been around hormonal and enzymic levels involving inhibitory or enhancing activities on plant growth hormones like auxins and gibberellins.

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