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.

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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 paralopsis* 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.

*Corresponding author. E-mail: g_ameh@yahoo.com.*
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 (BDCP), 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 paralopsis* 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.

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Inhibition zone diameter (IZD) (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella paratyphi</td>
<td>15</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>16</td>
</tr>
<tr>
<td>Klebsilla pneumoniae</td>
<td>25</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>29</td>
</tr>
<tr>
<td>Candida paralopsis</td>
<td>21.8</td>
</tr>
<tr>
<td>Candida tropicalis</td>
<td>30</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100 mg/ml</td>
</tr>
<tr>
<td>S. paratyphi</td>
<td>+</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>+</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>25</td>
</tr>
<tr>
<td>C. albicans</td>
<td>+</td>
</tr>
<tr>
<td>C. paralopsis</td>
<td>+</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = No inhibition.

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 microorganisms. 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 indicating that micro organisms exhibit 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


