**Antibacterial Activity of Garlic Varieties (Ophioscordon and Sativum) on Enteric Pathogens**

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**Abstract:** The present study aimed at assessing the antibacterial activity of two varieties of garlic (ophioscordon and sativum) against enteric pathogens such as Escherichia coli, Proteus mirabilis, Salmonella typhi, Shigella flexneri and Enterobacter aerogenes. Aqueous extract of both the garlic varieties inhibited the growth of enteric pathogens at the concentrations of 200,300,400 and 500mg. However Enterobacter aerogenes was not susceptible to the aqueous extract of both the garlic varieties. Ethanolic extract of sativum was found to be highly effective against all the bacteria tested. HPTLC analyses of garlic varieties confirm the presence of allicin in various concentrations. Further analysis using GC-MS identified other compounds such as n-hexadecanoic acid, 3-deoxy-d-mannoic lactone, thymine and hexanedioic, bis (2-ethylhexyl) ester.

**Key words:** Antibacterial activity, HPTLC, GC-MS, allicin and palmitic acid

**INTRODUCTION**

Indiscriminate use of commercial antimicrobial drugs has lead to multiple drug resistance (Service, 1995). Plants are effective in the treatment of infectious diseases and many plant extracts have been shown to possess antimicrobial properties in vitro (Sofowora, 1983).

Garlic (Allium sativum) has a long folklore history as a treatment for cold, cough and asthma and is reported to strengthen the immune system (Borek, 2001. Allium sativum is broadly classified into two sub varieties- ophioscordon (hard neck garlic) and sativum (soft neck garlic). It has many medicinal effects such as lowering of blood cholesterol level (Yeh and Yeh, 1994), antiplatelet aggregation (Steiner et al., 1996), anti-inflammatory activity (Baek et al., 2001) and inhibition of cholesterol synthesis (Piscitelli et al., 2002). Garlic has long been known to have antibacterial (Ekweney and Elegalan, 2005; Cellini et al., 1996), antifungal (Yoshida et al., 1987), anticancer (Pan et al., 1985) and antiviral properties (Block, 1985). The main antimicrobial constituent of garlic has been identified as the oxygenated sulfur compound, thio-2-propene-1-sulfinic acid S-allyl ester, which is referred to as allicin (Cavallito and Bailey, 1944).

The aim of the present investigation is to study the comparative antibacterial effect of ethanolic and aqueous extract of the two sub varieties (ophioscordon and sativum) of garlic against enteric pathogens. Bioactive compounds present in two sub varieties were identified and analyzed using HPTLC and GC-MS analyses.

**MATERIALS AND METHODS**

**Preparation of plant extract:** Garlic varieties used in this study were purchased from local market, Tiruchirappalli, Tamil Nadu, India and shade dried at room temperature for 15 – 20 days. Both aqueous and ethanolic extract of garlic varieties were prepared using soxhlet apparatus (3cycles/hour). The extracts were dried in a rotatory evaporator (Buchii R 124, Germany), dissolved in DMSO and used for antibacterial analyses.

**Test organisms:** The bacterial strains used in this study were obtained from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh, India. The bacterial strains studied were Escherichia coli MTCC 520, Proteus mirabilis MTCC 743, Salmonella typhi MTCC 733, Shigella flexneri MTCC 1457 and Enterobacter aerogenes MTCC *111. The test bacteria were inoculated in to nutrient broth and incubated at 37°C for 8-10 h. All the chemicals and medium used in this study were supplied by Hi media Pvt. Ltd., Mumbai, India.

**Agar well diffusion assay:** The antibacterial activity of Allium sativum L (ophioscordon and sativum) was evaluated by agar well diffusion method as followed by Chung et al., (1990). Muller Hinton agar medium was prepared and poured into the petridishes. Then it was inoculated with a swab of bacterial culture (mid log phase) and spread throughout the medium uniformly with a sterile cotton swab. Using a sterile cork borer (10mm diameter) wells were made in the agar medium. The test
compound was introduced into the wells and all the plates were incubated at 37°C for 24 h. The experiment was performed five times under strict aseptic conditions. Sensitivity of the organism was determined by measuring the diameter of the zone of inhibition. Each assay was repeated for five times and the mean value was taken for analyses. The control experiment was carried out with antibiotics such as streptomycin sulphate and chloramphenicol (Table 1).

**RESULTS AND DISCUSSION**

From Table 1, it is very clear that both the garlic (aqueous) extracts showed growth inhibition activities at the concentrations of 200mg to 500mg. *Proteus mirabilis* was sensitive to aqueous extracts of *ophioscordon* at higher concentrations (400 and 500mg). *E. aerogenes* was not susceptible to the aqueous extract of both the garlic varieties, while *S. typhi* was susceptible to both the extracts of garlic varieties (Table 2). Ethanolic extract of *sativum* was highly effective against all the bacterial species that was taken for the study whereas *Escherichia coli*, *S. typhi* and *S. flexneri* were sensitive to ethanolic extract of *ophioscordon*. Al-Delaimy and Ali (1970) reported that 4% (w/v) fresh garlic in extract inhibited the growth of *S. aureus, E. coli, S. typhi* and *S. dysenteriae*. Garlic is rich in anionic components such as nitrates, chlorides and sulfates as well as other water soluble components common in most plants which may...
be responsible for its antibacterial activity (Astal, 2004). Since each extract showed a unique range of zone of inhibition, to reason out the cause and to compare their effects the extracts were subjected to HPTLC analysis.

HPTLC analyses (Fig. 1) revealed allicin was the major compound present in different concentration of each extract, in accordance with previous study (Canizares et al., 2004). Allicin concentrations vary between aqueous and ethanolic extracts of *sativum* (14.64% and 15.61%) and *ophioscordon* (5% and 9%). These observations when correlated with antibacterial nature of sub-varieties of garlic extract revealed that allicin concentration increases the antibacterial property of garlic. Allicin present in the ethanolic extract of *sativum* inhibited almost all the organism with a significant zone of inhibition. The other compounds

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**Fig 1:** HTPCL analyses of garlic varieties (ophioscordon and sativum) a1-aqueous extract of sativum; a2-ethanolic extract of sativum; a3-aqueous extract of ophioscordon; a4-ethanolic extract of ophioscordon

**Fig 2:** GCMS analysis of ethanolic extract of sativum
present in the extracts may also be responsible for the antibacterial nature. This is accordance with Wills (1956), who studied the effect of allicin solutions on the growth of both gram-positive and gram-negative bacteria.

To further study the nature of components present in the ethanolic extract of *Sativum* GC-MS was performed (Fig. 2). The major compounds of GC-MS analysis and their retention time were listed in Table 3. These include allicin (5.37%), palmatic acid (4.52%), 3-deoxy-D-manno lactone (3.86%), thyme (3.5%) and hexanedioic acid bis (2-ethylhexyl) ester (1.87%).

Allicin is the major compound in the GC-MS analysis. We however report high levels of palmatic acid (4.52%) in garlic extract and it also posses antibacterial activity as reported by Yf et al., (2002) in *Pentanisia prunelloides*. Presence of these phytochemical components confirms the long-standing concept of garlic used as antibacterial agent. The phytochemical constituents are varied between the garlic varieties, which could be the reason for the variation in their antimicrobial properties.

<table>
<thead>
<tr>
<th>SNO</th>
<th>Retention time (min)</th>
<th>Compound</th>
<th>Peak area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.40</td>
<td>Allicin</td>
<td>5.37</td>
</tr>
<tr>
<td>2</td>
<td>26.07</td>
<td>Palmitic acid</td>
<td>4.52</td>
</tr>
<tr>
<td>3</td>
<td>17.44</td>
<td>3-Deoxy-D-manno lactone</td>
<td>3.86</td>
</tr>
<tr>
<td>4</td>
<td>6.16</td>
<td>Thyme</td>
<td>3.50</td>
</tr>
<tr>
<td>5</td>
<td>33.16</td>
<td>Bis(2-ethylhexyl ester)</td>
<td>1.87</td>
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</table>

REFERENCES


