ANTIMICROBIAL EFFECT OF GARLIC AND GINGER ON Staphylococcus aureus FROM CLINICAL SPECIMENS IN MADONNA UNIVERSITY TEACHING HOSPITAL, NIGERIA

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ABSTRACT

Antimicrobial properties of garlic (Allium sativum) and ginger (Zingiberofficinale) were investigated for their microbial activity against both clinical and laboratory isolates of Staphylococcus aureus using disc diffusion method. Ten isolates were obtained from the Department of Medical Microbiology, Medical Laboratory science Madonna University Teaching Hospital, Elele. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by the broth dilution method. The antibiotic susceptibility test against the test organisms were performed by disc diffusion method. The result from this study showed garlic and ginger extract were effective against Staphylococcus aureus used in this study. The inhibitory effect exerted by garlic extract was highest at the concentration of 100µg/ml with mean zone inhibition of 11.5 ± 0.69mm which was statistically significant (p<0.05) from the control 21.6 ± 1.07mm. Also the inhibitory effect exerted by garlic was least at the concentration of 12.5µg/ml with mean zone of inhibition of 3.5 ± 0.46mm, while concentration at 50µg/ml showed mean zone of inhibition of 8.9 ± 1.11mm and concentration at 25µg/ml showed mean zone of inhibition of 5.6 ± 0.55mm. The inhibitory effect exerted by ginger was highest at the concentration of 100µg/ml with mean zone of inhibition of 16.0 ± 0.21mm which was statistically significant (p<0.05) from the control 21.6 ± 1.07mm. Also the inhibitory effect exerted by ginger was least at the concentration of 12.5µg/ml with mean zone of inhibition of 4.5 ± 0.68mm, while concentration at 50µg/ml showed mean zone of inhibition of 11.7 ± 1.05mm and concentration at 25µg/ml showed mean zone of inhibition of 8.0 ± 0.89mm. Staphylococcus aureus was found to be more sensitive to ginger than garlic with highest mean zone inhibition of 16.0 ± 0.21mm for ginger and 11.5 ± 0.69mm for garlic at 100µg/ml. Also there were lower minimum inhibitory concentrations with 25µg/ml for garlic and 12.5µg/ml for ginger on Staphylococcus aureus than the minimum bactericidal concentrations with 50µg/ml for garlic and 25µg/ml for ginger indicating that the extracts could be bactericidal in action. Therefore garlic and ginger extracts can be used as a source of antibiotic substances for possible treatment of staphylococcal infection though not replacing the use of antibiotics but to prevent multidrug resistance.

INTRODUCTION

Natural products of animals, plants and microbial sources have been used by man for thousands of years either in the pure or crude extracts to cure many diseases[1]. Garlic (Allium sativum) has an important dietary and medicinal role for centuries. Its therapeutic uses include beneficial effects on the cardiovascular system, antibiotics, anticancer, anti-inflammatory, hypoglycemic, and hormone-like effects. Garlic extract have been used to treat infections for thousands of years. Its typical pungent odour antibacterial activity depend on alliin, which is produced by enzymatic (alliin-lyase) hydrolysis of alliin after cutting and crushing of the cloves[2].

Ginger (Zingiberofficinale) is a medicinal plant that has been widely used all over the world, since antiquity, for a wide array of unrelated ailments including arthritis, cramps, rheumatism, sprains, sore throats, muscular aches, pains, constipation,
vomiting, hypertension, indigestion, dementia, fever and infectious diseases[3]. Ginger has direct antimicrobial activity and thus can be used in treatment of bacterial infections[4]. Ginger belongs to Zingiberaceae family[5]. The Zingiberaceae plants have strong aromatic and medicinal properties and are characterized by their tuberous or non tuberous rhizomes[6]. Ginger is relatively inexpensive due to their easy availability, universally acceptable and well tolerated by the most people. In many countries including Bangledesh, ginger is used in boiled food preparation.

Staphylococcus aureus is the most medically important member in terms of pathogenicity of the group. Two other less important members are Staphylococcus epidermis and Staphylococcus saprophyticus[7]. It is commonly present in the upper respiratory tract and intestinal tract. They grow on a variety of media and ferment many carbohydrates without gas. Colonies may be white, grey or golden yellow. The species name aureus is derived from the Latin word, ‘aureus’ meaning golden. They are halophilic (salt loving)[8].

The increased usage of antibiotics has induced micro organisms to acquire resistance factors which have become a public health challenge[9]. As a result there is an urgent need to find the alternative of chemotherapeutic drugs in diseases treatment particularly those of plants origin which are easily available and have considerably less side effects[10]. The use of higher plants and their extracts for treating infectious diseases has long been practiced in many different forms including: powder, liquid or mixtures which could be raw or boiled such as, liniments, ointments and incisions which have caused a turbid appearance in the tube.

Sterility of the Extracts
One (1) ml of the extracts was added separately in two test tubes containing 5ml of sterile nutrient broth. They were then incubated at 37°C for 24hours. The extracts were cleared after incubation indicating the absence of contaminant which could have caused a turbid appearance in the tube.

Preparation of the Antimicrobial Disc
Discs of 6mm in diameter were punched out using Whatman No. 1 filter paper with the aid of a paper punch and placed in Bijou bottles. The discs were then sterilized by autoclaving at 121°C for 15mins after which they were allowed to cool.

Stock solutions of these garlic cloves and garlic rhizomes ethanolic crude extracts (that were recovered) were prepared by dissolving 0.5g (i.e. 50mg) of each of the two plant extracts in 5ml of dimethyl sulphoxide. Therefore, each stock solution had a concentration of 10000mg/ml. From these stock four different concentrations of each plant extract were prepared. These were 12.5µg/ml, 25µg/ml, 50µg/ml and 100µg/ml which finally yielded disc potencies of 0.125µg/disc, 0.25µg/disc,0.5µg/disc and 1.0µg/disc respectively. This was followed by introducing 100 sterile discs into each concentration. The discs were allowed to absorb the solution and kept for further analysis. Each paper disc was capable of absorbing 0.01ml

Collection, Inoculation and Isolation of Pure Culture
A total of 10 samples was collected, 6 fresh urine samples was collected using a sterile universal container. 3 high vaginal swabs and 1 wound swabs were collected using sterile swab sticks from patients attending Madonna university teaching hospital (MUTH).

MacConkey agar, Chocolate agar, Blood agar and Mannitol agar were prepared and poured into a sterile petri dish when cooled and refrigerated and the amount needed was dried in hot air oven. Fresh urine samples, was cultured on MacConky agar using a sterile wire loop, it was streaked using the standard method, while high vaginal swab and wound swab was cultured into chocolate and MacConkey agar.

Using sterilized wire loop, single discrete colonies were sub cultured on fresh mannitol plate and incubated at 37°C for 24hours, after which the purified strains of Staphylococcus Aureus which fermented the mannitol were inoculated on nutrient agar slants and preserved at 4°C in the refrigerator.

Identification of Isolate
Isolates were identified macroscopically, microscopically and biochemically[13].

Alcoholic extracts of Allium sativum (garlic) and Zingiberofficinale
Garlic and ginger extract was investigated for antimicrobial activity against Staphylococcus aureus using agar diffusion method and broth dilution method. The antimicrobial susceptibility test was performed by disc diffusion method. Minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) were determined by broth dilution method[13].
Aliumsativum (garlic) and Zingiberofficinale (ginger)

**Sensitivity Testing of Bacterial Strain**

Pure culture of *Staphylococcus aureus* was subjected to antimicrobial susceptibility using the disc diffusion method. Using a sterile wire loop, pure colonies of the test organism was aseptically inoculated in 3mls of sterile normal saline and compared with McFarland standard and was allowed to incubate for 5minutes. A sterile swab stick was dipped into the bacterial suspension, excess inoculum was reduced by pressing the swab stick against the wall of the test tube. Inoculum was spread on the surface of Mueller Hinton agar and was allowed to stay for 5 minutes to dry. A sterile forceps was used to pick the impregnated extract disc unto Mueller Hinton agar and was incubated at 37°C for 24hrs. The area of inhibition was examined and measured in milliliters.

**Serial dilution of Aliumsativum (garlic) and Zingiberofficinale (ginger)**

Serial tube dilution technique was used to determine the minimum inhibitory concentration (M.I.C.). The garlic cloves and ginger rhizomes crude extracts were serial diluted in the range from 12.5 to 100µg/ml. The tubes were inoculated with 100µl of bacterial culture at a concentration of 10 6cells/ml. Standard antibiotic ciprofloxacin was included in the assay for comparison. Peptone water with the inoculum only was used as a growth control. All experiments were carried out in triplicates. The tubes were incubated aerobically at 37°C for 18hours.

**Determination of Minimum Inhibitory Concentration (M.I.C) and Minimum Bactericidal Concentration (M.B.C)**

Antimicrobial agents were diluted in peptone water. Each dilution was inoculated with standard inoculum of test organism. After appropriate incubation, the lowest concentration in the tube that showed visible inhibition of growth was recorded as the minimum inhibitory concentration (M.I.C). Dilution with test organism were then subcultured on blood agar medium without antimicrobial agent and incubated for 24hrs at 37°C. The minimum bactericidal concentration (M.B.C) is the lowest concentration of antimicrobial agent that produces no bacterial growth on a solid medium. M.I.C and M.B.C were expressed as microgram / milliliters.

**Statistical Analysis**

Data obtained from this study was analysed statistically using the statistical package for social sciences (SPSS) version 20 for window 8.1. The results were expressed in mean ± standard deviation. Comparison of mean was analysed using one way analysis of variance(ANOVA) and values were considered significant at p<0.05 and not-significant at p>0.05.

**RESULT**

Table 1 shows the mean values for zones of inhibition (mm) in the isolates (*Staphylococcus aureus*) subjected to various concentrations (mg/ml) of garlic (*Allium sativum*) ethanolic extract.

In the isolates (*Staphylococcus aureus*), there was a significant change (p<0.05) in the zones of inhibition (mm) with garlic extract as the concentration changes.

Table 2 shows the mean values for zones of inhibition (mm) in the isolates (*Staphylococcus aureus*) subjected to various concentrations (mg/ml) of ginger (*Zingiberofficinale*) ethanolic extract.

In the isolates (*Staphylococcus aureus*), there was a significant change (p<0.05) in the zones of inhibition (mm) with ginger extract as the concentration changes.

Table 3 shows the comparison between the mean values for zones of inhibition (mm) in the isolates (*Staphylococcus aureus*) subjected to various concentrations (µg/ml) of garlic (*Allium sativum*) and ginger (*Zingiberofficinale*) ethanolic extract. The inhibitory effect exerted by ginger (*Zingiberofficinale*) was seen to be greater than that exerted by garlic (*Allium officinale*) with ginger (*Zingiberofficinale*).

<table>
<thead>
<tr>
<th>Concentrations (µg/ml)</th>
<th>Garlic</th>
<th>Ginger</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>11.5 ± 0.69º</td>
<td>16.0 ± 0.21º</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>50</td>
<td>8.9 ± 1.11º</td>
<td>11.7 ± 1.05º</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>25</td>
<td>5.6 ± 0.55º</td>
<td>8.0 ± 0.89º</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>12.5</td>
<td>3.5 ± 0.46º</td>
<td>4.5 ± 0.68º</td>
<td>P&lt;0.05</td>
</tr>
</tbody>
</table>

Key = P<0.05 ...............Significant from the control
Garlic has traditional dietary and likely to be effective against multi-drug resistant bacteria. Consequently, the minimum bactericidal concentration (MBC) result also showed a lower MBC value for garlic.

Table 4 shows the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of garlic (Allium sativum) and ginger (Zingiber officinale) against Staphylococcus aureus. Result showed a lower MIC value for garlic compared to garlic. Consequently, the minimum bactericidal concentration (MBC) result also showed a lower MBC value for garlic.

Table 4 | Minimum Inhibitory Concentration (M.I.C.) and minimum bactericidal concentration of Garlic (Allium sativum) and Ginger (Zingiber officinale) against Staphylococcus aureus

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>MIC (µg/ml)</th>
<th>MBC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Garlic</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>Ginger</td>
<td>12.5</td>
<td>25</td>
</tr>
</tbody>
</table>

Table 6 shows the percentage susceptibility and resistance of Staphylococcus aureus to extracts of garlic and ginger. The percentage susceptibility was 100% for both extracts of garlic and ginger.

Table 6 | Percentage Susceptibility and Resistance of Staphylococcus aureus to Ethanolic Extract of Garlic (Allium sativum) and Ginger (Zingiber officinale)

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Staphylococcus aureus (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Garlic (Allium sativum)</td>
<td>S(%)</td>
</tr>
<tr>
<td>Ginger (Zingiber officinale)</td>
<td>10(100)</td>
</tr>
</tbody>
</table>

Factorial analysis of variance (ANOVA) was performed to determine if there was a significant difference in results. Significant difference in results may be due to difference in species of garlic and ginger or different strains of Staphylococcus aureus used.

In this study, extracts of garlic (Allium sativum) and ginger (Zingiber officinale) had antimicrobial effect on S. aureus, with garlic exerting a higher inhibitory effect at a concentration of 100µg/ml with mean zone of inhibition of 11.5 ± 0.69mm which was statistically significant (p<0.05) from the control 21.6 ± 1.07mm and a lowest inhibitory effect at a concentration of 12.5µg/ml with mean zone of inhibition of 3.5 ± 0.46mm; while garlic exerted highest inhibitory effect at a concentration of 100µg/ml with mean zone of inhibition of 16.0 ± 0.21mm which was statistically significant (p<0.05) from the control 21.6 ± 1.07mm and lowest inhibitory effect at a concentration of 12.5µg/ml with mean zone of inhibition of 4.5 ± 0.68mm. This is not in line with the work of [22], who reported that garlic exerted highest inhibitory effect at 200µg/ml with mean zone of inhibition of 14.55 ± 0.20mm and lowest inhibitory effect at a concentration of 25µg/ml with mean zone of inhibition of 10.30 ± 0.42mm; while garlic exerted the highest inhibitory effect at 200µg/ml with mean zone of inhibition of 13.55 ± 0.20mm and lowest inhibitory effect at 25µg/ml with mean zone of inhibition of 9.30 ± 0.32mm. This difference in results may be due to difference in species of garlic and ginger or different strains of Staphylococcus aureus used.

Ginger extract exerted more effect than garlic extract, having the highest mean zone of inhibition of 16.0 ± 0.21 at 100µg/ml concentration, while garlic extract had a mean zone of inhibition of 11.5 ± 0.69 at 100µg/ml concentration. This is in line with the work of [24], who reported a zone of inhibition of 16mm for ginger extract as against 10mm for garlic extract at 100µg/ml concentration.

Percentage susceptibility was seen to be 100% for both garlic and ginger extract on Staphylococcus aureus. This is in consonance with the work of [25] who reported 100% susceptibility for all Staphylococcus aureus isolates.

The minimum inhibitory concentration (M.I.C.) whichever was 25µg/ml and 12.5µg/ml for garlic and ginger extracts respectively, with ginger having the lower M.I.C. value. On the other hand, the minimum bactericidal concentration (M.B.C.) which is the lowest concentration which produces no bacterial growth of Staphylococcus aureus was 50µg/ml and 25µg/ml for garlic (Allium sativum) and ginger (Zingiber officinale) respectively, with ginger also having the lower M.B.C. value. This is in unison with the work of [25].

According to earlier reports, garlic has traditional dietary and medicinal applications as an anti-infective agent [14]. Allicin, the active ingredient of garlic, acts by partially inhibiting DNA and protein synthesis and also totally inhibiting RNA synthesis as a primary target [15]. Organosulfur compounds and phenolic compounds have been reported to be involved in the garlic antimicrobial activity [16;17;18;19;20;21].

The antimicrobial potency of ginger is believed to be due to tannins, saponins, phenolic compounds, essential oils and flavonoids [22]. It is interesting to note that even crude extracts of these plants showed good activity against multi-drug resistant strains where modern antibiotic therapy has limited effect.

In this study, extracts of garlic (Allium sativum) and ginger (Zingiber officinale) had antimicrobial effect on S. aureus, with garlic exerting a higher inhibitory effect at a concentration of 100µg/ml with mean zone of inhibition of 11.5 ± 0.69mm which was statistically significant (p<0.05) from the control 21.6 ± 1.07mm and a lowest inhibitory effect at a concentration of 12.5µg/ml with mean zone of inhibition of 3.5 ± 0.46mm; while garlic exerted highest inhibitory effect at a concentration of 100µg/ml with mean zone of inhibition of 16.0 ± 0.21mm which was statistically significant (p<0.05) from the control 21.6 ± 1.07mm and lowest inhibitory effect at a concentration of 12.5µg/ml with mean zone of inhibition of 4.5 ± 0.68mm. This is not in line with the work of [22], who reported that garlic exerted highest inhibitory effect at 200µg/ml with mean zone of inhibition of 14.55 ± 0.20mm and lowest inhibitory effect at a concentration of 25µg/ml with mean zone of inhibition of 10.30 ± 0.42mm; while garlic exerted the highest inhibitory effect at 200µg/ml with mean zone of inhibition of 13.55 ± 0.20mm and lowest inhibitory effect at 25µg/ml with mean zone of inhibition of 9.30 ± 0.32mm. This difference in results may be due to difference in species of garlic and ginger or different strains of Staphylococcus aureus used.
ginger and 100µg/ml for garlic extract, though with higher MIC and MBC values as compared with that in this study.

The result of this study shows that spices such as garlic and ginger though not replacing totally the use of standard antibiotics, can be used alternatively to prevent multiple drug resistance in the treatment of staphylococcal infections.

CONCLUSION AND RECOMMENDATION

From this study, it was observed that ethanolic extracts of garlic (Allium sativum) and ginger (Zingiber officinale) exhibited high inhibitory activity on the test organism. Under clinical supervision, Allium sativum and Zingiber officinale can be used for the treatment of staphylococcal infections. It is therefore recommended that further studies be carried out on the efficacy of garlic and ginger extracts, to enhance the primary health care delivery system in the developing countries. The importance of ethno-medical systems in health care delivery system cannot be over emphasized; hence intense research should be encouraged to enhance the health care system.

Significance Statement

This study discovered that ethanolic extracts of garlic (Allium sativum) and ginger (Zingiber officinale) exhibited high inhibitory activity on Staphylococcus aureus used in this study. This can be beneficial in the treatment of staphylococcal infections. This study will help the researchers to uncover the critical areas of traditional medicine practice in infectious disease therapy and combating antibiotic resistance that many researchers were not able to explore. Thus a new theory on the use of garlic (Allium sativum) and ginger (Zingiber officinale) in combating staphylococcal multidrug resistance may be arrived at.

References


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