ANTIMICROBIAL ACTIVITY OF AMINOGLYCOSIDE ANTIBIOTICS
COMBINED WITH CLOVE AND GINGER


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ABSTRACT
The present work was aimed at the antimicrobial activity of clove, ginger methanol, and ethanol extracts alone and in combination with aminoglycoside antibiotics such as Streptomycin, Kanamycin, Gentamicin, Amikacin were analyzed by the agar disc diffusion method against three gram negative microorganism like E. coli, Pseudomonas aeruginosa, Serratia marcescens. The zones of inhibition obtained from the different agar plate were measured by antibiotic zone reader. According to the zone of inhibition observed in the entire agar plates, the methanol extract of clove and ginger with gentamicin showed maximum zone of inhibitions 27 mm against S. marcescens. The methanol extract of clove and ginger with kanamycin showed zone of inhibition 23 mm against S. marcescens. The ethanol extract of ginger with Gentamicin showed zone of inhibition 30 mm against p. aeruginosa and methanol extract of ginger with Amikacin showed zone of inhibition 16 mm against p. aeruginosa. The methanol extract of ginger showed lowest antimicrobial activity 12 mm against E. coli. Combination of ginger and clove extracts with aminoglycoside antibiotics increased the antimicrobial activity than the clove, ginger extracts separately. This is the new method of approach to decrease the resistance of antibiotics against microorganisms. The large varieties of compounds produced by plants have proved their antimicrobials, resistance modifiers and in combination with antimicrobial chemotherapy which should form the subject of further extensive study.

KEYWORDS: Antimicrobial activity, Ginger, Clove extracts, Aminoglycoside antibiotics.
INTRODUCTION
The extensive use of antibiotics has made many microorganisms develop resistance to them. This has created enormous clinical problems in the treatment of diseases. Therefore, there is a need to develop alternative antimicrobial agents for the treatment of diseases. Non-antibiotic approaches to the treatment and prevention of infection includes the application of clove and ginger extracts combined with aminoglycoside antibiotics. The interest in the study of medicinal plants as a source of pharmacologically active compounds has increased worldwide. It is recognized that in developing countries like India, plants are the main medicinal source to treat infectious diseases. Approximately 20% of the plants found in the world have been subjected to pharmacological or biological test, and a substantial number of new antibiotics introduced in the market are obtained from natural or semi-synthetic resources. New antibiotics were produced by pharmacological industries in the last three decades. [1] However, these antibiotics have failed to discourage the growth of many bacteria that have genetic ability to transmit and acquire resistance to drugs.

Development of resistance to various antibiotics makes it necessary to select logically and rationally, a drug for successful treatment. Interest in the pharmaceutical importance of plants has led to the discovery and adaptation of plant extract which were commonly used in traditional medicine as alternative source of remedy. One strategy employed to overcome resistance mechanisms is the use of combinations of drugs and several plant extracts, which had exhibited synergistic activity against microorganisms.

Ginger (Zingiber officinale), in the family of Zingiberaceae is an important spice used as preservative in food and as an aphrodisiac. Some compounds found in its may act in stomach, facilitating digestion and inhibiting nausea and vomiting, and its major constituent is zingiberene. In India, small pieces of ginger rhizome are mixed with honey and used to treat coughs, sore throat, fever and colds. The characteristic odor and flavor of ginger root come from a volatile oil composed of shogaol and gingerols. Gingerols have been investigated for analgesic, sedative, antipyretic, antibacterial, and gastrointestinal tract motility effects. They have been found to inhibit Gram-positive and Gram-negative bacteria. Further, ginger was also well-regarded for its ability to fight inflammation, to clean colon, to reduce spasms and cramps, and to stimulate circulation. So, it was well justified for the India’s Ayurvedic and the ancient Chinese herbalists that had used ginger for 5,000 years as a medical panacea for curing diseases.
Cloves (Syzygium aromaticum) are the aromatic flower buds of a tree in the family of Myrtaceae, Indigenous to India, Indonesia, Zanzibar, Mauritius and Ceylon. Clove is one of the most valuable spices that have been used for centuries as food preservative and for many medicinal purposes. Cloves are used in Indian Ayurvedic medicine, Chinese medicine, and western herbalism and dentistry where the essential oil is used as an anodyne (painkiller) for dental emergencies. Cloves have many therapeutic uses: they control nausea and vomiting, cough, diarrhea, dyspepsia, flatulence, stomach distension and gastro intestinal spasm, relieve pain, cause uterine contractions and stimulate the nerves. Cloves are used as a carminative, to increase hydrochloric acid in the stomach and to improve peristalsis. Cloves are also said to be a natural anthelmintic. The essential oil is used in aromatherapy when stimulation and warming are needed, especially for digestive problems. Topical application over the stomach or abdomen are said to warm the digestive tract. Applied to a cavity in a decayed tooth, it also relieves toothache. Clove oil is widely used as a perfume and food flavouring as a medicine for the treatment of asthma, rheumatoid arthritis, acne, warts, scars and various allergic disorders as an analgesic, anti spasmodic, and as a general antiseptic in medical dental practices.

As clove and ginger both are used in Indian context in cities, as well as in villages. Both are easily available and consumed on daily basis by Indian people. Many studies have been conducted to explore medicinal uses of the clove and ginger for various illnesses.

The present study was aimed to determine the antimicrobial activity of aminoglycoside antibiotics with clove and ginger extracts against gram negative microorganism to decrease the resistance developed by the microorganisms.

MATERIALS AND METHODS

MATERIALS

Plant Materials

Clove (Syzygium aromaticum) and ginger (Zingiber officinale) used in the present study were purchased from the local market of Vijayawada.

Standard drug

The standard drugs used for this work are Amikacin (Nourish Pharmaceutical Pvt. Ltd), streptomycin (Abbott Healthcare Pvt. Ltd), Gentamicin (Vee Excel Drugs and Pharmaceuticals Pvt. Ltd), Kanamycin (Himedia Laboratories Pvt. Ltd).
Microorganisms
Gram negative microorganisms were used in these studies such as *E. coli* (NCIM 2256), *P. aeruginosa* (NCIM 2037) and *Serratia marcescens* (NCIM 2078).

Instruments
The instruments used for the work are incubator, Refrigerator, Laminar air-flow chamber, Autoclave, Hot air oven, precision electric balance, micropipette (100 to 1000 µl), Inoculating loop etc.

Chemicals
The chemicals used for this work are Ethanol, Methanol, peptone, sodium chloride, and beef extract (Research Lab Fine Chem. industries), Mueller-Hinton Agar, (Titan Biotech Ltd).

METHODS

Extraction and Storage of Plant Material
Freshly collected plant materials such as clove, ginger were powdered and stored at a room temperature. Methanol and ethanol solvents were used for the extraction of ginger and clove powders. 15 g of dried clove powder was extracted with 125 ml of ethanol, and methanol separately by soxhlet apparatus for 4 h (or) till the plant material gets colorless. Methanolic and ethanolic extracts of clove powder was prepared and stored in the refrigerator at 2-5°C until it was used for the antimicrobial studies.

Similarly, 15 g of dried ginger powder was extracted with 125ml of ethanol and methanol separately by soxhlet apparatus for 4 h (or) till the plant material gets colorless. Methanolic and Ethanolic extracts of ginger powder were prepared and stored in refrigerator at 2-5 °C. Totally 4 extracts were prepared with two different solvents. Then the combination of these extracts with aminoglycoside antibiotics was prepared by mixing extracts and antibiotics in 1:1 ratio.

Screening of Antimicrobial Activity

Media for Test Organisms
19 g of Mueller-Hinton agar medium was added to 250 ml of distilled water and autoclaved at 121°C for 15 min at 15 lbs. After cooling the medium, it was inoculated with gram negative microorganism and poured into sterile plates and set aside until to get solidified.
Preparation of Inoculum
A loopful of inoculum was taken from a pure culture of *E. coli* bacteria and inoculated into 10 ml of Mueller-Hinton broth (Hi Media, Mumbai, India). A similar procedure was adopted to prepare the inoculum of other bacterial species i.e., *P. aeruginosa*, *Serratia marcescens*. The broth suspension was then incubated at 37 °C for 3 h and utilized for antibacterial assays.

Agar Disc Diffusion Techniques
These discs were dipped aseptically in four distinct antibiotics such as Streptomycin, Gentamicin, Amikacin, Kanamycin and Methanol, Ethanol extracts of clove and ginger. As well as the combination of clove and ginger extracts with antibiotics in 1:1 ratio and placed over Mueller-Hinton Agar plates seeded with respective pathogens. The plates were incubated in an upright position at 37 °C for 24 hrs. The diameter of inhibition zones formed was measured in mm and the results were recorded. Discs with 7 mm diameter are considered as having no antibacterial activity. Diameter between 7 mm and 12 mm were considered as moderately active and those with greater than 12 mm in diameter of zone of inhibition were considered as highly active. For alcoholic extracts, alcohol served as negative control and for methanol extracts; methanol was used as negative control.

RESULTS AND DISCUSSION
Anti-microbial activity of Aminoglycoside antibiotics such as Amikacin, Streptomycin, Gentamicin and Kanamycin individually and with clove, ginger extracts against three Gram – ve microorganisms and the zone of inhibitions were tabulated and graphical representation shown in Table No: 1 to 4 and Graph No: 1 to 4 respectively.

Table No: 1 Antimicrobial activity of Aminoglycoside Antibiotics against Gram negative Microorganisms

<table>
<thead>
<tr>
<th>S. No</th>
<th>Name of Antibiotic</th>
<th>Name of Microorganisms (Zone of Inhibition in mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>E. coli</em></td>
</tr>
<tr>
<td>1.</td>
<td>Amikacin</td>
<td>14</td>
</tr>
<tr>
<td>2.</td>
<td>Streptomycin</td>
<td>12</td>
</tr>
<tr>
<td>3.</td>
<td>Gentamicin</td>
<td>15</td>
</tr>
<tr>
<td>4.</td>
<td>Kanamycin</td>
<td>14</td>
</tr>
</tbody>
</table>
Table No: 2 Antimicrobial activity of Clove and Ginger Extracts against Gram negative Microorganisms

<table>
<thead>
<tr>
<th>S. No</th>
<th>Name of the Plant with Extract</th>
<th>Name of the Microorganisms (Zone of Inhibition in mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>E. coli</td>
</tr>
<tr>
<td>1.</td>
<td>Clove Methanol</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Clove Ethanol</td>
<td>22</td>
</tr>
<tr>
<td>2.</td>
<td>Ginger Methanol</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Ginger Ethanol</td>
<td>15</td>
</tr>
<tr>
<td>3.</td>
<td>Positive control Methanol</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Positive control Ethanol</td>
<td>6</td>
</tr>
</tbody>
</table>

Fig. 2: Zone of Inhibition of Clove and Ginger Extracts against Gram negative Microorganisms
Table No: 3 Antimicrobial activity of Aminoglycoside Antibiotics with Clove Extracts against *E. coli*

<table>
<thead>
<tr>
<th>S. No</th>
<th>Name of the Antibiotic with clove</th>
<th>Methanol Extract (Zone of Inhibition in mm)</th>
<th>Ethanol Extract (Zone of Inhibition in mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Amikacin</td>
<td>16</td>
<td>17</td>
</tr>
<tr>
<td>2.</td>
<td>Streptomycin</td>
<td>16</td>
<td>13</td>
</tr>
<tr>
<td>3.</td>
<td>Gentamicin</td>
<td>17</td>
<td>15</td>
</tr>
<tr>
<td>4.</td>
<td>Kanamycin</td>
<td>15</td>
<td>13</td>
</tr>
</tbody>
</table>

Fig. 3 Zone of Inhibition of Antibiotics with Clove Extracts against *E. coli*

Table No: 4 Antimicrobial activity of Aminoglycoside Antibiotics with Ginger Extracts against *E. coli*

<table>
<thead>
<tr>
<th>S. No</th>
<th>Name of the Antibiotic with Ginger</th>
<th>Methanol Extract (Zone of Inhibition in mm)</th>
<th>Ethanol Extract (Zone of Inhibition in mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Amikacin</td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td>2.</td>
<td>Streptomycin</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>3.</td>
<td>Gentamicin</td>
<td>15</td>
<td>18</td>
</tr>
<tr>
<td>4.</td>
<td>Kanamycin</td>
<td>17</td>
<td>16</td>
</tr>
</tbody>
</table>

Fig. 4 Zone of Inhibition of Antibiotics with Ginger Extracts against *E. coli*
Fig. 5 Zone of Inhibition of Amikacin with Clove and Ginger Methanolic Extracts

Fig. 6 Zone of Inhibition of Streptomycin with Clove and Ginger Methanolic Extracts

Fig. 7 Zone of Inhibition of Amikacin with Ginger Methanolic Extracts

Fig. 8 Zone of Inhibition of Gentamicin with Ginger Methanolic Extracts
All the solvents extracts showed varying degree of antibiotic activity on the microbial activity on the tested gram negative microorganisms.

According to the zone of inhibition observed in the entire agar plates, the methanol extract of clove and ginger with antibiotics showed maximum zone of inhibitions 27 mm against *S. marcescens*. The methanol extract of clove and ginger with kanamycin showed zone of inhibition 23 mm against *S. marcescens*. The ethanol extract of ginger with Gentamicin showed zone of inhibition (30 mm) against *p. aeruginosa* and methanol extract of ginger with Amikacin showed zone of inhibition (16 mm) against *p. aeruginosa*. The methanol extract of ginger showed lowest antimicrobial activity (12 mm) against E. Coli.

The present study was conducted to observe the combine studies of ginger and clove extracts with aminoglycoside antibiotics against gram negative microorganisms. The disc diffusion method was used in this study. The ethanol and methanol extract of ginger and clove extracts with aminoglycoside antibiotics showed more antimicrobial activity against gram negative microorganisms than the clove and ginger extracts separately. This is the effective method to decrease the resistance of antibiotics against microorganisms.

**CONCLUSION**

The increased resistance to antimicrobial agents among clinical isolates is a serious problem that dramatically raises the cost of health care worldwide. To enhance the susceptibility of these microorganisms by the combination of herbal products such as ginger and clove extracts. Combination of ginger and clove extracts with aminoglycoside antibiotics increased the antimicrobial activity than the clove, ginger extracts separately. This is the new method of approach to decrease the resistance of antibiotics against microorganisms.

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**REFERENCES**


