Antibacterial Activity of Mentha Piperita and Allium Sativum Against Some of Gram-ve Bacteria

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ABSTRACT

The aqueous extracts of Peppermint (Mentha piperita) and garlic (Allium sativum) with five concentrations (0.1, 0.2, 0.3, 0.4 and 0.5mg/ml) of each one were tested for antibacterial activities against pathogenic bacteria like: Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Proteus vulgaris. The testing was performed by well diffusion method. Both extracts of (peppermint, garlic) showed clear antimicrobial activity against pathogenic bacteria, and this activity was enhanced with the increasing of concentrations belongs to them. The concentration (0.5mg/ml) of both extracts gave highest activity against these bacteria; therefore we screened a mixture of equivalent ratio 1:1 of both extracts in this concentration which has exhibited a significant result (average zone of inhibition 27 mm). Antimicrobial activity of both extracts was compared with that for a number of antibiotics that include: ampicillin, cephalexin, erythromycin, amoxicillin, gentamicin, tetracyclin, chloromphenicol, nalidixic acid and trimethoprim by using antibiogram test.

INTRODUCTION

The use of plants in medicine goes as far back as thousands of years and still continues today, many plants are used for the treatment of different diseases and many possess antimicrobial activities [1].

Mentha piperita (peppermint) is a medicinally important plant that belongs to the family Labiate, an aromatics plants have traditionally been used in folk medicine as well as to extend the shelf life of foods, showing inhibition against bacteria, fungi and yeasts [2]. Peppermint has a high menthol content, and is often used as tea and for flavouring ice cream, confectionery, chewing gum, and toothpaste[1]. The oil also
contains menthone and menthyl esters, particularly menthyl acetate [3]. Peppermint extracts are bacteriostatic against Staphylococcus aureus, Salmonella typhimurium, E.coli and Listeria monocytogenes [4]. Peppermint is also found to have antiviral and fungicidal activity [5]. Menthol and peppermint oil are fungicidal against Candida albicans, Aspergillus albus and Dermatophytic fungi [6].

Allium sativum, (garlic) plant's bulb is the most commonly used part of the plant, with the exception of the single clove types; the bulb is divided into numerous fleshy sections called cloves[7]. The cloves are used for consumption (raw or cooked), or for medicinal purposes, and have a characteristic pungent, spicy flavor that mellows and sweetens considerably with cooking [7]. Allium sativum has been consumed as a spice and medicine for thousands of years, ancient Egyptians were known to use it for the treatment of diarrhoea; in ancient Greece it was used for intestinal and lung disorders [8].

The antimicrobial activity is, however, diminished upon boiling, which is attributed to its key component allicin, which is denatured at high temperature [9]. It has been proposed that the development of resistance to betalactam antibiotics is 1000 fold easier than the development of resistance to allicin making garlic a prime candidate for therapeutic use [10]. Even today, garlic is popular in use as an alternative remedy in infectious diseases such as otitis media [11]. Garlic extracts are also known to be effective against Helicobacter pylori, the cause of gastric ulcers [12]. The aim of study to determine the antibacterial activity of aqueous extract of both Garlic and Peppermint against some pathogenic bacteria.

MATERIALS AND METHODS
PREPARATION OF EXTRACTS
Apparently healthy plants were collected, washed thoroughly in tap water and dried at dark room temperature for 15 days. The garlic and peppermint were powdered and extracted separately following the published procedure [13]. The powdered material was soaked in Distilled water by keeping it in a shaker for 3 days at 30 °C. The extracts were filtered through Whatman No.1 filter paper and the filtrate was concentrated in vacuum using a rotary evaporator in order to reduce the crude extract and then autoclaved at 121°C and 15 lb pressure for 20 min. The extract was cooled and immediately assayed for antibacterial activity. It is a known fact that the loss of antibacterial activity of natural products by heating may be due to volatilization and/or the physical and chemical changes that take place during heating.
Antibacterial activity of garlic extracts at 80°C to 90°C for 5 minutes completely destroyed [12].

**PREPARATION OF BACTERIAL SOLUTION**

The microorganisms were tested including: *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Proteus vulgaris* which stored in Nutrient agar slant at 4°C obtained from Biotechnology department – Applied sciences - University of technology. Each organisms (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Proteus vulgaris*) were inoculated separately onto Nutrient Broth (Hi-media) at 37°C for overnight and were stored in Nutrient agar slant at 4°C and sub-cultured fortnightly. The bacterial cells were harvested by centrifuging at 5000g for 15 min. The pellet formed was washed twice with PBS (Phosphate Buffer Saline) and (10 mM Sodium Chloride, pH 7.4) and the cells were counted by haemocytometer (*neubauer counting chamber*). The bacterial cells were diluted to approximately $10^5$ CFU/ml before use [14].

**WELL DIFFUSION TECHNIQUE:**

Screening of antibacterial activity was performed by well diffusion technique [15]. The Mueller Hinton Agar (MHA) (Hi-media) plates were seeded with 0.1 ml of the standardized inoculums of each testing organism. The inoculums were spread evenly over plate with sterile an L-shaped rod glass spreader. The seeded plates were allowed to dry in the incubator at 37°C for 20 minutes. A standard cork borer of 8 mm diameter was used to cut uniform wells on the surface of the MHA and 100 µl of each fresh vegetable extracts were placed in the well. The inoculated plates were incubated at 37°C for 24 hours and the inhibition zones were measured to the nearest millimeter (mm).

A mixture of equivalent ratio 1:1 of both extracts (peppermint and garlic) in the concentration (0.5mg/ml) which has exhibited a significant result by mixing equivalent ratio 1:1 in sterile beaker then placed 100µl in the wells. The inoculated plates were incubated at 37°C for 24 hours and the inhibition zones were measured to the nearest millimeter (mm).

Antibiogram test in which small discs containing different antibiotics are dropped in different zones of the culture on an agar plate, which is a nutrient-rich environment in which bacteria can grow. The antibiotic will diffuse in the area surrounding each tablet, and a disc of bacterial lysis will become visible.

**RESULTS AND DISCUSSION**

The aqueous extract of peppermint (*Mentha piperita*) with five concentrations (0.1, 0.2, 0.3, 0.4 and 0.5mg/ml) was actively against the strains of the bacteria that are common cause of infections. The
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antibacterial activity was expressed at varying degrees with the activity being both strain and dose dependent (Table-1). We observed maximum activity at 0.5mg/ml concentration against *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Proteus vulgaris* (Figure-1).

Table-1: The mean of inhibition zone of the aqueous extract of peppermint (M) against *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Pseudomonas aeruginosa*

<table>
<thead>
<tr>
<th>Concentration(mg/ml)</th>
<th>0.1</th>
<th>0.2</th>
<th>0.3</th>
<th>0.4</th>
<th>0.5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>bacteria species</strong></td>
<td>Zone of inhibition(mm)</td>
<td>-</td>
<td>4</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>2</td>
<td>5</td>
<td>6</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td><em>Klebsiella pneumonia</em></td>
<td>4</td>
<td>6</td>
<td>7</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em></td>
<td>2</td>
<td>4</td>
<td>7</td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>2</td>
<td>4</td>
<td>7</td>
<td>10</td>
<td>14</td>
</tr>
</tbody>
</table>

The cold equates extract of *Mentha piperita*(M) shows significant activity (Figure -1) Upon chemical analysis, the extracts were found to possess glycosides and alkaloids. In addition, plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids and flavonoids which have been found *in vitro* to have antimicrobial properties [16]. In addition to these properties, it has also been used as appetite stimulant, a treatment for gastrointestinal infection and to lower blood sugar in diabetics. Its use for the treatment of certain types of cancer and viral infections has also been reported[17] (Derrida, 2003).
Figure-1: The antimicrobial activity of aqueous extract of peppermint (M) against *Escherichia coli*, *Klebsiella pneumonia*, *Proteus vulgaris* and *Pseudomonas aeruginosa*

The present work was similar to [16], shows that the compounds from *Mentha piperita* possess potent antimicrobial activity. Besides, extract of the entire plant has shown antiprotozoal activity against *Entamoeba histolytica* and has demonstrated antibacterial properties against *Helicobacter pylori*, the bacteria causing stomach ulcer.[18].

The water extract of garlic (G) with five concentrations tested against pathogenic bacteria, all bacterial strains showed promising sensitivity to water extract of garlic (Table-2). When crushed, *Allium sativum* yields allicin, a powerful antibiotic and antifungal compound (phytoncide) [19]. *Allium sativum* may have other beneficial properties, such as preventing and fighting the common cold.[20]

Table-2: The mean of inhibition zone of The aqueous extract of garlic (G) against *Escherichia coli*, *Klebsiella pneumonia*, *Proteus vulgaris* and *Pseudomonas aeruginosa*

<table>
<thead>
<tr>
<th>Concentration(mg/ml)</th>
<th>0.1</th>
<th>0.2</th>
<th>0.3</th>
<th>0.4</th>
<th>0.5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>bacteria species</strong></td>
<td>Zone of inhibition(mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td></td>
<td>4</td>
<td>6</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td><em>Klebsiella pneumonia</em></td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em></td>
<td>-</td>
<td>4</td>
<td>8</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>-</td>
<td>4</td>
<td>7</td>
<td>9</td>
<td>12</td>
</tr>
</tbody>
</table>
The main antimicrobial effect of allicin is due to its oxidative interaction with important thiol containing enzymes [21]. The active allicin molecule also has a very short half-life, keeping the defence mechanism rapid and much localized, preserving the rest of the alliin in the clove for future attacks [21].

Figure-2: The antimicrobial activity of aqueous extract of garlic (G) against *Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris and Pseudomonas aeruginosa*

Allicin also contains the sulfur containing compounds alliin, ajoene, diallylsulfide, dithiin, S-allylcysteine, and enzymes, vitamin B, proteins, minerals, saponins, flavonoids, and maillard reaction products, which are non-sulfur containing compounds[22]. The antibacterial property of garlic has been tested in many studies and *in vitro* experiments have shown inhibition of 14 species of bacteria including *Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa* [15]. A striking aspect of the activity of garlic is the apparent inability of most bacteria to develop resistance to it because its mode of action is completely different from that of other antibiotics[10].

These results encourage us to screen a mixture of equivalent ratio 1:1 of both extracts (peppermint and garlic) in the concentration (0.5mg/ml) which has exhibited a significant result by mixing equivalent ratio 1:1 in sterile beaker then placed 100µl in the wells. The inoculated plates were incubated at 37°C for 24 hours and the inhibition zones were measured to the nearest millimeter (mm). (Table-3). As we mentioned earlier peppermint and garlic has a many effective compound and the key component allicin in garlic which is denatured at high
temperature, so when we got a mix of the two extractions perhaps an interaction between the components and the formation of more efficient vehicles, which led to greater inhibition of bacterial growth.

Table-3: The mean of inhibition zone of the aqueous extract of peppermint and garlic (GM) against Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris and Pseudomonas aeruginosa

<table>
<thead>
<tr>
<th>peppermint and garlic concentration (0.5mg/ml)</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E. coli</td>
</tr>
<tr>
<td></td>
<td>27</td>
</tr>
</tbody>
</table>

Figure-3: The antimicrobial activity of aqueous extract of peppermint and garlic (GM) against Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris and Pseudomonas aeruginosa

Antimicrobial activity of both (peppermint and garlic) extracts was compared with that for a number of antibiotics (A*) that known for their ability of inhibition pathogenic bacteria which causes injuries inflammation and infections include ampicilin, cephalexin, erythromycin, amoxicillin, gentamicin, tetracyclin, chloromphenicol, nalidixic acid and trimethoprim by using antibiogram test. (Table-4). A* = antibiotic disc.
Table-4: The mean of inhibition zone of antibiotics (A) against *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Pseudomonas aeruginosa*

<table>
<thead>
<tr>
<th>Bacteria sp.</th>
<th>Amoxicillin</th>
<th>Tetracycline</th>
<th>Erythromycin</th>
<th>Cephalexin</th>
<th>Ampicillin</th>
<th>Gentamicin</th>
<th>Chloramphenicol</th>
<th>Trimethoprim</th>
<th>Nalidixic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>R*</td>
<td>4</td>
<td>R</td>
<td>R</td>
<td>10</td>
<td>14</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td><em>Klebsiella pneumonia</em></td>
<td>R</td>
<td>12</td>
<td>R</td>
<td>R</td>
<td>12</td>
<td>18</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em></td>
<td>R</td>
<td>10</td>
<td>R</td>
<td>10</td>
<td>12</td>
<td>18</td>
<td>4</td>
<td>4</td>
<td>R</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>R</td>
<td>R</td>
<td>8</td>
<td>R</td>
<td>14</td>
<td>20</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
</tbody>
</table>

R* = Resistance

An antimicrobial activity of aqueous extract of peppermint was better than of tetracyclin and gentamicin against *E. coli* while the antimicrobial activity of latest concentration of garlic extract against *E. coli* was better than the effect of same antibiotic. The effect of chloramphenicol against *Pseudomonas aeruginosa*, was better than that of both extracts concentrations, while the mixture of (with 0.5mg/ml concentration) both extracts had significant antimicrobial activity against *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Pseudomonas aeruginosa*, which affected by chloromphenicol, gentamicin and tetracyclin. The resistance of pathogenic bacteria to amoxicillin, erythromycin and ampicillin was noticed. (Figure-4)

Figure- 4: The antimicrobial activity of some antibiotics (A*) against (1) *Escherichia coli*, (2)*Klebsiella pneumoniae*, (3)*Proteus vulgaris* and (4)*Pseudomonas aeruginosa*
Because garlic and peppermint are known to act synergistically with antibiotics, and resistance has not been reported for them (garlic and peppermint), more dose-response preclinical studies and eventually clinical studies should be done to assess the use of garlic /peppermint combination for bacteria that are difficult to eradicate. In view of the strong antibiotic properties and the complete absence of development of resistance

Finally, it can be concluded that the active chemical compounds present in *Mentha piperita* and *Allium sativum* should certainly use in treatment of various bacterial infections. The results from the present study are very encouraging and indicate this herb should be studied more extensively to explore its potential in the treatment of infectious diseases as well, besides, the same may also be used for self medication in domestic settings.

REFERENCES