Antimicrobial Activity of Ethanolic Extracts From Some Medicinal Plant

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Abstract: Six ethanolic extracts from plants of Ginger (Zingiber officinale), Cinnamon (Cinnamomum verum), black cumin (Nigella sativa), Clove (Syzygium aromaticum) black pepper (Piper nigrum) and chamomile (Anthemis nobilis) were purchased from local market in Dammam, Saudi Arabia were assayed for the in vitro antimicrobial activity against gram-positive standard bacteria represented by Staphylococcus aureus and Bacillus subtilis and gram-negative standard bacteria represented by Escherichia coli and Pseudomonas aeruginosa and yeast represented by Candida albicans using hole-plate diffusion method. Clove was strongly inhibited the growth of B. subtilis, Ps. aeruginosa and C. albicans, whereas Cinnamon was strongly inhibited the growth of B. subtilis and C. albicans only. The other plants have no inhibition activity or have stimulation activity on test organisms. Staphylococcus aureus and Escherichia coli were resistant to all the plants extracts. Ps. aeruginosa was resistant to almost all of the plants extracts except clove. Ginger and chamomile showed stimulation activity on B. subtilis and C. albicans. The inhibitory concentration of each extract for given microorganisms. Both clove and cinnamon extracts showed remarkable effect on B. subtilis and C. albicans at very low concentration MIC, 3.125-6.25 µg/ml and 12.5-25, respectively. In addition, clove extract have marked inhibitory effect on Ps. aeruginosa at low concentration MIC 25 µg/ml.

Key words: ethanolic extracts, Clove, Cinnamon, yeast, bacteria.

INTRODUCTION

New antibiotics were produced by pharmacological industries in the last three decades (Al-Juraifani, A.A., 2011). However, these antibiotics have failed to discourage the growth of many bacteria that have genetic ability to transmit and acquire resistance to drugs (Cohen, M.L., 1992). Thus, infections with these bacteria are associated with high morbidity and mortality especially with immunocompromised patients (Driscoll, J.A., S.L. Brody and M.H. Kollef, 2007; Del Toro, M.D. et al., 2006). In addition, many researches have established the side effect of overuse and misuse antibiotic which can harm vital organs like liver, kidneys and some cells such as the pancreas and spleen as well as their impact on the immune system. The known success of traditional medicine has guided the search for new chemotherapeutic alternatives to eliminate the infections caused by drug-resistant microbes and to reduce the harm caused by antibiotic (Bocanegra-Garcia, V. et al., 2009; Giamarellou, H., 2006).

Medicinal Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, flavonoids, phenols and quinones (Al-Zubaydi, S.R., M.A. Al-Hmdany and S.J. Raesan, 2009; Leon, J., E. Rojo and J. Sanchezerranzo, 2001; Cowan, M.M., 1999), which have been used worldwide in traditional medicine to treat several diseases and infection (Saad, B., H. Azaizeh and O. Said, 2005; Kalemba, D. and A. Kunicka, 2003; Jain, S.C. et al., 1996). Many studies all over the world have been showed that these plants and their extract have multi-antimicrobial properties (Al-Juraifani, A.A., 2011; Bocanegra-Garcia, V. 2009; Bakht, J. 2011; Boklari, F.M., 2009). While 25 to 50 % of current pharmaceuticals are derived from plants, none is used as antimicrobials (Cowan, M.M., 1999).

Biological effects of these plants on prokaryotic and eukaryotic organisms have been discussed by few studies (Al-Zubaydi, S.R., M.A. Al-Hmdany and S.J. Raesan, 2009; Bakkali, F. 2008). However, Plants have an almost infinite ability to synthesize compounds that have diverse bioactive properties that we cannot synthesize. Moreover, a large number of plant species have not been studied for potential medicinal value or described.

Therefore, this study was conducted to evaluate the antimicrobial activities and biological effect of most used plant in Saudi Arabia that used as drinks, food spices and flavoring on gram-positive standard strains bacteria represented by Staphylococcus aureus and Bacillus subtilis and gram-negative standard represented by Escherichia coli and Pseudomonas aeruginosa and yeast represented by Candida albicans.

MATERIALS AND METHODS

Plant Material:

Six plants were used ginger (Zingiber officinale), cinnamon (Cinnamomum verum), black cumin (Nigella sativa), clove (Syzygium aromaticum) black pepper (Piper nigrum) and chamomile (Anthemis nobilis) they...
The well diffusion assay technique (Chung, K.T. et al., 1998) was used, 500 µl of microbes cultures age 18 - 24 h were added to Petri plates and nutrient agar (NA) were poured. After media were solidified, holes were made by using 5 mm cork borer each hole was filled with 50 µl of plant extract. The inoculated agar plates were left in refrigerator for one hour for proper diffusion then plates were incubated, at 37 °C for the bacteria and 30 °C for the yeast, for 24 h. Negative and positive controls were used. The zones of inhibition were then recorded in millimeters.

**Microorganisms:**
Four bacterial species, Gram-positive *Staphylococcus aureus* and *Bacillus subtilis*, gram-negative *Pseudomonas aeruginosa* and *Escherichia coli* and yeast represented by *Candida albicans*. These microbes were obtained from the microbiology department of Dammam University.

**Screening of Antibacterial Activities:**
The well diffusion assay technique (Chung, K.T. et al., 1998) was used, 500 µl of microbes cultures age 18 - 24 h were added to Petri plates and nutrient agar (NA) were poured. After media were solidified, holes were made by using 5 mm cork borer each hole was filled with 50 µl of plant extract. The inoculated agar plates were left in refrigerator for one hour for proper diffusion then plates were incubated, at 37 °C for the bacteria and 30 °C for the yeast, for 24 h. Negative and positive controls were used. The zones of inhibition were then recorded in millimeters.

**Determination of Minimum Inhibitory Concentrations (MIC):**
The antimicrobial activity of the plant extract, that shows antimicrobial activity, were determined by 2-fold dilution methods as described by (Omura, S. et al., 1993) and MICs were read in µg/ml after over night incubation at 37 °C. All experiments were made in replicate.

**RESULT AND DESICCATION:**
The effects of the six-plant extract on the organisms were summarized in Table 1. Many investigations were carried out to discover the antimicrobial effect of plant extract. The results showed that the extracts from clove and cinnamon had an antimicrobial activity against some tested bacteria (Table 1). While the extracts from chamomile, ginger, black cumin and black pepper did not show any antimicrobial activity. However, chamomile and ginger showed stimulations effect on *B. subtilis* and *C. albicans* that may be due to the nutrients in these herbs. This does not diminish the importance of these plants for the treatment of many diseases (Saad, B., H. Azaizeh and O. Said, 2005). These results are in accordance with many researchers, especially the impact of clove and cinnamon (Ghaly, M.F. 2010; Eruteya, O.C. and S.A. Odunfa, 2009; Nascimento, G.G.F. 2000) while the effect of chamomile, ginger, black cumin and black pepper were various. (Onyeagba, R.A. 2004) Found ginger ethanolic extracts had no activity against *Bacillus spp.*, *S. aureus* and *E. coli*. Whereas, (Pattaratanawadee, E. et al., 2006; Sebiomo, A. et al., 2011) found ginger ethanolic extracts inhibit the growth of *S. aureus* and *E. coli*. (Salman, M.T., R.A. Khan and I. Shukla, 2008) found black cumin oil inhibit the growth of *S. aureus* and *P. aeruginosa* while *E. coli* was resistant. (Masood, N., A. Chaudhry and P. Tariq, 2006) found black pepper had antibacterial activity against *P. aeruginosa*, *S. aureus* and *E. coli*. Moreover, (Ertürk, Ö., 2006) found *P. aeruginosa* was the most sensitive bacterial strain to black peeper. (McKey, D.L. and J.B. Blumberg, 2006) Reported that Chamomile has moderate antimicrobial activity.

It was observed that the antimicrobial effect of plant extract varies from one plant to another in different regions of the world. This may be due to many factors such as, the effect of climate, soil composition, age and vegetation cycle stage, on the quality, quantity and composition of extracted product, different bacterial strains (Masotti, V. et al., 2003; Angioni, A. 2006). Moreover, different studies found that the type of solvent has an important role in the process of extracting (Al-Zubaydi, S.R., M.A. Al-Hmdany and S.J. Raesan, 2009; Bakht, J. 2011; Boklari, F.M., 2009; Bedi, N. 2010).

Result showed that the clove extract effect was stronger than cinnamon. Both of them have essential oil that cause membrane disruption to the bacteria (Table 1) and cause mitochondrial damage to the yeast (Armstrong, J.S., 2006; Bakkali, F. 2005). The difference between their effects may be due the quantity of the phenolic compounds, eugenol, (Table 2) this finding is in agreement with (Al-Zubaydi, S.R., M.A. Al-Hmdany and S.J. Raesan, 2009; Bakkali, F. et al., 2008) who had found that the biological effects of the essential oils depends on the type and quantity of active compounds. Thus, eugenol of clove and cinnamon could be responsible for their antimicrobial activity against *B. subtilis*, *Ps. aeruginosa* and *C. albicans*. Eugenol is phenol and it is one of the...
aromatic compounds containing many free phenol groups and the antimicrobial activity increased by increasing these groups (Al-Zubaydi, S.R., M.A. Al-Hmdany and S.J. Raesan, 2009). In addition, this result revealed that eugenol had a broad antimicrobial activity when appear in high quantity.

The result showed that the spores form B. subtilis, which more resistant to environmental conditions than any other tested bacteria, was the best-inhibited microorganism with a mean inhibition zone of 6.2 ± 0.7 mm by clove extract and 5.8 ± 0.2 mm by cinnamon extract. E. coli and St. auerus, which already known to be multi-resistant to antibiotics, were resistant to all tested plant extracts. While Ps. aeruginosa, which also resistant to different antibiotics (Giamarellou, H., 2006) was the least inhibited with a zone of 3.2 ± 0.2 by clove extract. This result is very interesting, because therapeutic agent means failed to control this bacterium, which was isolated from hospital environment.

Results of this study demonstrated that the gram-negative bacteria were more resistant to the plant extract than gram-positive bacteria such as Ps. aeruginosa exhibited more resistant than B. subtilis when they were tested with Cinnamon extract. Because lipopolysaccharide (LPS) layer of gram- negative bacteria in outer membrane have a high hydrophobicity which acts as a strong permeability barrier against hydrophobic molecules (Smith-Palmer, A., J. Stewart and L. Fyfe, 1998). Hydrophobic molecules can pass through cell wall of gram-positive bacteria easier than the gram- positive bacteria because cell wall of the gram- positive bacteria contained only peptidoglycan (Nikaido, H. and M. Vaare, 1985; Lambert, R.J.W. 2001). These results agree with those reported by (Lan-ciotti, R. 2004) who confirmed that the antimicrobial effects of essential oil constituents are dependent on their hydrophobicity.

In addition, C. albicans was strongly influenced with a mean inhibition zone of 4.8 ± 0.2 mm by clove extract and 3.8 ± 0.3 mm by cinnamon extract. This result is very interesting because C. albicans has been the most extensively studied pathogen in antifungal resistance because of their morbidity and mortality associated with infections in immunocompromised patients (Casalinuovo, I.A., P. Di Francesco and E. Garaci, 2004; Redding, S. et al., 1993).

The results revealed variability in the inhibitory concentration of each extract for given microorganisms. Both clove and cinnamon extracts showed remarkable effect on B. subtilis and C. albicans at very low concentration MIC, 3.125-6.25 µg/ml and 12.5-25, respectively. In addition, clove extract was found to have marked inhibitory effect on Ps. aeruginosa at low concentration MIC 25 µg/ml. This result is very interesting because the possible toxic effects of active compound may be minimized when used in very low concentration (Table 4).

**Conclusion:**

The results of this work suggest that the compound extracted from clove and cinnamon have a broad spectrum of antimicrobial activity and this effect is increased by increasing the quantity of this compound, which can be used as an alternative for antibiotics. Therefore, pharmacological test is necessary to isolate and characterize their active compounds. Moreover, these plants extract should be investigated in vivo to better understand their safety, efficacy and properties.

<table>
<thead>
<tr>
<th>Table 1: Effect of plant extract on test organisms.</th>
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<tbody>
<tr>
<td><strong>Mean diameter of Inhibition zone (mm) ± S.D</strong></td>
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<tr>
<td><strong>plant extract</strong></td>
</tr>
<tr>
<td>Cloves</td>
</tr>
<tr>
<td>Cinnamon</td>
</tr>
<tr>
<td>Chamomile</td>
</tr>
<tr>
<td>Ginger</td>
</tr>
<tr>
<td>Black cumin</td>
</tr>
<tr>
<td>black pepper</td>
</tr>
<tr>
<td>Ethanol (96%) and distilled water (20:80 v:v) (Negative control)</td>
</tr>
<tr>
<td>Streptomycin 10 mcg (Positives control)</td>
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</tbody>
</table>

- had no effect, + had stimulation effect.

<table>
<thead>
<tr>
<th>Table 2: scientific name, common name, part used, active substance and their Mechanism.</th>
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</thead>
<tbody>
<tr>
<td><strong>Scientific name</strong></td>
</tr>
<tr>
<td>Syzygium aromaticum</td>
</tr>
<tr>
<td>Cinnamomum verum</td>
</tr>
<tr>
<td>Zingiber officinale</td>
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<tr>
<td>Nigella sativa</td>
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</tbody>
</table>
Intercalate into cell wall and/ or DNA.

Table 3: Antimicrobial components of plant extract.

<table>
<thead>
<tr>
<th>plant extract</th>
<th>Proximate essential oil content (%)</th>
<th>Antimicrobial components</th>
<th>reference</th>
<th>Type of essential oil (aromatic compounds)</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>clove</td>
<td>16.0 – 18.0</td>
<td>Eugenol</td>
<td>[39]</td>
<td>phenol</td>
<td>[15]</td>
</tr>
<tr>
<td>cinnamon</td>
<td>0.5 – 0.2</td>
<td>Cinnamaldehyde, Eugenol</td>
<td>[39]</td>
<td>Aldehyde, phenol</td>
<td>[15]</td>
</tr>
</tbody>
</table>

Table 4: Minimal Inhibitory Concentration (MIC) of plant extracts.

<table>
<thead>
<tr>
<th>plant extract</th>
<th>MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B. subtilis</td>
</tr>
<tr>
<td>Clove</td>
<td>3.125</td>
</tr>
<tr>
<td>Cinnamon</td>
<td>6.25</td>
</tr>
</tbody>
</table>

REFERENCES


