Evaluation the Antibacterial Activity Of Aniseed: In Vitro Study

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Abstract

Multiple drug resistance in human pathogenic microorganisms has built-up due to random use of commercial antimicrobial drugs usually used in the conduct of infectious diseases and the development of antibiotic resistance is multifactorial. This situation has requisite scientists to search for novel antimicrobial substances from a diversity of sources as narrative antimicrobial chemotherapeutic agents. The aim of present study is to show the antibacterial activity of Aniseed on selected Gram-positive and Gram-negative bacteria with minimal inhibitory concentrations. The bacterial strains involved in this study were Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae and streptococcus pneumoniae. The aqueous extraction of the water-soluble ingredients was conceded out by grounded 4 gm of leaves then consecutive drenched for 2hrs using 10 ml of distilled water in a 250 ml sterile tapering flask Well Diffusion method and Minimum Inhibitory Concentration (MIC) determined the antibacterial potency. Results showed that aniseed produced significant antibacterial activity against common pathogenic G+ and G- bacteria p?0.05 in comparison with negative control. In conclusion the aniseed aqueous extracts have great potential as antimicrobial components against microorganisms and they can be used in the treatment of infectious diseases caused by bacteria.

Materials and Methods

This study was conceded out in Department of Pharmacology, College of Medicine, Almustansiriya University, Baghdad – Iraq, 2012. It is approved by scientific judges and licensed by board of medical College. The aqueous extraction of the water-soluble ingredients was conceded out by grounded 4 gm of leaves then consecutive drenched for 2hrs using 10 ml of distilled water in a 250 ml sterile tapering flask. The extracts were clean using Whatman filter paper No 1. The filtrates were stored in universal bottles and refrigerated at 4°C prior to use. Then 1ml used for diffusion method [12]. The bacterial strains involved in this study were Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae and streptococcus pneumoniae. All the bacterial strains were grown and maintained in nutrient agar.
**Well Diffusion method:** Microbial type culture were maintained on nutrient agar slants and 0.2 ml of overnight grown cultures of each organism was dispensed into 20 ml of sterile nutrient broth and incubated for 4 hrs at 37°C to standardize the culture to 10^6 CFU/ml. Then 10 μl of aniseed extract (40 mg/0.1 ml) was soaked by sterile filter paper discs. The sterile filter paper discs were saturated with aniseed extract placed on the surface of the medium and incubated at 37°C for 24 hrs. The assessment of antibacterial activity was measured around the disc. [13]

**Minimum Inhibitory Concentration (MIC):** The MIC was defined as the lowest concentration of the compound to inhibit the growth of microorganisms. The minimum inhibitory concentration (MIC) values were determined by broth dilution assay. The cultures were ordered at 24hr broth cultures of selected bacteria. Serial dilution of concentrated aqueous aniseed extract for 2, 4, 8, 16, 32 and 64 mg/ml respectively to be used for MIC. The positive control was ciprofloxacin while distilled water regarded as negative control. [14]

**Results**

Aniseed aqueous extract produced significant antibacterial activity against the selected G+ and G- bacteria in this study table (1). *p*<0.05 in comparison with negative control.

**Discussion**

International consideration in the utilize of medicinal and aromatic plants is increasing and positive effects of plant based medicines and other plant based crop are being rediscovered. Ethnobotanical in sequence is leading to the detection of a novel phytopharmaceuticals phytoproducts. Nonetheless, legalization and utilization as a phytopharmaceutical product require a great deal basic and practical research in order to put this reserve on the same level as the patented antimicrobial products so achieving acceptance via the medical system, and fulfilling the requisite of efficiency, safety and dominance [15, 16, 17]. In this study aniseed (Pimpinella Anisum) aqueous extract produced significant antibacterial effects on Staphylococcus aureus, Escherichia coli and Klebsiella pneumonia but showed minimal effects on Pseudomonas aeruginosa. The resistance of Pseudomonas aeruginosa can be explained by that this bacterium is one of the lowest antibiotic susceptibility, which is related to a intensive action of multidrug efflux pumps with chromosomally encoded antibiotic resistance genes, and the low permeability of the bacterial cellular envelopes and so Pseudomonas aeruginosa simply develops acquired resistance via mutation in chromosomally encoded genes or through the horizontal gene transfer of antibiotic resistance determinants and some new studies have shown phenotypic resistance associated to biofilm formation or to the appearance of small-colony variants may be significant in the reaction of Pseudomonas aeruginosa to antibiotics treatment. [18] Additionally; regulation of gene expression can occur via cell-cell communication or quorum sensing (QS) via the induction of small molecules called autoinducers and quorum sensing is known to organize appearance of a numeral of virulence factors. Garlic experimentally blocks quorum sensing in Pseudomonas aeruginosa. [19, 20] and because Pimpinella Anisum not containe the active constituents of garlic this may explained the resistant of Pseudomonas aeruginosa against aniseed extracts. Phytochemical analysis of aniseed active extracts established the presence of phytoconstituents like tannin, saponins, flavonoids and alkaloids [21]. A number of the most significant bioactive phytochemical constituents were terpenoids and phenolic agents as reported previously. Antibacterial properties of several herbs extracts have been accredited to some of these phytoconstituents [22, 23]. The results of present study are in conformity with the sequence of other studies and the antimicrobial achievement of tannin derived from Pimpinella Anisum might be associated to their ability to the inhibition of microbial adhesions, enzymes cell transport proteins and cytoplasmic molecules [24, 25] and flavonoids action is approximately surely due to their capacity to involved with extracellular and soluble proteins and with bacterial cell wall and the lipophilic flavonoids may also disrupt microbial cell membrane [26]. Bio-autography study of thin layer chromatographic plate showed clear zones containing active constituents that inhibited the growth of Klebsiella pneumoniae over the region containing the aniseed extract and in preponderance of the plants tannins were experimental as most active constituents for their antimicrobial activity. [27] Aniseed aqueous extract was generate to be the mainstream potent antibacterial extract, as well as the activity against both Gram+ve and Gram-ve bacteria suggest its possible use as a broad spectrum antibacterial agent.[28] Consequently; Pimpinella Anisum aqueous extract was selected in this study as an alternative of alcoholic extract. Furthermore; the results of present
study are in contrasting with the study carried out by Elgayyar et al. 2001 in which aniseed has exhibited broad antibacterial spectrum against Gram positive and Gram negative bacteria including Pseudomonas aeruginosa, Staphylococcus aureus and Yersinia enterocolitica [29] this may be explained through small sample size or practice of highly resistant strain of Pseudomonas aeruginosa in this study. The mechanisms through which microorganisms continue to resist the action of antimicrobial agents are insufficiently implicit and stay behind debatable. Alternatively, the chemical constituents of this extracts may have a primary role in vivo expectation of diseases caused by bacteria, fungi and yeast. [30, 31] Darwish and Aburjai study 2010 showed that the activity of cephalosporine on the resistant strain was improved while used in combination with aniseed extract the augmentation of the activity of cephalosporine was more noticeable beside the standard strain this might indicated that the aniseed permissible better penetration of the drug throughout the outer layers to the cell wall, which is the target site for this antibiotic. This might also point to that the Pimpinella Anisum acts by another mechanism such as blocking the inhibitory effect of the enzymes at bacterial cell wall. [32] These results suggest that the aniseed extracts possibly will be used as a natural antibacterial managerial for human and infectious diseases and any of these substances in aniseed may be accountable for these effects, further detailed studies of aniseed are required to decide which of their components are more liable for its antimicrobial effect and to elucidate their biological actions.

Conclusions

The aniseed extracts have great potential antimicrobial effects against microorganisms and it can be used in the treatment of infectious diseases caused by bacterial pathogenic strains and supplementary effort is required to split the secondary metabolites from the extracts studied in order to test specific antibacterial activity and the underlying mechanisms.

References

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20. Akinmoladun AC, Ibukun EO, Afor E, Obuotor EM, Farombi EO. Phytochemical constituents and
Illustrations

Illustration 1

Table (1). Antimicrobial activity of Aniseed in comparison with positive control using agar disc diffusion method (inhibition zone in mm mean±SD) and MIC of Aniseed.

<table>
<thead>
<tr>
<th>Bacterial types</th>
<th>Aniseed (mean±SD)</th>
<th>Ciprofloxacin (mean±SD)</th>
<th>MIC (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>20±0.83*</td>
<td>23 ± 0.82</td>
<td>4</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>11±0.54*</td>
<td>15 ± 1.03</td>
<td>16</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>6 ± 0.07</td>
<td>22 ± 1.14</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>23±1.57*</td>
<td>26 ± 0.72</td>
<td>4</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>22±0.05*</td>
<td>25±0.34</td>
<td>4</td>
</tr>
</tbody>
</table>
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