Antibacterial Activity of Citrus Juices against Methicillin Resistant Staphylococcus aureus

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Abstract
This study aimed to see the inhibitory activity of Citrus Juices against methicillin resistant Staphylococcus aureus, and to compare with the inhibitory activity of some antibiotics. Several Citrus juice concentrations of 100%, 75%, 50% and 25% (v/v) were diluted with distilled water after filtration through bacterial filter (Millipore). Result indicated that the best antibacterial activity was observed in 100%, 75%, 50% and 25% (v/v) concentrations for the three types of Citrus juices only but there were no antimicrobial activities in 100%, 75%, 50% and 25% concentration for the one type of Citrus juices that C. tangerina (mandarin orange) on same bacteria, on the other hand all the 30 MRSA isolates showed a high level of resistance to aminoglycosides group, with rate reached to 5 isolates (16.6%) for streptomycin, 11 isolates (36%) for amikacin, 15 isolates (50%) for gentamicin, 19 isolates (63.3%) for kanamycin. All the isolates showed sensitive for streptomycin antibiotic, the isolates showed these resistance to all antibiotics amikacin, gentamicin, kanamycin.

Keywords: Citrus Juices, Antibacterial, Staphylococcus aureus

Introduction
Bacteria are responsible for high mortality rates in numerous developing countries with as many as 50,000 people dying daily as a consequence of infections (Sapkota et al. 2012). Bacterial resistance has appeared for every major class of antibiotics (Lambert, P.A. 2005). Staphylococcus aureus causes a variety of suppurrative (pus-forming) infections and toxinoeses and more serious infections such as pneumonia, mastitis, meningitis, and urinary tract infections. S. aureus is a major cause of hospital acquired (nosocomial) infection of surgical wounds and infections associated with indwelling medical devices. 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. The extract of different parts of following plants have been used in the present project work to check their antibacterial effectiveness against human bacterial pathogens (Sapkota et al. 2012). The juice of citrus fruit shows its actions as a cytotoxic (Xu et al. 2003) and an antimicrobial against upper respiratory tract bacterial pathogens (Guthrie et al. 1998; Hollman et al. 1995; Kawaii et al. 1999; Lam et al. 1989). Howed that the flavonoids, limonoids, and ascorbic acid are groups of citrus phytochemicals and micronutrients which are responsible for the antiinflammatory and antitumor activities. The citrus limonoids inhibit the chemically induced colon carcinoma in addition to the potential use of citrus flavonoids in cancer treatment (Tanaka et al., 1998 Rooprai et al. 2001; So et al. 1996).

The aim of this study is to determine the efficiency of Citrus juice extract of Citrus fruits C. limon (lemon), C. sinensis (orange), C. aurantium (bitter orange) C. tangerina (mandarin orange) using different concentration against gram positive bacteria.

Materials and Methods
Preparation of crude extract
Fresh fruits C. limon (lemon), C. sinensis (orange), C. aurantium (bitter orange) C. tangerina (mandarin orange) were collected from local market Baghdad - Iraq. Taxonomic identification of the plant was done. Pericarp of ripened fruit was collected and washed with sterile distilled water. Samples crushed into parts and squeezed to remove the crude extract, the crude extract were filtered through filter paper in to vials (Mbata et al. 2006).

Antimicrobial Assay
The agar diffusion technique (well diffusion method) was used to screen the antibacterial activity. 50 ul of the different concentrations (25%, 50%, 75% and 100% w/v) of the Fresh fruits crude extract samples were separately placed in the different punched wells with 1 ml sterile syringe. The plates were allowed to stay for 15 mins for pre-diffusion to take place followed by an overnight incubation that lasted for 24 hrs at 37°C. In-vitro the antibacterial activity was evaluated by measuring the diameter of the inhibition zone formed around the wells (Abdul sattar et al. 2010).

Bacteriological study
The gram positive (Staphylococcus aureus) bacterial isolates from the skin and urinary tract infection identification from previous study

Sensitivity to antimicrobial agents
Also, solidified plates which had been flooded with different test organisms were allowed to dry at 37°C for 30
mins and conventional antibiotics (gentamicin, streptomycin, kanamycin and amikacin) disks were placed on them, by using the Kirby-Bauer standardized single disc method. The plates were incubated at 37°C for 24 hrs. The diameters of the zones of inhibition were measured using a caliper and also recorded (Bauer et al. 1966).

Results and Discussion
Preliminary screening employing the well diffusion assay was utilized to compare the antimicrobial activity of Iraqi Citrus juice diluted with distilled water. The best antibacterial activity was observed in 100%, 75%, 50% and 25% (v/v) concentrations for the three types of Citrus juices only but there were no antimicrobial activities in 100%, 75%, 50% and 25% concentrations for the one type of Citrus juices that C. tangerina (mandarin orange) on same bacteria. The large zone of inhibition of C. limon (lemon) 30, 28, 25 and 21 mm by 100%, 75%, 50% and 25% (v/v) concentrations respectively. The zone of inhibition C. aurantium (bitter orange) 27, 23, 20, 18 mm by 100%, 75%, 50% and 25% (v/v) concentrations respectively. But inhibition effect of C. sinensis (orange) 20, 17, 16, 14 mm by 100%, 75%, 50% and 25% (v/v) concentrations respectively as shown in Table (1) and figure (1).

Table 1: Antimicrobial activity of Iraqi Citrus juices diluted with distilled water on Staphylococcus aureus

<table>
<thead>
<tr>
<th>Citrus juices</th>
<th>Crude juices Concentration 100%</th>
<th>Concentration 75%</th>
<th>Concentration 50%</th>
<th>Concentration 25%</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. limon (lemon)</td>
<td>30</td>
<td>28</td>
<td>25</td>
<td>21</td>
</tr>
<tr>
<td>C. aurantium (bitter orange)</td>
<td>27</td>
<td>23</td>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td>C. sinensis (orange)</td>
<td>20</td>
<td>17</td>
<td>16</td>
<td>14</td>
</tr>
<tr>
<td>C. tangerina (mandarin orange)</td>
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</tbody>
</table>

In this study, the Citrus juices of lemon, (orange) and bitter orange (C. limon, C. sinensis and C. aurantium) showed good antibacterial activities against Staphylococcus aureus. The sensitivity pattern of the organism to these extracts was comparable to the values obtained by (Al-Ani et al. 2010), similar result were obtained by (Lee et al. 1987). One study found that citric and lactic acids and dlimonene were less effective as antimicrobial compounds (Winniczuk et al. 1997). This antibacterial activity including citric acid, limonene, linalool, linalyl
acetate, turpinol and cymen (Lee et al. 1987). Have recently reported the presence of limonoids in C. species, which can be considered responsible for activity against many clinically, isolated bacterial strains. Limonoids obtained from C. limon showed good antibacterial and antifungal activity (Giuseppe et al. 2007; Roy et al. 2006). Some studies have related the stage of growth to the production of phenolic compounds by fruits which showed to be powerful antioxidants and free radical scavengers, those compound being able to induce reactions of electron transfer and do react with vital nitrogen compound in the microbial cell such as nucleic proteins and acids (Tao et al. 2007). The complicated mixtures of those compounds represented the strongest barrier to infection and may contribute to the differences in their bactericidal activity. Flavonoids also have been reported to act as antioxidants in various biological systems. The antioxidant activities of C. flavonoids exhibited a potent antibacterial activity which is probably due to their ability to complex with bacterial cell walls and disrupt microbial membrane (Giuseppe et al. 2007; Guthrie et al. 1998; Hollman et al. 1995; Kawai et al. 1999; Lam et al. 1989). showed that the flavonoids, limonoids, and ascorbic acid are groups of citrus phytochemicals and micronutrients which are responsible for the antiinflammatory and antitumor activities. Recently these flavonoids have been implicated in cytoprotective activity (Johann et al. 2007). The phytochemical analysis showed the presence of phenols, flavonoids, glycosides, steroid, saponin, and reducing sugar in citrus fruit juices (Bansode et al. 2012). One study found the use of different concentrations of Citrus juice extracts had an effective antibacterial activity against Staphylococcus aureus, Proteus vulgaris and Pseudomonas aeruginosa (Al-Ani et al. 2010). They can be used in the treatment of infectious diseases caused by resistant microbes, as good antibacterial effect, sterility, and no or minimal side effects of fruit juice in comparison to many antibacterial drugs (Ali, Z.M. 2010).

In this study, all Staphylococcus aureus isolates were identification from previous study which gave positive phenotypic tests for methicillin resistance MRSA. This antimicrobial susceptibility test performed for all isolates against four different antibiotic (gentamicin, streptomycin, kanamycin and amikacin) according to the recommendation of (CLSI 2009). In general, all the 30 MRSA isolates (figure 2), showed a high level of resistance to aminoglycosides group, with rate reached to 5 isolates (16.6%) for streptomycin, 11 isolates (36%) for amikacin, 15 isolates (50%) for gentamicin, and 19 isolates (63.3%) for kanamycin. All the isolates showed sensitive for streptomycin antibiotic, the isolates showed these resistance to all antibiotics amikacin, gentamicin, kanamycin.

**Figure (2): The Susceptibility of MRSA isolates to antibiotics.**

In general this result is less than results of (Kim et al. 2004) who reported that more than 90% of the MRSA isolates were multidrug resistant to kanamycin, (98%) tobramycin, (95%) gentamicin and (90%) amikacin. This low susceptibility is attributable to a concerted action of multidrug efflux pumps with chromosomally-encoded antibiotic resistance genes (e.g. mexAB, mexXY etc) (Poole, K. 2004).

**REFERENCES**

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