

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 concentration of 100%,75%,50% and 25%, which 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) concentrationsfor the three types of Citrus juices only but there were no antimicrobial activities in 100%,75%,50%and 25% concentrating for the one types 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,11isolates(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 withas many as 50,000 people dying daily as aconsequence 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 ofsuppurative (pus-forming) infections andtoxinoses and more serious infections such aspneumonia, mastitis, meningitis, and urinary tract infections. *S. aureus* is a majorcause of hospital acquired (nosocomial) infection of surgical wounds and infectionsassociated with indwelling medical devices Plants are rich in a wide variety of secondarymetabolites such as tannins, terpenoids,alkanoids and flavonoids which have beenfound invitro to have antimicrobial properties.The extract of different parts of followingplants have been used in the present projectwork to check their antibacterial effectivenessagainst 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 concentra-tion 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* (mandarinorange) were collected from local market Baghdad - Iraqi .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).

AntimicrobialAssay

The agar diffusion technique (well diffusion method) wasused to screen the antibacterialactivity.50 ulof the different concentrations (25%, 50%, 75% and 100% w/v) of the Fresh fruitscrudeextract samples were separately placed in thedifferent punched wells with 1 ml sterile syringe. The plates were allowed to stay for 15 mins for pre-diffusion to takeplace followed by an overnight incubation that lasted for 24 hrs at 37 C.In-vitro the anti-bacterial 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 infectionidentification 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) concentrationsfor the three types of Citrus juices only but there were no antimicrobial activities in 100% ,75%,50% and 25% concentrating for theone types 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 mmby 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) 20,17,16,14mm by100%,75%,50% and 25% (v/v) concentrations respectivelyas shown in Table (1) and figure (1).

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

Citrus juices	Crude juices Concentration 100%	Concentration 75%	Concentration 50%	Concentration 25%
	Zone of inhibition (mm)			
***C. limon* (lemon)**	30	28	25	21
***C. aurantium* (bitter orange)**	27	23	20	18
***C.sinensis* (orange)**	20	17	16	14
***C.tangerina* (mandarin orange)**	-	-	-	-

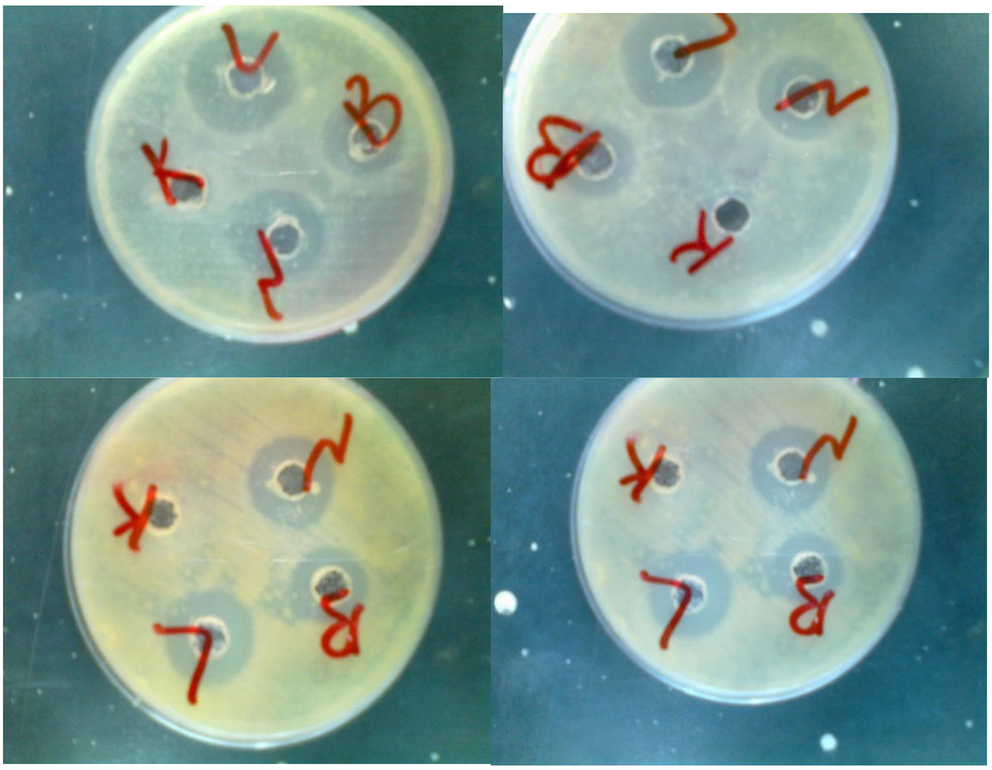
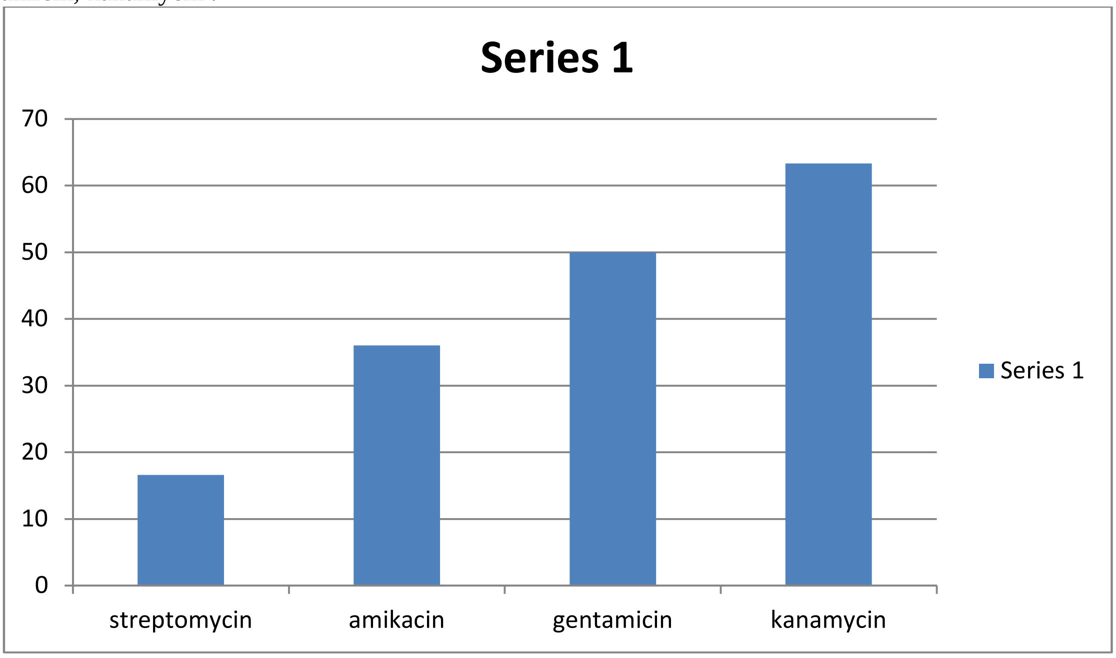


figure (1):The zone of inhibition of concentrations Citrus juice on *Staphylococcus aureus*.
L= lemon,N= bitter orange,B= orange,K= mandarin orange

In this study, the Citrus juices of lemon, (orange) and bitter orange (*C. limon ,C.sinensisand C. auran-tium*) 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,l inalyl 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 toinfection and may contribute to the differences in their bactericidalactivity. 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;Kawaii *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 Is 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 methecillin 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, 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 antibiotcs.

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 effluxpumps with chromosomally-encoded antibiotic resistance genes (*e.g. mexAB*, *mexXYetc*)(Poole,K.2004).

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