Original Article

IN VITRO ACTIVITY OF CERTAIN DRUGS IN COMBINATION WITH PLANT EXTRACTS AGAINST *STAPHYLOCOCCUS AUREUS* INFECTIONS

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ABSTRACT

Objectives: This study has been done to evaluate the interaction between ethanolic extracts of *Rhus coriaria* (seed), *Psidium guajava* (Leaf), *Lawsonia inermis* (Leaf) and *Sacropoterium spinosum* (seed) and antimicrobial drugs including oxytetracycline HCl, enrofloxacin, gentamicin sulphate and sulfadimethoxine against four clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA).

Methodology: Evaluation of the interaction between ethanolic extracts and different antimicrobial agents has been done using well-diffusion method.

Results: It showed that ethanolic extracts increase the inhibition zones of oxytetracycline HCl, gentamicin sulphate, and sulfadimethoxine, while combinations between these plant extracts and enrofloxacin decrease inhibition zone.

Conclusion: This study probably suggests the possibility of concurrent use of these antimicrobial drugs and plant extracts in combination in treating infections caused by *S. aureus* strains or at least the concomitant administration may not impair the antimicrobial activity of these antibiotics.

KEYWORDS: Ethanolic plant extracts, Antibiotics, Drugs, Synergism, Antagonism, *Staphylococcus aureus*, MRSA.

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INTRODUCTION

Infectious diseases still represent an important cause of morbidity and mortality among humans, especially in developing countries. Even though pharmaceutical companies have produced a number of new antibacterial drugs in the last years, resistance to these drugs by bacteria has increased and has now become a global concern. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs used as therapeutic agents.[1] *Staphylococcus aureus* is recognized as one of the major causes of infections in humans occurring in both the community and the hospital. Methicillin-resistant and multidrug resistant staphylococci have become a major nosocomial

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pathogen.[2] Therefore the importance of identifying new effective antimicrobial agents cannot be overemphasized. Among the potential sources of new agents, medicinal plants have long been investigated. In rational drug therapy, the concurrent administration of two or more drugs is often essential and sometimes mandatory in order to achieve the desired therapeutic goal or to treat co-existing diseases. However, the drug interaction may have different effects on the host as well as the infecting microorganism. The potential benefits of using combined antimicrobial therapy can be treatment of mixed infections, therapy of severe infections in which a specific causative organism is known, enhancement of antibacterial activity, reducing the time needed for long-term antimicrobial therapy and prevention of the emergence of resistant microorganisms.[3,4]

Drug synergism between known antimicrobial agents and bioactive plant extracts is a novel concept and has been recently reported.[1,5-14] Three of the plants used in this study are medicinal plants (*Rhus coriaria*, *Psidium guajava*, and *Lawsonia inermis*) and the fourth (*Sacropoterium spinosum*) which is considered as animal food. In this *in vitro* study, we attempted to evaluate the possible antimicrobial interaction between ethanolic extracts of these plants and certain known antimicrobial drugs (oxytetracycline HCl, enrofloxacin, gentamicin sulphate and sulfadimethoxine) against four strains of methicillin-resistant *S. aureus* (MRSA) using the well diffusion method.

METHODOLOGY

Plant material and extract preparation: The plant materials used in this study consisted of *R. coriaria* (seed), *P. guajava* (Leaf), *L. inermis* (Leaf) and S. *spinosum* (seed), which are growing in Palestine. Ethanolic extracts were prepared as described previously.[15-17] Plant materials were dried in an open air protected from direct exposure to sunlight and 30g of dried plant materials were separately powdered and extracted with 80% ethanol; the extracts were filtered through Whatman No. two filter paper under suction. Extracts were concentrated to dryness in vacuum. Then, 100mg of the dry residue was dissolved in one ml of sterile distilled water.

Bacterial strains: Four methicillin-resistant *S. aureus* (MRSA) strains were isolated from clinical specimens (urine and semen). These isolates were identified as *S. aureus* according to colonial and microscopic morphology, growth on Mannitol Salt agar, 5% blood sheep agar, positive catalase, and coagulase production. Methicillin resistance was carried out in the microbiology laboratories of An-Najah National University, Palestine, using the disk diffusion method.[18] Methicillin (5µg) disks (Oxoid) were used, and inhibition zones were determined in accordance with procedures of the National Committee for Clinical Laboratory Standard (NCCLS).[19] A reference strain [*Bacillus subtilis* ATCC6633] was also tested.

Antimicrobial drugs: Four drugs were evaluated for synergism assays. These included oxytetracycline HCl (10%), enrofloxacin (10%), gentamicin sulphate (50%) and sulfadimethoxine as sodium (40%). All these antimicrobial agents were produced by Jerusalem Pharmaceutical CO. Balsam Branch, and were diluted to a final concentration 100µg/ml.

Antimicrobial tests: Antibacterial activity was measured using a well diffusion method according to the National Committee for Clinical Laboratory Standard.[19] Briefly, Petri plates containing 20ml of Mueller Hinton agar medium were inoculated with a 24 hour culture of the bacterial strains. Wells (6mm diameter) were punched in the agar and filled with 30ìl of plant extracts or antibiotics and in case of combination 30µl of each has been added into well. Triplicates of each plate have been done. The plates were incubated at 37°C for 24 hour. The antibacterial activity was assessed by measuring the diameter of the area in which bacterial growth was inhibited around the well. The average of three replicates for each extract, antibiotic and combination were calculated.

RESULTS

Antimicrobial mechanisms of the drugs used here were variable and the nucleic acid synthesis inhibitor did not show synergistic effect. However, competitive inhibitor (sulfadimethoxine as sodium) and protein synthesis inhibitors (oxytetracycline HCl, gentamicin sulphate) showed strongest synergistic interaction. The data pertaining to the antimicrobial potential of the plant extracts and antibacterial drug combination against *S. aureus* using well diffusion method are presented in Table-I.

DISCUSSION

Antimicrobial drugs effective for treatment of patients infected with MRSA are limited. Thus, it is important and valuable to find compounds that potentiate antimicrobial activity of antibiotics on MRSA. Our results in this report indicated that all the extracts of the plants studied showed an increase in the antimicrobial activity of certain drugs that can be used against *S. aureus*, and synergistic interaction of plant extracts is possible with antimicrobial drugs. These results are consistent with previous reports which showed that some plant extracts can increase the activity of antimicrobial drugs *in vitro* against bacteria.[1,8-12]

Competitive inhibitor (sulfa drugs as sulfadimethoxine) and protein synthesis inhibitors (aminoglycosides as gentamicin sulphate and tetracyclines as oxytetracycline HCl) showed high synergism rate with plant extracts, while nucleic acid synthesis inhibitor (quinolones as enrofloxacin) did not show this effect. These results are in agreement with that reported previously, which showed that protein synthesis inhibitors showed strongest synergistic interaction and nucleic acid synthesis inhibitor showed weak synergism with plant extracts.[8,9] This high synergism rate shows the need for more studies concerning the molecular basis of these interactions to understand the synergistic mechanism which is fundamental to development of pharmacological agents to treat bacterial infections using medicinal plants.

Here we recommended the evaluation of the exact drug-plant ratio at which the interaction in maximal between the plant extract and antimicrobial drug. A wider study with increase in the number of drugs in each group, increased number of clinical isolates, and the identification of the effective compounds in the crude extract are also necessary. It may be deduced that numerous compounds within the crude extract may have interfered with the actions of one another. Once they were separated by various methods, the inhibiting effect of one on the other can be reduced significantly.

CONCLUSION

The results presented in this report were encouraging, although clinical controlled studies are needed to define the real efficacy and possible toxic effects *in vivo*. This study probably suggests the possibility of concurrent use

Table-I: Synergistic effect between antimicrobial drugs and plant extracts against 4 *Staphylococcus aureus* strains by the well-diffusion method.

Drug target	*Drug*	*R. coriaria*	*P. guajava*	*L. inermis*	*S. spinosum*	*Synergism rate (extract /drug)*	*Mean*
Protein synthesis	Oxytetracyclin HCl	S	S	S	S	4	3.5
	Grntamicin	S	A	S	S	3	
Nucleic acid synthesis	Enrofloxacin	A	A	A	A	0	0
Competitive inhibition	Sulphadimethoxin	S	S	S	S	4	4
Total	4	3	2	3	3		

(S): Synergism; (A): Antagonism

of these antimicrobial drugs and extracts in combination in treating infections caused by *S. aureus* strains or at least the concomitant administration of these plants and antimicrobial drugs may not impair the antimicrobial activity of these antibiotics.

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