African Journal of Basic & Applied Sciences 2 (1-2): 25-29, 2010
ISSN 2079-2034

Synergistic Effect of S*alvadora persica* Extracts, Tetracycline and Penicillin Against S*taphylococcus aureus*

[1]*Zafar Ahmed,* [1]*Shaukat Saeed Khan,* [1]*Mahnaaz Khan,*
[1]*Arshiya Tanveer and* [2]*Zahoor Ahmad Lone*

[1]Department of Microbiology, Saifia Science College, Bhopal - 462001 India
[2]Department of Botany, Govt. Science and Commerce Benazeer College,
Near M.V.M College, Bhopal - 462008 India

Abstract: In the present investigation inhibitory effect of two antibiotics viz., Penicillin and Tetracycline against *Staphylococcus aureus* was observed separately and in combination (synergistic). It was found that the zone of inhibition was 23mm for tetracycline and 18 mm for penicillin. However, their synergistic effect was much more effective and caused an inhibition that measured 27 mm in diameter. Apart from this these antibiotics were also applied in combination with various extracts (ethanolic) of *Salvadora persica*, a medicinal plant of repute. The highest inhibition was noticed (31.5 mm) when S.aureus was exposed to tetracycline + Salvadora stem extract. It was followed by tetracycline + leaf extract combination of *Salvadora persica* with a zone of inhibition of 30.0 mm. The combination of stem and leaf extract with penicillin could not produce the same inhibitory effect as that of tetracycline and Salvadora stem and leaf extracts. However, penicillin and stem extract of Salvadora combination resulted in a ZOI which was 21.0 mm, which surpassed 18 mm ZOI recorded for both penicillin and stem extract of *S.persica* applied separately.

Ket words: Missing

INTRODUCTION

Different antibiotics exercise their inhibitory activity on various pathogenic organisms either by killing them outright (microbicidal action) or by arresting the growth of the causal organism (microbistatic action). The impact of antibiotic on a pathogen is specific which differs from pathogen to pathogen and vice-versa. Some studies in recent years have suggested the use of combination of antibiotics, the synergistic effect of which often surpasses their individual inhibitory activity. It was therefore thought imperative to use an antibiotic in combination with stem and leaf extracts of *Salvadora persica*, a common toothbrush plant with a number of therapeutic attributes to it. The synergistic effect from the combination of antibiotics with plant extracts against resistant bacteria leads to new ways for the treatment of infectious diseases. The increasing and indiscriminate use of antibiotics has led to the development of bacterial resistance to antibiotics. The use of synergistic combinations in antimicrobial chemotherapy is often used commercially for the treatment of various infections [1]. The *in vitro* antibacterial activity of ethanolic leaf extract of *Vangueria spinosa* alone and in combination with antibiotics (doxycycline and ofloxacin) by means of fractional inhibitory concentration indices (FICI) as well as by the use of time-kill assays against one Gram-positive bacterium (*Staphylococcus aureus*) and three Gram-negative bacteria (*Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) was studied by him. MIC/MBC values for ethanolic leaf extract of *V. spinosa* against all the tested bacteria ranged between 25.5 - 52.6/22.4 - 60.5 µg/ml, for doxycycline 4.0/4.0 - 4.5 µg/ml and for ofloxacin 0.625 - 2.5/1. 25 - 5.0 µg/ml respectively. The average log reduction in viable cell count in time-kill assay ranged between 2.4 log10 - 4.5 log10 cfu/ml after 1 h of interaction and between 3.9 log10 -5.0 log10 cfu/ml after 3 h interaction in 1 × MIC to 4 × MIC. When leaf extract and antibiotics were combined, the average log reduction in viable cell count for doxycycline from 1.5 log10 - 5.18 log10 cfu/ml and for ofloxacin 3.06 log10- 5.39 log10 cfu/ml. Synergistic

Corresponding Author: Zahoor Ahmad Lone, Department of Botany, Govt. Science and Commerce Benazeer College, Near M.V.M College, Bhopal - 462008 India

actions were observed in all the cases except against *P. aeruginosa* which showed an additive effect for ofloxacin and plant extract combination.

Searches for substances with antimicrobial activity are frequent and medicinal plants have been considered interesting by some researchers since they are frequently used in popular medicine as remedies for many infectious diseases. Synergism between 13 antimicrobial drugs and 8 plant extracts - "guaco" (*Mikania glomerata*), guava (*Psidium guajava*), clove (*Syzygium aromaticum*), garlic (*Allium sativum*), lemongrass (*Cymbopogon citratus*), ginger (*Zingiber officinale*), "carqueja" (*Baccharis trimera*) and mint (*Mentha piperata*) against *Staphylococcus aureus* strains was studied by Betoni *et al.* [2]. The results showed that clove, guava and lemongrass presented the highest synergism rate with antimicrobial drugs, while ginger and garlic showed limited synergistic activity.

To control clinical bacterial isolates implicated in urinary tract infections, Ghaly *et al.* [3] carried out their study to investigate the synergistic effect of antibiotics and plant extract. Antibiotic sensitivity test for Gram-positive and negative bacteria showed that the antibiotic ofloxacin is more effective against clinical bacterial isolates (58%) followed by amikacin (54%), chloramphenicol (52%), norfloxacin (51%) and azithromycin (48%). The effect of medicinal plant extracts against highly resistant bacterial isolates showed that, the clove, rosemary, peppermint, hibisicus, thyme and cinnamon showed strong inhibitory action against tested isolates. The combination between ofloxacin and amikacin with clove plant extract clearly showed synergistic effect against tested clinical bacterial isolates.

In the same year Kumar *et al.* [4] investigated synergistic activity of methanolic extract of *Thespesia populnea* (Malvaceae) flowers with oxytetracycline. The MIC values were found to be less with oxytetracycline alone and it was found to be still lesser with the methanolic extract of *T. populnea*. However, the MIC was found to be the least with combination of oxytetracycline and methanolic extract of *T. populnea*. Moreover, the therapeutic efficacy was found to be higher even in low concentration.

The synergistic antimicrobial activity of tea and various antibiotics against enteropathogens was studied by Tiwari *et al.* [5]. Tea extract showed synergistic activity with chloramphenicol and other antibiotics like gentamycin, methicillin and nalidixic acid against test strains. Esimone *et al.* [6] also evaluated the interaction between tea extracts and penicillin G against *Staphylococcus aureus.* Few studies have found that the efficacy of antimicrobial agents can be improved by combining them with crude plant extracts against different pathogens including *S. aureus*, *P. aeruginosa*, *E. coli*, Extended Spectrum $-lactamases-producing multidrug-resistant *E. coli* and vancomycin-resistant enterococci (*Enterococus fecalis*) [7]. This study has been done to evaluate the interaction between water extracts of *Psidium guajava*, *Rosmarinus officinalis*, *Salvia fruticosa*, *Majorana syriaca*, *Ocimum basilicum, Syzygium aromaticum*, *Laurus nobilis* and *Rosa damascena* alone and then synergy testing of these extracts with known antimicrobial agents of different mechanisms (oxytetracycline HCl and gentamicin sulfate; penicillin G and cephalexin; Sulfadimethoxine and enrofloxacin) using both well-diffusion and microdilution method. The results of the conducted experiments using well-diffusion method demonstrated that these plants showed *in vitro* interactions between antimicrobial agents and plant extracts and were additive against the five strains of *S. aureus*, while using micro dilution method showed synergistic effects between combination of antibiotics and plant extracts with significant reduction in the MICs of the test antibiotics against these strains of *S. aureus*. Also the results showed that synergism effect between antimicrobial agent and plant extract occurred in both sensitive and resistant strains but the magnitude of minimum fold inhibition in resistant strains especially MRSA strain was higher than the sensitive strains. Therefore, the present study was undertaken to investigate synergistic activity of *Salvadora persica* with tetracycline and penicillin against *Staphylococcus aureus*. *Salvadora persica* contains a number of medicinally beneficial properties including abrasives, antiseptics, astringent, detergents, enzyme inhibitors and fluoride [8]. Penicillin is usually effective against G +ve bacteria and tetracycline is a broad spectrum antibiotic.

MATERIALS AND METHODS

Preparation of Plant Extracts: Fresh and healthy leaves and stems of *Salvadora persica* were collected, sun-dried and powdered in a grinder and later subjected to extraction in a Soxhlet. The solvent used was ethanol. The extracts were then subjected to solvent recovery with the help of Soxhlet and reflux condenser. After a thick extract was left in the flask, it was transferred to small specimen tubes [9].

Antibiotics: Solutions of known concentrations (50000 ppm and 40000 ppm) of tetracycline and penicillin respectively were prepared.

The above known volumes of antibiotic solutions and plant extracts (leaf and stem extract of *S. persica*) were prepared alone and in combination. Filter paper discs were impregnated in each solution for about 6 h and then air dried. Pure culture of *Staphylococcus aureus* was sub-cultured on nutrient agar media in pre-sterilized Petri plates. The filter paper discs were then transferred to freshly inoculated Petri plates carefully. The plates were then incubated at 35°±2°C for 24 h to test efficacy of antibiotics and *Salvadora persica* extracts alone and in combination against *Staphylococcus aureus*. After 24 h of incubation the plates were then examined for zone of inhibition, if any [10].

RESULT AND DISCUSSION

Many plants have been used for the purpose of food, shelter, clothing, medicine, cosmetics, manufacturing useful products like papers for printing media, environmental improvement, etc. This has helped in the betterment of our life quality. Synergistic effects resulting from the combination of antibiotics with various plant extracts has been studied and experimented by a number of scientists. Ibezim *et al.* [11] studied the interaction between some flouroquinolones and extracts of *Kola nitida* seed. Esimone *et al.* [12] evaluated the *in vitro* interaction of some penicillins (amoxicillin, ampicillin and benzylpenicillin) and caffeine against *Staphylococcus aureus.* He found that at 5 and 10 mg/ml, caffeine decreased the MIC of amoxicillin by 22 and 25 times respectively, while that of ampicillin was decreased by 6 and 8 times. The MIC of benzylpenicillin against *Staphylococcus aureus* was, however, increased by 59 and 40 times at caffeine concentrations of 5 and 10 mg/ml respectively. The inhibition zone diameter increment above 19 % (index of synergism in OLISD method) was recorded only for amoxicillin at amoxicillin concentrations of 7.81, 15.3, 31.25 and 62.5 mg/ml.

Cai *et al*. [13] determined the antibacterial activity of Allicin with β -Lactams against *Staphylococcus* spp. and *Pseudomonas aeruginosa.* Allicin is one of the most effective compounds isolated from garlic showing antibacterial activity. The results showed that allicin alone did not have good antibacterial activity (MIC90 > 512m g/ml) but it facilitated antibacterial activity of all three β -lactams (cefazolin/oxacillin and cefoperazone) tested at subinhibitory concentrations.

Table 1: Antibacterial efficacy of antibiotics and plant extracts alone and in combinations against *Staphylococcus aureus*

S.No.	Antibiotic/ Plant Extract	Zone of Inhibition (mm)
1	Tetracycline	23.0
2	Stem extract of Salvadora	18.0
3	Penicillin	18.0
4	Leaf extract of Salvadora	10.5
5	Penicillin + Tetracycline	27.0
6	Tetracycline + Stem extract of Salvadora	31.5
7	Tetracycline + Leaf extract of Salvadora	30.0
8	Penicillin + Stem extract of Salvadora	21.0
9	Penicillin + Leaf extract of Salvadora	16.0

In the present investigation the inhibitory effect of tetracycline, a broad spectrum antibiotic; penicillin, an antibiotic that acts against G +ve bacteria and a medicinal plant, *Salvadora persica,* was investigated against *Staphylococcus aureus*. In the experiment Kirby Bauer disc diffusion test was applied. After 24 h of incubation at a temperature of 35°± 2°C it was found that all the antibiotics and the plant extracts as well as their combination proved inhibitory against *S.aureus* but to varying degrees the zone of inhibition ranged from 10.5 mm to 31.5 mm respectively under the influence of leaf extract of *Salvadora persica* and the combination of tetracycline with stem extract of *S.persica*. It was closely followed by tetracycline + leaf extract of this plant with the zone of inhibition of 30.0 mm. The third highest inhibition was recorded for the combination of penicillin and tetracycline, the synergistic effect of these antibiotics produced a ZOI of 27.0 mm.

Sometimes the use of single antibiotic does not produce the desired or the effective inhibitory effects and to overcome this, combination of drugs often exercise their synergistic effect which surpasses their individual performance. A perusal of Table 1 clearly indicates that penicillin alone could cause the inhibition which was 18 mm, whereas tetracycline alone resulted in the formation of 23 mm inhibition zone. But when combined, these two antibiotics surpassed both these values of inhibitory zone and synergistically resulted in the formation of 27 mm inhibition zone. Similar was the case with the combination of tetracycline with stem (31.5 mm) and leaf extracts (30.0 mm) which exhibited synergistic effect. Penicillin alone produced 18 mm zone of inhibition but in combination with stem extract of *S.persica* the ZOI was 21 mm. However, the combination of the penicillin and leaf extract of *S.persica* resulted in the formation of 16 mm ZOI which is more than the leaf extract used alone (10.5 mm) but less than when penicillin was used independently.

The synergistic effect may be due to certain complex formation which becomes more effective in the inhibition of a particular species of microorganisms either by inhibiting the cell wall synthesis or by causing its lyses or death. Thus it is concluded that to control a particular disease *in vitro* experiments should be carried out with various antibiotics and their combination as well as antibiotics and plant extracts. So that a right combination may be administered to the patient for early and safe recovery from a specific ailment. All the combinations do not produce synergistic effect and therefore a number of combinations are required to be tested. Example - the penicillin and leaf extract of *S.persica* exhibited lesser zone of inhibition and therefore less degree of affectivity than the effect of penicillin used alone (Table 1).

The effect of combinations of the crude methanolic extract of the leaves of *Helichrysum pedunculatum* and eight first-line antibiotics were investigated by Aiyegoro *et al.* [14] by time kill assays against a panel of bacterial strains that have been implicated in wound infections. The plant extract showed appreciable antibacterial activities against the test bacteria with zones of inhibition ranging between 18 and 27 mm and minimum inhibitory concentrations (MICs) varying between 0.1 and 5.0 mg/ml. The MICs of the test antibiotics ranged between 0.001 and 0.412 mg/ml and combination of the plant extract and the antibiotics resulted in reduction of bacterial counts by between 0 and 6.63 Log_{10} cfu/ml. At V_2 MIC, 56.81% synergy; 43.19% indifference and no antagonism were observed and at MIC levels, 55.68% synergy; 44.32% indifference and no antagonism were observed when the extracts were combined with eight different antibiotics.

REFERENCES

1. Chatterjee, Saroj Kumar, Indranil Bhattacharjee and Gautam Chandra, 2009. In vitro synergistic effect of doxycycline and ofloxacin in combination with ethanolic leaf extract of *Vangueria spinosa* against four pathogenic bacteria. Indian J. Med. Res., 130: 475-478.
2. Betoni, Joyce Elaine Cristina, Rebeca Passarelli Mantovani, Lidiane Nunes Barbosa, Luiz Claudio Di Stasi and Ary Fernandes Junior, 2006. Synergism between plant extract and antimicrobial drugs used on *Staphylococcus aureus* diseases. Mem Inst Oswaldo Croz, Rio de Janeiro, 101(4): 387-390.
3. Ghaly, M.F., M.A. Shalaby, S.M.S. Shash, M.N. Snehata and A.A. Ayad, 2009. Synergistic effect of antibiotics and plant extract to control clinical bacterial isolates implicated in urinary tract infections. J. Applied Sci. Res., 5(10): 1298-1306.
4. Kumar, Arthanari Saravana, K. Venkateshwaran, J. Vanitha, V.S. Saravanan, M. Ganesh, M. Vasudevan and T. Sivakumar, 2009. Synergistic activity of methanolic extract of *Thespesia populnea* (Malvaceae) flowers with oxytetracycline. Bangladesh J. Pharmacol., 4: 13-16.
5. Tiwari, R.P., S.K. Bharti, H.D. Kaur, R.P. Dikshit and G.S. Hoondal, 2005. Synergistic antimicrobial activity of tea and antibiotics. Indian J. Med. Res., 122: 80-84.
6. Esimone, C.O., I.R. Iroha, F.C. Ibezim, C.O. Okeh and E.M. Okpana, 2006. *In vitro* evaluation of the interaction between tea extracts and penicillin G against *Staphylococcus aureus.* Afr. J. Biotechnol., 5: 1082-1086.
7. Adwan, Ghaleb and Mohammad Mhanna, 2008. Synergistic effects of plant extracts and antibiotics on *Staphylococcus aureus* strains isolated from clinical specimens. Middle-East J. Sci. Res., 3(3): 134-139.
8. Almas, Khalid, 2002. The effect of *Salvadora persica* extract (Miswak) and chlorahexidine gluconate on Human Dentin: A SEM Study. J. Contemporary Dental Practice, 3(3): 27-35.
9. Khan, Shaukat Saeed, A.H. Ansari, Dolly Malhotra, Mahnaaz Khan and Arshiya Tanveer, 2008. *In vitro* antibacterial activity of *Nigella sativa* L. against *Staphylococcus aureus* and *Pseodomonas aeruginosa*. Ultra Chemistry, 4(2): 307-310.
10. Bauer, A.W., W.M. Kirby, J.C. Sherris and M. Truck, 1966. Antibiotic susceptibility testing by a standardized single disk method. Am J. Clin. Pathol., 45: 493-496.
11. Ibezim, E.C., C.O. Esimone, P.O. Nnamani, I.V. Onyishi, S.A. Brown and C.E. Obodo, 2006. *In vitro* study of the interaction between some flouroquinolones and extracts of *Kola nitida* seed. Afr. J. Biotechnol., 5(19): 1781-1784.
12. Esimone, C.O., F.B.C. Okoye, C.S. Nworu and C.O. Agubata, 2008. *In vitro* interaction between caffeine and some penicillin antibiotics against *Staphylococcus aureus.* Trop J. Parm. Res., 7(2): 969-974.

13. Cai Yun, Rui Wang, Fei Pei and Bei-Bei Liang, 2007, Antibacterial activity of Allicin alone and in combination with β - lactams against *Staphylococcus* spp. and Pseudomonas aeruginosa, J. Antibiot., 60(5): 335-338.

14. Aiyegoro layinka, A., J. Afolayan Anthony and I. Okoh Anthony, 2009. Synergistic interaction of *Helichrysum pedunculatum* leaf extracts with antibiotics against wound infection associated bacteria. Biol. Res., 42(3): 327-338.