



# International Journal of Pharmacology

ISSN 1811-7775

**science**  
alert

**ansinet**  
Asian Network for Scientific Information

## Effects of *Scrophularia striata* Ethanolic Leaves Extracts on *Staphylococcus aureus*

<sup>1</sup>A.M. Bahrami and <sup>2</sup>Valadi Ali

<sup>1</sup>School of Veterinary Sciences, Ilam University, Ilam, Iran

<sup>2</sup>School of Microbiology, Azad University, Ilam, Iran

**Abstract:** The *in vitro* antibacterial activity of ethanolic leaf extract of *Scrophularia striata* 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 Gram-positive bacterium (*Staphylococcus aureus*). Antibacterial activity was assayed by using the microdilution method. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined for the ethanolic leaf extract of *Scrophularia striata* alone and also in combination with antibiotics using the fractional inhibitory concentration (FIC) and time-kil assay method. Synergism was also tested using checker board dilution method. MIC/MBC values for ethanolic leaf extract of *Scrophularia striata* against all the tested bacteria ranged between 25.5-52.6/22.4-60.5  $\mu\text{g mL}^{-1}$ , for doxycycline 4.0/4.0-4.5  $\mu\text{g mL}^{-1}$  and for ofloxacin 0.625-2.5/1.25-5.0  $\mu\text{g mL}^{-1}$ , respectively. The average log reduction in viable cell count in time-kill assay ranged between 2.4-4.5  $\log_{10}$  cfu  $\text{mL}^{-1}$  after 1 h of interaction and between 3.9-5.0  $\log_{10}$  cfu  $\text{mL}^{-1}$  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 1.5-5.18  $\log_{10}$  cfu  $\text{mL}^{-1}$  and for ofloxacin 3.06-5.39  $\log_{10}$  cfu  $\text{mL}^{-1}$ .

**Key words:** *Scrophularia striata*, *Staphylococcus aureus*, ethanolic extract

### INTRODUCTION

Nature has served as a rich repository of medicinal plants for thousands of years and an impressive number of modern drugs have been isolated from natural sources, notably of plant origin (Cowan, 1999).

In recent years, prophylactic usage of natural products and tendency to resort to alternative medicine has increased rapidly (Ozaslan *et al.*, 2009) known as ahvaz, kerman and Ilam in Iran, issued as antipyretic, a remedy for kidney diseases, car-diotonic (Tadeg *et al.*, 2005) hypoglycemic, antidiabetic and antiinflammatory activity, respectively. The use as an antiseptic (Viegi *et al.*, 2003) anti-inflammatory and cicatrising is some regions of central Italy (Nebel *et al.*, 2006) and diuretic in typhoid fever, galact-orrhea, leuckorrhea, throat diseases, inflammation of mouth, lungs, large intestine, bladder and heart and as a remedy for tumors, abscesses, cancer of the lung, goiter and achingbones (Perry and Metzger, 1980). It has also been reported to exhibit hypoglycemic activity in normal fasting and alloxanized rats (Shabana *et al.*, 1990). A literature survey revealed that no phytochemical and pharmacological work has been done so far on the plant.

In this study the antibacterial activity of *Scrophularia striata* ethanolic leaf extract was assessed

*in vitro* when used alone and in combination with doxycycline and ofloxacin against *staphylococcus aureus*.

### MATERIALS AND METHODS

This project was conducted in Medical Research Laboratory, University of Ilam, during March 2009-August 2009.

**Plant material:** The leaves of *S. striata* were collected at Ilam (a town in Ilam Province, the Western of Iran). The leaves were initially rinsed with distilled water and dried on paper towels in laboratory at (40±1)°C for 24 h. Ilam University properly identified the plant and voucher samples were preserved for reference in the herbarium of Department of Pharmacognosy, School of Pharmacy, Tehran (293-0107-18).

**The preparation of extract:** After drying, the leaves were ground in a grinding machine. Exposure to sunlight was avoided to prevent the loss of active components; 200 mL of ethanol was mixed with 50 g of dried powdered leaf and the mixtures left for 24 h in tightly sealed vessels at room temperature, protected from sunlight and stirred thoroughly several times with sterile glass rod. The mixtures were filtered by filter paper and the residues

adjusted to the required concentration (50 mL of ethanol for the residue of 50 of powdered leaves) with the extraction fluid for further extraction. This was repeated thrice and a clear colorless supernatant extraction liquid was finally obtained.

**Antibiotics and bacterial strains:** Doxycycline was purchased from Amin Limited, Tehran, Iran and ofloxacin from Aventis Pharma Limited, Tehran, Iran. Four bacterial strains were used, three of these *Staphylococcus aureus* MTCC 2940, were obtained from The Microbiology Laboratory of Tehran Medical College, Tehran, Center of Iran. The bacteria were grown in nutrient broth Hi-Media, M003 at 37°C and maintained on nutrient agar Hi-Media, M011 slants at 4°C.

Determination of minimum inhibitory and bactericidal concentrations (MIC and MBC). MICs were determined by the broth microdilution method according to the standard methods of National Committee for Clinical Laboratory Standards (1997).

The MIC was the lowest concentration of antibiotic that yielded no visible growth after incubation at 37°C for 24 h (Sung *et al.*, 2006). where as the MBCs were determined as the lowest concentration *S. striata* of ethanolic leaf extract that killed 98.8% of the test bacteria.

**Checker board titration:** The antibacterial effects of combining *S. striata* ethanolic leaf extract with antibiotics (doxycycline and ofloxacin) were assessed using a checker board titration (Jung *et al.*, 2005; Climo *et al.*, 1999). Combinations of doxycycline and ofloxacin with ethanolic leaf extract of *S. striata* were tested at concentrations of 0 to 32 µg mL<sup>-1</sup> for doxycycline and 0 to 10 µg mL<sup>-1</sup> for ofloxacin. The Fractional Inhibitory Concentration Index (FICI) was determined as the sum of the FICs of each drug, which in turn was defined as the MIC of each drug when used in combination, divided by the MIC of the drug when used alone. An FIC index of <0.5 was defined as synergy, >0.5 to 4.0 was defined as additive or indifferent and >4.0 as antagonistic (Climo *et al.*, 1999).

**Time-kill assay:** The Time-kill assay was performed by the broth macro dilution technique. The extract and antibiotics were incorporated into 50 mL of nutrient broth

at MIC, 2×MIC and 4×MIC, respectively. Controls consisting of nutrient broth incorporated with the extract and the respective antibiotic alone at the test concentrations were included in each experiment. The test and control flasks were inoculated with each test organism to a final inoculum density of approximately 10 cfu mL<sup>-1</sup> (Chatterjee *et al.*, 2007) Immediately after inoculation, aliquots (100 µL) of the negative control flasks were taken serially diluted in sterile saline and plated on nutrient agar in order to determine the zero hour counts. The test flasks were incubated at 37°C with shaking at 120 rpm. Bacterial counts were taken at 0, 0.5, 1, 2, 3, 6, 12 and 24 h by plating 0.1 mL aliquots and serially diluting each onto Mueller-Hinton agar in duplicates. After incubation, the numbers of colonies were enumerated and the mean counts (cfu mL<sup>-1</sup>) for each test and controls were determined and expressed as log<sub>10</sub>. The interactions were considered synergistic if there was a decrease of >2 log<sub>10</sub> cfu mL<sup>-1</sup> in colony counts after 24 h by the combination compared to the most active single agent (Pankey *et al.*, 2005). Additivity or indifference was described as a <2 log<sub>10</sub> cfu mL<sup>-1</sup> change in the average viable counts after 24 h for the combination, in comparison with the most active single drug. Antagonism was defined as a >2 log<sub>10</sub> cfu mL<sup>-1</sup> increase in colony counts after 24 h by the combination compared with that by the most active single agent alone (Jeong *et al.*, 2006; Pankey *et al.*, 2005).

## RESULTS

The extract leaves *S. striata* showed antibacterial activity. The MIC and MBC values of the leaf extract ranged from 50.6 and 60.3 µg mL<sup>-1</sup> and were higher than those of antibiotics (Table 1). The ethanolic extract showed strongest activity was seen against Doxycycline and Ofloxacin (MIC = 50.6) and (MBC = 60.3), respectively.

The FICI was determined as the sum of the FICs of each drug, which in turn was defined as the MIC of each drug when used in combination, divided by the MIC of the drug when used alone. An FIC index of <0.5 was defined as synergy, >0.5 to 4.0 was defined as additive or indifferent and >4.0 as antagonistic. The activity of the antibiotics against a Gram-positive (*S. aureus*) bacteria

Table 1: Minimum inhibitory and bactericidal concentrations (MIC and MBC) of the antibiotics and ethanolic leaf extract of *S. striata*

Name of the strains	MIC (µg mL <sup>-1</sup> )			MBC (µg mL <sup>-1</sup> )		
	Doxycycline	Ofloxacin	<i>S. striata</i> ethanol extract	Doxycycline	Ofloxacin	<i>S. striata</i> ethanol extract
<i>S. aureus</i>	4	2.40	50.6	3.9	4.9	60.3
<i>E. coli</i>	4	1.24	32.1	3.9	2.4	39.0

Table 2: Fractional inhibitory concentration (FIC) and FIC index (FICI) values for the combination between the ethanolic leaf extract of *S. striata* and antibiotics

Name of the strains	FIC ( $\mu\text{g mL}^{-1}$ )				FICI		Results	
	Doxycycline	Ofloxacin	<i>S. striata</i> ethanol extract+doxycycline	<i>S. striata</i> ethanol extract+ofloxacin	<i>S. striata</i> ethanol extract+doxycycline	<i>S. striata</i> ethanol extract+ofloxacin	<i>S. striata</i> ethanol extract+doxycycline	<i>S. striata</i> ethanol extract+ofloxacin
<i>S. aureus</i>	0.124	0.24	0.180	0.180	0.314	0.430	Synergistic	Synergistic
<i>E. coli</i>	0.24	0.24	0.154	0.154	0.404	0.404	Synergistic	Synergistic

Table 3: Determination of synergy between plant extract and antibiotics using the time-kill assay

Concentration of extract ( $\text{g mL}^{-1}$ )	Viable cell reduction per exposure time(h) ( $\log_{10}$ )							
	<i>S. aureus</i>				<i>E. coli</i>			
	0.5	1	2	3	0.5	1	2	3
MIC	-1/4	-3/0	-4/1	-4/6	0	-3/0	-4/1	-4/6
2×MIC	-2/3	-3/8	-5/4	-4/7	-1/5	-3/8	-4/5	-5/0
4×MIC	-3/0	-4/4	-4/6	-4/6	-3/0	-4/1	-4/5	-5/0
Negative control	0	+1/5	+2/4	+3/0	+1/5	+3/0	+4/4	+4/7
Ofloxacin	0	-2/4	-3/8	-4/4	-1/5	-3/5	-4/5	-5/0
Doxycycline	-1/5	-3/5	-4/2	-4/7	0	-3/5	-4/2	-5/0

was increased by the presence of sub-inhibitory concentration. The FIC indices of the Gram-positive bacterium were 0.314 and 0.43, respectively (Table 2).

Table 3 shows determined of synergy between plant extract and antibiotics using the time-kill assay. The Time-kill assay was performed by the broth macrodilution technique. The extract and antibiotics were incorporated into 50 mL of nutrient broth at MIC, 2×MIC and 4×MIC, respectively. the net  $\log_{10}$  increase (+) or reduction (-) in viable bacterial cell counts ( $\text{cfu mL}^{-1}$ ) for all the strains following exposure to MIC, 2×MIC and 4×MIC of ethanolic leaf extract of *S. aureus* for different periods of time (0.5-24 h).

As cent percent death for all the strains was noted within 3 h of exposure time, the data up to 3 h were shown.

### DISCUSSION

In this study the antibacterial activity of *Scrophularia striata* ethanolic leaf extract was assessed *in vitro* when used alone and in combination with doxycycline and ofloxacin against *Staphylococcus aureus*. The MIC and MBC values of the leaf extract ranged from 50.6 and 60.3  $\mu\text{g mL}^{-1}$  and were higher than those of antibiotics. The activity of the antibiotics against a gram-positive (*S. aureus*) bacteria was increased by the presence of sub-inhibitory concentration.

Doxycycline and ofloxacin are commonly used antibiotics for treatment of diseases caused by several bacteria (Jacobs, 1995). In order to assess the effects of combinations of plant leaf extract and antibiotics, the MIC values of the antibiotics were determined as these provide the reference point for defining the interactions (Vacher *et al.*, 2007).

The activity of the plant against both gram-positive and gram-negative bacteria may be indicative to the presence of broad spectrum antibiotic compounds or simply general metabolic toxins in the plant, in addition to the plant (fruits, leaves and root) content of pharmacological active metabolites like furostanol and spirostanol saponins.

The rate of kill of test bacterial cells varied with concentrations of extract, duration of exposure and the bacterial strains tested. Overall, the result indicates that the extract may be used to prepare potent antibacterial preparations, at least *in vitro*.

Synergistic effects resulting from the combination of antibiotics with extracts have been documented earlier (Muroi and Kubo, 1996). Data from the literature as well as present results reveal the potential of plants for therapeutic treatment. Therefore, more studies need to be conducted to search for such compounds and plant extracts before being used in new therapeutic treatments, should have their toxicity tested *in vivo*.

In conclusion, our preliminary results showed that the combined use of ethanolic leaf extracts of *S. aureus* and antibiotics may be helpful for treating infectious diseases. Further *in vivo* studies need to be done to confirm these findings.

### CONCLUSION

The results suggest that *S. aureus* leaves extracts have significant antibacterial activity on *S. aureus*.

### ACKNOWLEDGMENTS

This research has been done by supporting from the Ilam University grant No. 3345671 in March 2009.

### REFERENCES

- Chatterjee, S.K., I. Bhattacharjee and G. Chandra, 2007. Bactericidal activities of some common herbs in India. *Pharma. Biol.*, 45: 350-354.
- Climo, M.W., R.L. Patron and G.L. Archer, 1999. Combinations of vancomycin and beta-lactams are synergistic against *Staphylococci* with reduced susceptibilities to vancomycin. *Antimicrob Agents Chemother.*, 43: 1747-1753.

- Cowan, M.M., 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev., 12: 564-582.
- Jacobs, M.R., 1995. Activity of quinolones against mycobacteria. Drugs, 49: 67-75.
- Jeong, L.M., B.D. Ho, L.D. Ha, J.K. Hyo, O.D. Hwan and H.S. Do, 2006. Reduction of *Bacillus cereus* in cooked rice treated with sanitizers and disinfectants. J. Microbiol. Biotechnol., 16: 639-642.
- Jung, H.J., K.S. Choi and D.G. Lee, 2005. Synergistic killing effects of synthetic peptide P20 and cefotaxime on methicillin-resistant nosocomial isolates of *Staphylococcus aureus*. J. Microbiol. Biotechnol., 5: 1039-1046.
- Muroi, H. and I. Kubo, 1996. Antibacterial activity of anacardic acid and totarol, alone and in combination with methicillin, against methicillin-resistant *Staphylococcus aureus*. J. Appl. Bacteriol. 80: 387-394.
- National Committee for Clinical Laboratory Standards, 1997. Methods for Dilution Antimicrobial Susceptibility Test for Bacteria that Grow Aerobically. 4th Edn., National Committee for Clinical Laboratory Standards, Wayne, PA.
- Nebel, S., A. Pieroni and M. Heinrich, 2006. Ta chòrta: wild edible greens used in the Graecanic area in Calabria, Southern Italy. Appetite, 47: 333-342.
- Ozaslan, M., M.E. Zumurtdal, K. Daglioglu, I.H. Kilic and I.D. Karagoz *et al.*, 2009. Antitumoral effect of *L. inermis* in mice with EAC. Int. J. Pharmacol., 5: 263-267.
- Pankey, G., D. Ashcraft and N. Patel, 2005. *In vitro* synergy of daptomycin plus rifampin against *Enterococcus faecium* resistant to both linezolid and vancomycin. Antimicrobial Agents Chemother., 49: 5166-5168.
- Perry, L.M. and J. Metzger, 1980. Medicinal Plants of Southeast Asia. MI Press, Cambridge, London, UK., pp: 385.
- Shabana, M.M., Y.W. Mirhom, A.A. Genenah, E.A. Aboutabl and H.A. Amer, 1990. Study into wild Egyptian plants of potential medicinal activity. Ninth Communication: Hypoglycaemic activity of some selected plants in normal fasting and alloxanised rats. Arch. Exp. Vet. Med., 44: 389-394.
- Sung, W.S., H.J. Jung, I.S. Lee, H.S. Kim and D.G. Lee, 2006. Antimicrobial effect of furaneol against human pathogenic bacteria and fungi. J. Microbiol. Biotechnol., 16: 349-354.
- Tadeg, H., E. Mohammed, K. Asres and T.G. Mariam, 2005. Antimicrobial activities of selected traditional Ethiopian medicinal plant used in the treatment of skin disorders. J. Ethnopharmacol., 100: 168-175.
- Vacher, S., J.L. Pellegrin, F. Leblanc, J. Fourche and J. Maugein, 2007. Comparative antimycobacterial activities of ofloxacin, ciprofloxacin and grepafloxacin. J. Antimicrobial Chemother., 44: 647-652.
- Viegi, L., A. Pieroni, P.M. Guarrera and R. Vangelisti, 2003. A review of plants used in folk veterinary medicine in Italy as basis for a databank. J. Ethnopharmacol., 89: 221-244.