research article | International Journal of Plant Sciences, (January to June, 2010) Vol. 5 Issue 1 : 262-265

Antibacterial activity of plant extracts on methicillin-resistant *Staphylococcus aureus* (MRSA)

INAMPUDI SAILAJA, P.G. REDDY, IVVALA ANAND SHAKER AND ABJIT AWARI

Accepted : November, 2009

SUMMARY

Methanolic and aqueous leaf extracts of three plants, *Leucas longifolia, Oscimum bascilicum* and *Lantana camara*, were evaluated for their potential antibacterial activity against methicillin resistant *Staphylococcus aureus* (MRSA). The extracts of all three plants demonstrated positive antibacterial activity. The extract of *L. camara* exhibited high antibacterial activity having minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values. Combination of extracts of plants showed synergistic antibacterial activity. Methanolic extracts presented more growth inhibitory action than aqueous extracts.The extracts were found to be bacteriostatic at lower concentration and bactericidal at higher concentration.

Key words : *Leucas longifolia, Ocimum bascilicum, Lantana camara*, Methanolic extract antibacterial activity, Methicillin-Resistant *Staphylococcus aureus* (MRSA)

Bacteria have the genetic ability to transmit and acquire resistance to drugs resulting in the emergence of new multi drug resistant bacterial strains (Cohen 1992).

This fact has become a cause for concern, eventhough pharmacological industries have produced a number of new antibiotics during the last three decades. (Abu-Shanab *et al.*, 2004).

Plants are a rich source of wide variety of secondary metabolites such as tannins, terpenoides, alkaloids, flavonides, phenols, essential oils, which have been found to have antimicrobial properties (Trease and Evans 1972; Evans *et al*.,1986; Cowan, 1999) and have the potential to act against multi drug resistant bacteria (Firas and Hassan, 2008).

Plants based antimicrobials may become the base for the development of new drugs or be used for the treatment of disease. (Trease and Evans, 1972).

Interest in plants with antimicrobial properties is revived as a result of current problems associated with the use of antibiotics. Dependence on traditional medicine for curing a variety of diseases has increased particularly in developing countries.

In recent years several workers, Ikram and Inamul (1984), Naqvi *et al.(*1991), Samy *et al*. (1998), Dorman and Deans (2000), Gislene *et al*. (2000), Samy and Ignacimuthu (2000), Srinivasan *et al*. (2001), Kapoor *et al*. (2007), Nair and Chanda (2007), Seema *et al*. *(*2007), Sengottuvel *et al*. (2007), Jeya and Veerapagu (2008), Farooz Ahmad Dar *et al*. (2008) screened many plants for antibacterial properties.

Methicillin-resistant *Staphylococcus aureus* (MRSA) pathogenic bacteria is now common in many areas of the world. The frequencies of infections and outbreaks due to MRSA have continued to increase. MRSA is often multi drug resistant and therapeutic options are limited. (Fluit *et al*., 2001, Fridkin *et al.*, 2005, Schito 2006). The effect of plant extract on *Staphylococcus aureus* was evaluated by Ayandele and Adebiyi (2007) and Firas and Hassan (2008).

Therefore the present investigation was carried out to evaluate the effect of leaf extracts of *Leucas longifolia, Ocimum bascilicum, Lantana camara* on the growth MRSA.

MATERIALS AND METHODS

Fresh and mature leaves of *L. longifolia*, *O.bascilicum* and *L.camara* were collected washed first in tap water and then again in sterile distilled water and kept for drying in shade. The dried leaves were then ground into fine powder in a blender and then sieved through 2-micron sieve.

10 g of the powder was extracted in 100 ml methanol and distilled water in Soxhlet apparatus. The crude extracts

Correspondence to:
P.G. REDDY, P.G. Department of Botany, Padmashri Vikhe Patil College, Pravaranagar, Loni, AHMEDNAGAR (M.S.) INDIA
Authors' affiliations:
INAMPUDI SAILAJA, Department of Biochemistry and Molecular Biology, College of Agriculture Biotechnology, Loni, AHMEDNAGAR (M.S.) INDIA
IVVALA ANAND SHAKER, Department of Biochemistry, Pravaranagar Institute of Medical Sciences, Loni, AHMEDNAGAR (M.S.) INDIA
ABJIT AWARI, Department of Microbiology, Pravaranagar Institute of Medical Sciences, Loni, AHMEDNAGAR (M.S.) INDIA

were then kept in oven at 37^{0}C for the solvents to evaporate to get the residue for further use.

The residue was mixed in appropriate amount of DMSO (Dimethyl Sulphoxide) so as to get the concentration of 10 mg / ml which served as the stock from which different concentrations *viz*., 25µl, 50µl, 75µl, 100µl, 125µl were used for determining the effect on the growth of MRSA bacterial strain.

The MRSA strains used in this study were clinical isolates from patients presenting with symptoms of *S. aureus* associated diseases. The isolates were identified as *S. aureus* according to colonial and microscopic morphology, positive catalase test and coagulase production. All *S. aureus* isolates tested were found to be methicillin resistant. Antibacterial activity

The antibacterial activity was determined by the well diffusion method (NCCLS 1993, Perez *et al.*, 1990). The MRSA isolates were transferred into 5 ml of tryptic soya broth (TSB) in test tube and kept for 12 hours for the inoculum buildup. The turbidity of bacterial suspension was adjusted to reach an optical comparison to that of a 0.5 OD (McFarland standard), so that the suspension contains approximately 1 to 2 x 10^8 cfu /ml. This suspension was streaked evenly on the sterile Mueller-Hinton Agar medium in the Petriplates.

6 mm diameter wells were bored in the inoculated agar medium and 25µl, 50 µl, 75µl, 100µl, and 125µl, of the methanolic and aqueous extract from stock solution was introduced in separate wells to test the antibacterial activity of the plants extract. The plates were allowed to stand at room temperature for 1 h for extract to diffuse into the agar and then incubated at 37^0 C for 24 h. Subsequently, the plates were examined for bacterial growth inhibition and the diameter of zone of inhibition was measured to the nearest millimeter. The synergistic effect of combination of all three plants extracts was also noted. .

The Minimum Inhibitory Concentration (MIC) was determined by micro-broth dilution methods (NCCLS, 2000). The extract was reconstituted serially and diluted 2- fold in Mueller-Hinton broth. Each reconstituted dilution in tubes (replicated twice) was inoculated with 5 x 10^5 cells (cfu / ml) of the MRSA strain and incubated at 37^0 C for

18 h. MIC was taken as the highest dilution (least concentration) of extract showing the inhibition of growth. Minimum bactericidal concentration (MBC) was determined by sub culturing the inoculum of each test dilution on to a fresh extract-free solid medium and incubating further for 18-24 h. The highest dilution that yielded no single bacterial colony on a solid medium was taken as MBC.

RESULTS AND DISCUSSION

Methanolic as well as aqueous extracts of *Leucas longifolia, Ocimum bascilicum* and *Lantana camara* presented notable antibacterial activity against the MRSA test organism. But the extract in organic solvent (Methanol) showed more growth inhibition activity on the bacteria than the aqueous extract (Table 1). Similar observations were reported by Firas and Hassan (2008) and Goyal *et al*.(2008).

This suggests that organic solvent to be a better medium for extraction of antimicrobial active substances. The extract of *L camara* exhibited more antibacterial activity than other two plants extracts. However, combination of all three plants methanolic as well as aqueous extracts presented greater antibacterial activity than individual plant extract thus demonstrating a very good synergistic effect.

The antimicrobial activity of the extracts and their potency were quantitatively assessed by determining the MIC and MBC, respectively values (Table 2). The methanolic extracts of all three plants showed lower MIC and MBC values than aqueous extract.

The MIC and MBC values obtained for the extracts varied from plant to plant extract . MIC values of methanolic and aqueous extracts of *L. camara* were as

Table 1 : Antibacterial activity of the crude plant extracts on MRSA

Plant extract	Zone of inhibition (mm)									
	Aqueous extract					Methanolic extract				
	25 µl	50 µl	75 µl	100 µl	125 µl	25 µl	50 µl	75 µl	100 µl	125 µl
Leucas longifolia	4	5	6	7	8	8	10	12	14	17
Ocimum bascilicum	3	6	8	10	12	6	8	10	13	16
Lantana camara	6	7	9	11	15	10	14	18	23	27
Leucas longifolia + Ocimum bascilicum + Lantana camara	7	9	12	15	18	15	20	25	30	35

Table 2 : Minimum inhibiti\ory concentration (MIC) and minimum bactericidal concentration (MBC) values in µg/ml of the plant extracts

Plant extracts	Aqueous extract		Methanolic extract	
	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)
Leucas longifolia	14.08	15.30	10.30	11.32
Ocimum bascilicum	16.45	18.86	12.66	14.50
Lantana camara	7.10	8.42	4.30	6.32
Leucas longifolia + *Ocimum bascilicum* + *Lantana camera*	5.54	6.32	3.06	4.70

low as 4.30ug / ml. and 7.10 ug / ml. while that of *O. bascilicum* were as high as 12.66 ug / ml and 16.45, respectively.

Similarly MBC values of methanolic and aqueous extracts of *Lantana camara* were as low as 6.32 ug / ml. and 8.42 ug / ml. while that of *O.basilicum* were as high as 14.50 ug / ml and 18.86 ug / ml. But extracts of all three plants when combined together showed the lowest MIC and MBC values.

The zone of inhibition increased with the increase in the concentration of plant extract. The MIC and MBC values obtained for the extracts against the MRSA also support the findings.

It is interesting to note that the MIC values of the plant extracts obtained in this study were lower than the MBC values, which explains that the plant extracts were bacteriostatic at lower concentration and bactericidal at higher concentration. Combination of extracts exhibited more inhibitory action on the growth of MRSA with lowest MIC and MBC values.

The increasing occurrence particularly in hospitals, of *S. aureus* resistant not only to methicillin but to a wide range of antimicrobial agents, including all kinds of b-lactams, has made therapy more difficult. (Fluit *et al.*, 2001).

Although strategies have been proposed in an attempt to control the spread (Adwan *et al.*, 2005), there is a need to search for new sources of agents to treat MRSA infections.

The positive growth inhibition activity of leaf extracts of plants against hospital strains of MRSA observed in the present study stimulates the investigation of many more plants for their antibacterial properties and compounds to cure the diseases caused by a number of multi drug resistant microorganisms.

Acknowledgement :

The authors are thankful to the Principals of the concerned colleges for encouragement and providing necessary facilities for the work.

REFERENCES

Abu-Shanab, B. Adwan, G. and Abu-Safiya, D.(2004). Antibacterial activities of some plant extract utilized in Palestine in popular medicine. *Turkish. J. Biol.,* **28**: 99-102.

Adwan, K. Abu-Hasan, N. and Adwan, G. (2005). Nosocomial infection caused by methicillin-resistant Staphylococcus aureus in Palestine. *J. Microbiol. Drug Resistance.,* **11**: 75-77.

Ayandele, A.A. and Adebiyi, A.O. (2007). The phytochemical analysis and antimicrobial screening of extracts of *Olax subscorpioidea*. *African J. Biotech.,* **6**(7): 868- 870.

Cohen, M.L.(1992). Epidemiology of drug resistance: implications for a post-antimicrobial era. *Sci.,* **257**: 1050-1055.

Cowan, M.M.(1999). Plant products as antimicrobial agents. *Clinical Microbiol. Rev.,* **12**: 564-582.

Dar, Farooz Ahmad, Katyar, Keerti, Singh, Lokendra and Kunal Kishor. (2008) Antimicrobial activity of root extracts of *Withania somnifera* against pathogenic bacteria. *Adv. Plant. Sci.,* **21**(2): 385 – 387.

Dorman, H.J.D. and Deans, S.G. (2000). Antimicrobial agents from plants. *J. Appl. Microbiol.,* **88** (2): 308-316.

Evans, J.S. Pattison, E. and Morris, P. (1986). Antimicrobial agents from cell culture in Secondary metabolites in plant cell culture. Eds: P.Morris, A.Stafford and M.Fowler. Cambridge University Press. London.

Firas, A., Al-Bayati and Hassan, F. Al-Mola.(2008) Antibacterial and antifungal activities of different parts of *Tribulus terrestris* L. growing in Iraq. *J. Zhejiang. Univ. Sci. B.,* **9** (2): 154-159.

Fluit, A.C. Wielders, C.L.C. and Verhoef, J. (2001).Epidemiology and susceptibility of 3,051 *Staphylococcus aureus* isolates from 25 university hospitals participating in the European SENTRY Study. *J. Clincal Microbiol.*, **39**: 3727-3732.

Fridkin, S.K., Hageman, J.C.and Morrison (2005). Active Bacterial Core Surveillance Program of the Emerging Infections Program Network. Methicillin-Resistant *Staphylococcus aureus* disease in three communities. *New. England J. Med.*, **352**:1436-1444.

Goyal, Pankaj, Khanna, Arjun, Chauhan, Abhishek, Chauhan, Garima and Kaushik, Purshottam (2008). *In vitro* evaluation of crude extracts of *Catharanthus roseus* for potential antibacterial activity. *Internat. J. Green Pharm.*, **2**(3): 176- 181.

Gislene, G.F.N., Locatelli, J. Paulo, C. Freitas, P.C. and Silva, G.L. (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria. *Brazilian. J. Microbiol*., **31** (4): 247 - 256.

Ikram, M. and Inamul, H.(1984). Screening of medicinal plants for antimicrobial activities. *Fitoterapia*, **55** : 6-9.

Jeya, K.R. and Veerapagu, M.(2008). Studies on the antimicrobial activity of medicinal plants against *Listeria monocytogenes*. *Adv. Plant.Sci.*, **21**(2): 381-383.

Kapoor, B.B.S., Bhumika and Khatri, J.S.(2007).Antimicrobial activities of some medicinal tree species of Hanumangarh dist of Rajasthan. *J. Phytol. Res.*, **20**(2): 325- 326.

Nair, R. and Chanda Sumitra, V. (2007). Antibacterial activities of some medicinal plants of the western region of India. *Turkish. J. Bot*., **31**: 231-236.

Naqvi, S.A.H., Khan, M.S.Y. and Vohra, S.B. (1991). Antibacterial, antifungal and antihelminthic investigations of Indian medicinal plants. *Fitoterapia*., **62** : 221-226.

NCCLS (1993). *Performance standards for antimicrobial disc suspectibility tests*. Approved Standard NCCLS Publication M2- A5, Villanova, PA, USA.

NCCLS (2000). *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically*; Approved Standard Fifth Edition. NCCLS document M7-A5. Wayne, PA, USA.

Perez, C. Paul, M. and Bazerque, P.(1990). Antibiotic assays by agar well diffusion method. *Acta. Biol. Med. Exp.*, **15** : 113-115

Samy, P.R. Ignacimuthu, S. and Sen, A. (1998). Screening of 34 Indian medicinal plants for antibacterial properties. *J. Ethnopharmacol.,* **62** (2) : 173-181.

Samy, P.R. and Ignacimuthu, S. (2000). Antibacterial activities of some folk lore medicinal plants used by Tribals in Western Ghats of India. *J. Ethnopharmacol.,* **69**(1): 63-71.

Seema, J.P., Nitin Venugopalan and Pradeep, S. (2007). Screening for antibacterial activity of weeds. *Internat. J. Microbiol.*, **4**(1): 1-11.

Sengottuvel, R., Srinivasan, K., Mohanasundari, C., Natarajan, D. and Perumal, G. (2007). Screening of antimicrobial properties of leaf extracts of *Smilax zeylanica* and *Phyllanthus wightianus. Adv.Plant Sci.*, **20** (1): 273-275.

Schito, G.C. (2006). The importance of the development of antibiotic resistance in *Staphylococcus aureus*. *Clin Microbiol. Infect*., **1**: 3-8.

Srinivasan,D. Nathan, S., Suresh, T. and Lakshman Perumalswamy, D. (2001). A ntimicrobial activity of certain Indian medicinal plants used in folklore medicine. *J. Ethnopharmacol.*, **74** (3): 217-220.

Trease, G. and Evans, W. (1972). *Pharmacognosy*, Univ. Press, Aberdeen, Britain. pp:161-163

****
*****