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Efficacy of leaf extracts of selected medicinal plants against multi drug resistant strains of *Staphylococcus aureus*

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Abstract

Antibacterial activities of the crude extracts of the leaves of the medicinal plants *Allium cepa*, *Aloe vera, Cassia fistula*, *Lawsonia inermis, Moringa oleifera, Piper betle, Psidium guajava, Punica granatum* and *Tectona grandis* were determined against a multi drug resistant strain of *Staphylococcus aureus* by using disc diffusion and bioautography methods. The leaf extracts of *M. oleifera, L. inermis, P. guajava, C. fistula* and *T. grandis* showed antibacterial activities with the zone of inhibition ranging from 6 to 20 mm. TLC – Bioautography analysis indicated that the antimicrobial active compounds in *M. oleifera, L. inermis* and *P. guajava* might be similar.

Keywords : antibacterial activity, bioautography, medicinal plants, multidrug resistance, *Staphylococcus aureus*

INTRODUCTION

Hospital infections by *Staphylococcus aureus* is common in post operative wound infections and other hospital cross infections. Strains of *S. aureus* have been reported to develop resistance to drugs. For e.g., during the 1960s many isolates of *S. aureus* were found to be resistant to the semi-synthetic beta-lactams (methicillin, cloxacillin, flucloxallin, oxacillin), which became known as Methicillin-Resistant *S. aureus* (MRSA). They are also resistant to cephalosporin and clindamycin. The glycopeptide compound, Vancomycin, which destroys the integrity of the bacterial cell wall, remains the first-line therapy against MRSA. However, the Vancomycin-Resistant *S. aureus* (VRSA) have also become prevalent in most countries. As such treating such *S. aureus* Multidrug- Resistant strains warrants novel therapy. The higher plants have been found to possess antimicrobial compounds that are active against human bacterial and fungal pathogens (Mitscher *et al*., 1987). Even though the antimicrobial spectra of the natural products isolated from higher plants are comparatively narrow, they are comparatively easy to synthesize and their potency is often reasonable. Bearing this in mind susceptibility patterns of a clinical isolate of Multidrug Resistant *S. aureus* to antibiotics and efficacy of leaf extracts of nine selected Indian medicinal plants against it have been evaluated.

MATERIALS AND METHODS

A total of 166 samples (wound and pus) were collected from Sri Ramachandra Medical College and Research Institute, Porur, Chennai, South India. Single colonies from subcultures of sixteen Multidrug-Resistant strains of *Staphylococcus aureus* were inoculated onto nutrient agar, labeled with numbers 1 to 16 and sent to the Department of Microbiology, Maulana Azad Medical College, New Delhi for Phage typing.

An isolate of *S. aureus* from these samples was biochemically identified and confirmed. Antimicrobial activity of the antibiotics ampicillin, cephalexin, cephotaxime, cloxacillin, erythromycin, gentamicin, methicillin, penicillin, netilmicin, ciprofloxacin, rifampicin, clindamycin and vancomycin against the isolate was determined by the standard disc diffusion method of Bauer *et al*. (1966).

Nine authenticated plant species namely *Moringa oleifera, Lawsonia inermis, Psidium guajava, Cassia fistula, Tectona grandis, Aloe vera, Piper betle, Allium cepa* and *Punica granatum* were collected in an around Sri Ramachandra Medical College and Research Institute, School of Biomedical Science, Thiruvanmiyur, Chennai, South India. The leaves were washed with 70% ethyl alcohol and then with double distilled water. They were then dried, weighed (1 g) and crushed by using mortor and pestle. Then the plant material was made in to paste with 2ml of different solvents (Hexane, Benzene, Methanol, Ethanol, Chloroform, Methanol: Chloroform (1:1) and stored in sterile screw capped bottles at 4°C overnight. The solvents were then evaporated at 60°C on a water bath. The residues after evaporation were dissolved in dimethyl sulfoxide (DMSO) and used for antimicrobial testing against Multidrug-Resistant *S. aureus* (Ali-Shtayeh *et al.,* 1998).

Determination of anti-*Staphylococcus aureus* activity

Mueller Hinton Agar plates were seeded with 8 hours broth culture of Multidrug-Resistant *S. aureus* clinical isolate. Using sterilized dropping pipettes, 80µl of plant

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extract and solvent blanks were impregnated into the strile 5 mm diameter discs. Discs were dried and dispensed on the solidified Mueller Hinton Agar, previously inoculated with the test organisms. The plates were incubated for 18-24 hours at 37°C. The anti-*S. aureus* activity was evaluated and the mean values of the inhibitions zones were calculated (Srinivasan *et al.,* 2001).

Thin Layer Chromatography (TLC) of plant extracts

Thin layer chromatography for the leaf extracts of plants that showed strong anti- *S. aureus* activity was carried out. Silica gel GF_{254} plates, 855 cm and 1 mm thick were used. Plant extracts (10 µl) were applied and the chromatogram were developed using chloroform: methanol: water (65:35:5) as solvent. TLC plates were run in duplicate and one set was used as the reference chromatogram. Methanol: sulphuric acid (95 : 5), were used as spray reagent and the spots and bands were visualized under UV light (254 and 366 nm). The other set was used for bioautography as described Ahmed and Beg (2001).

TLC–bioautography

Drug resistant strains of *S. aureus* were used as test organisms. Chromatogram developed as described above was placed in plate with cover and the inoculum of Multidrug-Resistant *S. aureus* (10^6 CFU / ml) in Mueller-Hinton agar (15ml) was distributed over the plates. After solidification of the medium, the TLC plate was incubated overnight at 37°C. Subsequently the bioautogram was sprayed with an aqueous solution of 0.1% 2, 3, 5-Triphenyl-tetrazolium chloride (TTC). Inhibition zone indicated the presence of active compounds (Ahmed and Beg, 2001).

RESULTS

Clinical isolates

Out of 166 samples of clinical isolates studied, 116 were from males and 50 samples were from females (Table 1). Among them 42 samples were *S. aureus* positive with 16 samples being Multidrug-Resistant *S. aureus* (Table 1). Among the sixteen isolates of Multidrug-Resistant *S. aureus*, six isolates were phage typed and four different groups of strains were identified. Susceptibility of Multidrug – Resistant *S. aureus* to clindamycin, rifampicin, ciprofloxacin and netilmicin, were 81.3%, 68.8%, 25.0% and 12.5%, respectively (Fig. 1). No strain observed in the present study was resistant to Vancomycin (Fig. 1).

Anti *Staphylococcus aureus* activity of plant extracts

The anti *S.aureus* potencies of the plant extracts are given in table 2. All the solvent extracts of leaves of *M. oleifera, L. inermis, P. guajava, C. fistula and T. grandis* showed anti *S. aureus* activity, while none of the extracts from the

Table 1. Sex-wise frequency of *S. aureus* isolates in the samples examined

Sex	No. of samples	No. of *Staphylococcus aureus* positive samples	No. of samples with multidrug resistant *Staphylococcus aureus*	% of samples with multidrug resistant *Staphylococcus aureus*
Male	116	28	10	35.71
Female	50	14	6	42.85
Total	166	42	16	38.09

Table 2. Anti -*Staphylococcus* activities of various solvent extracts of the medicinal plants studied. Different solvents used for each plate served as controls (- indicates no inhibition)

Plant species	Common name	Anti-*Staphylococcus* activity of solvent extracts[a] (zone of inhibition in mm)					
		B	H	E	M	C	M/C
Allium cepa	Onion	-	-	-	-	-	-
Aloe vera	Indian aloe	-	-	-	-	-	-
Cassia fistula	Indian laburnum	11	12	12	12	12	12
Lawsonia inermis	Henna	14	15	12	14	14	11
Moringa oleifera	Drumstick	14	14	16	14	14	15
Piper betle	Betel leaf	-	-	-	-	-	-
Psidium guajava	Guava	11	12	14	14	12	11
Punica granatum	Pomegranate	-	-	-	-	-	-
Tectona grandis	Teak	12	11	12	11	14	13

[a]B-benzene, H-Hexane, E-Ethanol, M-Methanol, C-Chloroform, M / C-Methanol; Chloroform

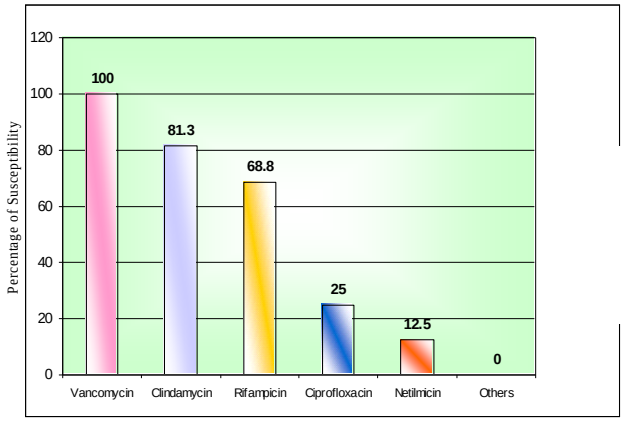

Figure 1. Susceptibility pattern of multidrug – resistant *Staphylococcus aureus* against various antibiotics

leaves of *A. vera, P. betle, A. cepa and P. granatum,* irrespective of the solvents, did show any inhibition (Table 2; Figs. 2 & 3).

Active components

TLC – bioautography was performed for the three highly active plant extracts (*M. oleifera, L. inermis* and *P. guajava*) in order to isolate the major active constituents responsible for anti-*Staphylococcus aureus* activity. In *M. oleifera* the active ingredient was present at an Rf value of 1.07 in benzene and hexane and in other solvents it was at 0.96. In *L. inermis* the Rf value of benzene, hexane, methanol: chloroform was 0.92, methanol 0.80, ethanol 0.89 and chloroform 0.90. Similarly in *P. guajava* the Rf value for benzene was 0.07, ethanol 0.84 and for other solvents it was 0.92.

DISCUSSION

Multi-drug Resistant *Staphylococcus aureus* (MRSA) has emerged and spread globally over the years since the first clinical use of methicillin. If MRSA has got introduced into hospitals, it usually becomes endemic, despite the implementation of infection control measures and also it has been reported to be an important cause of nasocomial infection (Coinbra *et al*., 2000). In this study a total of 42 isolates of *S. aureus* were selected from 166 clinical samples. Among them 16 samples (38%) were found to be Multidrug-Resistant *S. aureus*. The distribution pattern of the patients revealed its predominance among the males of the infected population. Earler Anupurba *et al.* (2003) have also reported the prevalence of the multi-drug resistant *S.aureus* among the male section of the patients in Uttar Pradesh, India.

The present study showed that MRSA is 100% sensitive to Vancomycin; but the drug is toxic and expensive. Moreover, of late the prevalence of Vancomycin Resistant *S. aureus* (VRSA) has also been found to be increasing in India (Anupurba *et al*., 2003).

Emergence of Multidrug-Resistant *S. aureus* in human as well as undesirable side effects of certain antibiotics have triggered immense interest in the search for new antimicrobial drugs of plant origin (Ahmed and Beg, 2001). In this study among the solvent extracts of nine traditionally used Indian medicinal plants that were tested against Multidrug-Resistant *S. aureus*, extracts of *M. oleifera, L. inermis* and *P. guajava* showed highest activities against MRSA. Comparatively methanol extracts gave better results than the other solvent extracts tested. The results suggest that the plant secondary metabolites could be effectively used for the treatment of Multidrug-Resistant *S. aureus*.

However, the intensity of antimicrobial activity observed in the present study was different from the earlier reports (Ahmed *et al*., 1998; Perumalsamy, *et al.,* 1998 ; Mehmood *et al*., 1999; Lopez *et al.,* 2001) on the extracts of same medicinal plants against different group of microorganisms. Such a difference in potency observed in the present study might be due to the age of the plant sample, differential sensitivity of the test strains and differences in the methods of extraction (Nimri and Meqdam, 1999).

The TLC separations of active compounds showed that some of the extracts exhibited same Rf value indicating the presence of more or less similar compounds that are responsible for the antimicrobial activity in the plants studied. Use of additional, more polar solvent systems that would allow better separation of zones is suggested.

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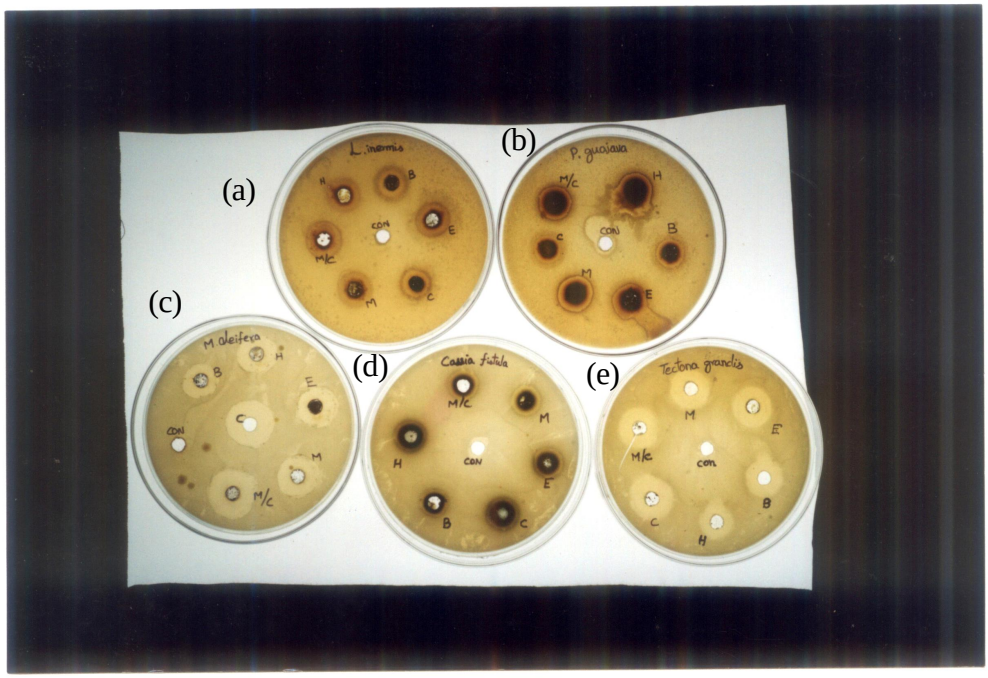

Figure 2. Anti *Staphylococcus aureus* activity of plant leaf extracts
a) *Lawsonia inermis* b) *Psidium guajava* c) *Moringa oleifera* d) *Cassia fistula* e) *Tectona grandis*
B – Benzene; H –Hexane; E-Ethanol; C-Chloroform; M-Methanol;
M / C-Methanol: Chloroform, Con-Control

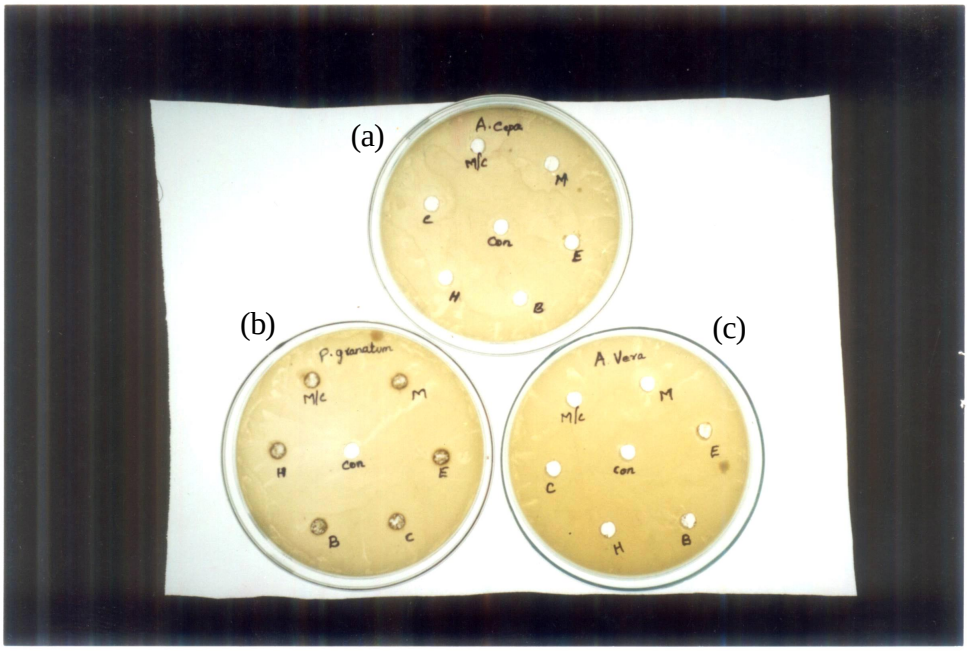

Figure 3. Anti *Staphylococcus aureus* activity of plant leaf extracts
a) *Allium cepa* b) *Punica granatum* c) *Aloe vera*
B – Benzene; H –Hexane; E-Ethanol; C-Chloroform; M-Methanol; M / C- Methanol: Chloroform, Con-Control

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