



**International Journal of Biology, Pharmacy
and Allied Sciences (IJBPAS)**

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

**ETHNIC CONSUMPTION OF PLANT LEAF EXTRACTS AND APPRAISAL OF
THEIR NUTRACEUTICAL EFFICACY AGAINST MULTIDRUG RESISTANT
*STAPHYLOCOCCUS AUREUS***

KAUSHIK S^{1,2*}, TOMAR RS¹, SHRIVASTAV V¹, SHRIVASTAV A² AND JAIN SK³

1: Amity Institute of Biotechnology, Amity University Madhya Pradesh, Gwalior (M.P.),
INDIA

2: College of Life Sciences, Cancer Hospital and Research Institute, Gwalior (M.P.), INDIA

3: Department of Microbiology, Vikram University, Ujjain (M.P.), INDIA

***Corresponding Author: E Mail: shuchi.kaushik2@gmail.com**

ABSTRACT

Nutraceuticals are natural bioactive chemical compounds that have health promoting, disease preventing or medicinal properties. Emergence of Multi Drug Resistant *Staphylococci* is increasing at alarming rates and diseases caused by these strains leave patients untreated due to lack of appropriate drugs. From ancient time, plants are rich source of effective and safe medicines. Therefore, it is of interest to explore the possibility of using phytochemicals as chemo preventive agents. The present study was designed to explore indigenous plant products for the development of effective formulation against multiple resistant *Staphylococcus aureus*.

The test bacteria were isolated and characterized by standard and NCCLS recommended microbiological techniques. A total of eighteen plant extracts were analysed for their antimicrobial activity. The selection of medicinal plants was based on their traditional uses in India. However most of these plants were not previously screened. Antibacterial activity of these components was performed by standard Kirby Bauer Disk Diffusion method approved by NCCLS and the inhibitory effect was analysed by calculating Zone of inhibition.

Among the eighteen plant extracts analysed we found highest activity in the Guava, Mango, Jamun and Pomengrate plant extracts, while most of the other plants were either showing very moderate/ least activity against test bacteria. Our recent experiment indicated that

phytochemicals extracted with methanol can be utilized as nutraceutical to lower the side effect of chemotherapy and as promising bio control agents.

Keywords: Multi-Drug Resistance, Antimicrobial, Nosocomial Infections, Zone of Inhibition

INTRODUCTION

Infectious diseases represent an important cause of morbidity and mortality among humans, especially in developing countries. The genus "*Staphylococcus*" is common inhabitant of the skin and mucous membrane and it accounts for a considerable proportion of human infections. Approximately 20–30% of the general population is "staph carriers" [1]. It is still one of the four most common causes of nosocomial infections, often causing post-surgical wound infections. The emergence of antibiotic resistance in this microorganism and their spread is threatening the medical community.

The resistance development in *Staphylococcus aureus* dates back to 1940s. Multiple drug resistance of *Staphylococcus aureus* is due to the presence of *mecA* gene coding for penicillin binding protein (PBP2a) with a low affinity for β -lactam antibiotics. This gene is carried on Staphylococcal Cassette Chromosome (SCC) *mec*, a unique mobile genetic element that harbors the methicillin resistant gene (*mecA*) and other antibiotic resistant determinants [2].

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. The increasing gain of resistance to available antimicrobials and side effects associated with the drugs have attracted the attention of scientific community towards the search and development of new cost effective drugs of natural and synthetic origin [3].

Varieties of aromatic medicinal plants are tested for treating infectious diseases [4]. The nature is having treatment of every disease in hidden form; many nutraceuticals have shown their beneficial effects on the treatment of various diseases. So the present study was designed to explore indigenous plant products for the development of effective formulation against MRSA.

MATERIALS AND METHODS

MRSA

Micro-organisms used in this study were isolated from patients between 5 to 75 years of age suffering from various infections. The present study comprised 872 cases among which 500 samples were taken from

patients, 266 cases were from healthy and 106 samples were taken from environment. Patients were examined by the doctors. Data were recorded, analysed and subjected to statistical analysis. Brain Heart Infusion agar and Mueller-Hinton agar were used for growing the organisms. Test bacteria were characterized by their biochemical characteristics as well as by standard microbiological techniques.

Plant Material and Extract Preparation

The selection of medicinal plants is based on their traditional uses in India [6]. However most of these plants were not previously screened against multi-drug resistant, pathogenic organisms.

Screening of Extracts

Extracts were screened for the antibacterial nature against MRSA by using disc diffusion method of [5]

RESULTS AND DISCUSSION

The threat to the human population is that reservoirs of drug-resistant bacteria is abundant. In the present study, *S. aureus* was indicated by the yellow halo produced around the colonies on Mannitol Salt Agar. This is caused by the ability of *S. aureus* to ferment mannitol to acids which is detected by a change of pH indicator from red to yellow [7].

This study also showed that antimicrobial resistance of *Staphylococcus aureus* was high and alarming. Even though

pharmaceutical companies have produced a number of new antibacterial drugs over the years, resistance to these drugs by *Staphylococcus aureus* has increased manifold and has now become a global concern. Therefore the identification of new effective antimicrobial agents is of paramount importance. Medicinal plants have long been investigated as the potential sources among new agents [8].

The results of the present study revealed the antimicrobial activity of plant extracts (Table 1, Figure 1 & Figure 2). The methanol extracts of Mango (*Mangifera indica*), Guava (*Psidium guajava*), Jamun (*Eugenia jambolana*) and Pomengranate (*Punica granatum*) were found to be active against most of the MRSA isolates, while few also well responded against methanolic extracts of Tulsi (*Oscimum sanctum*) & Neem (*Azadirachta indica*).

Most active antibacterial extract found was *Punica granatum*. It is a potent antimicrobial, immune-modulatory anti-diarrheal [9, 10]. We have also found good activity against MRSA with this extract. We also observed good antibacterial activity against test organism with *Mangifera indica* leaf extract. The seed extract of this plant effectively suppressed coagulase activity and mannitol fermentability of *Staphylococci* [11].

The antimicrobial activity of *Azadirachta indica* has also been reported many times and we also found it as effective antibacterial agent against test bacteria. Leaf

of *Azadirachta indica* has also been found to have bioactive compounds named cyclic trisulphide & cyclic tetra sulphide [12, 13].

Table 1: Antimicrobial Resistance Pattern of Test Bacterial Organism Against the Selected Plant Extracts Included in the Study

S. No.	PLANT EXTRACTS	RESISTANT	SENSITIVE
1	Mulethi	27 (29%)	67 (71%)
2	Ginger	71 (76%)	23 (24%)
3	Neem	19(20%)	75 (80%)
4	Marigold	30 (32%)	64 (68%)
5	Ashok	52 (55%)	42 (45%)
6	Guava	11 (12%)	83 (88%)
7	Amla	50 (53%)	44 (47%)
8	Mango	11 (12%)	83 (88%)
9	Jamun	11 (12%)	83 (88%)
10	Pomengranate	11 (12%)	83 (88%)
11	Methi	38 (40%)	56 (60%)
12	Dalchini	27 (29%)	67 (71%)
13	Aak	38 (40%)	56 (60%)
14	Ashwagandha	71 (76%)	23 (24%)
15	Jackfruit	27 (29%)	67 (71%)
16	Tulsi	19 (20%)	75 (80%)
17	Thuja	94 (100%)	0 (0%)
18	Lemon	75 (80%)	19 (20%)
	Control (Vancomycin 40µl)	9 (9.57%)	85 (90.4%)

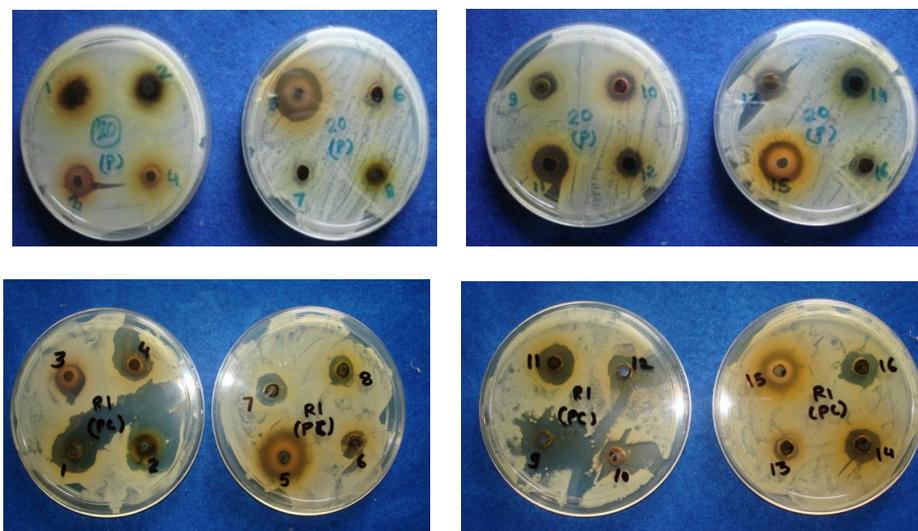


Figure 1: Antimicrobial Activity of Plant Extracts Against Test Bacteria

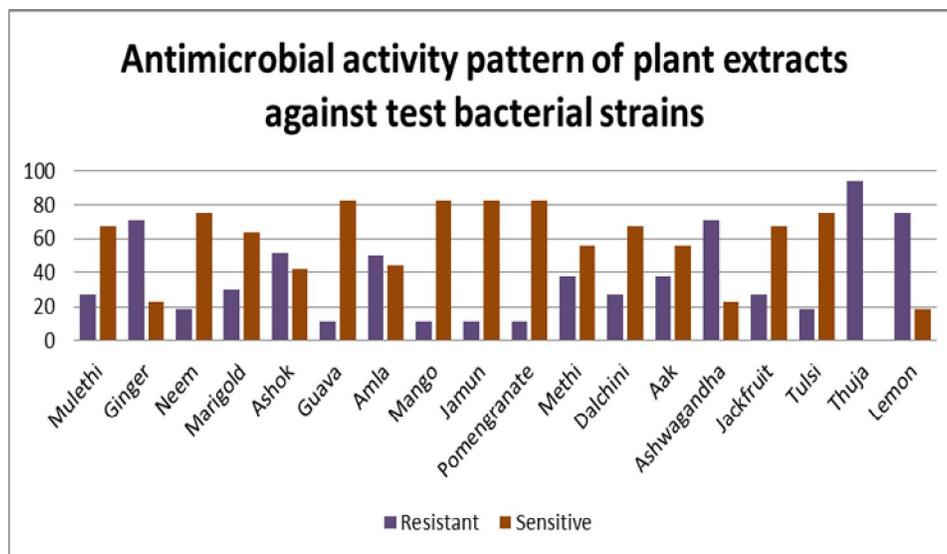


Figure 2: Showing the Antibacterial Pattern of Plant Extracts Against the Test Bacterial Strains

CONCLUSION

Our antimicrobial screening results justify the traditional uses of these plants in various ailments including infectious diseases. The active phytochemicals of these plants against multidrug-resistant bacteria has to be characterized and the efficacy of non-toxic extracts: preparations have to be evaluated *in vivo*. Study of the synergistic interaction of active phytochemicals with antibiotics is required to exploit these potential plant extracts in the combination therapy of infectious diseases caused by multi drug-resistant organisms.

The results presented in this report were encouraging, although clinical controlled studies are required to define the real efficacy and possible toxic effects *in vivo*.

REFERENCES

[1] Heyman D, Control of Communicable Diseases Manual,

18th Ed., Washington DC: American Public Health Association, 2004.

[2] Zhang K, McClure J, Elsayed S, Louie T and Conly JM, Novel multiplex PCR Assay for characterization and concomitant subtyping of staphylococcal cassette chromosome *mec* types I to V in methicillin resistant *Staphylococcus aureus*, J. Clin. Microbiol., 43, 2005, 5026-5033.

[3] Fine DH, Furgang D, Barnett ML, Drew C, Steinberg L, Charles CH and Vincent JW, Effect of an essential oil-containing antiseptic mouthrinse on plaque and salivary *Streptococcus mutans* levels, J. Clin. Periodontol., 27 (3), 2000, 157-61.

[4] Kalembe D and Kunicka A, Antibacterial and antifungal

- properties of essential oils, *Curr. Med. Chem.*, 10, 2003, 813-829.
- [5] Bauer AW, Kirby WMM, Sherris JC and Turck M, Antibiotic susceptibility testing by a standardized single disk method, *Am. J. Clin. Pathol.*, 45, 1996, 493-6.
- [6] Ahmad AZ and Beg J. *Ethnopharmacol.*, 74, 2001, 113-123.
- [7] Leboffe MJ and Pierce BE, *Microbiology: Laboratory Theory and Application*, Englewood, CO: Morton Publishing Company, 2002.
- [8] Prakash MV, Karthikeyan SK and Karmegam N, Synergistic activity of certain plant extracts against Methicillin resistant *Staphylococcus aureus* (MRSA), *J. Ecotoxicol. and Environmen. Monitoring*, 16, 2006, 387-389.
- [9] Kurokawa M, Ochiai H, Nagasaka K, Neki M, Xu H, Kadota S, Sutardjo S, Matsumoto T, Namba T and Shiraki K, Antiviral traditional medicines against herpes simplex virus (HSV-1) , poliovirus and measles virus in vitro and their therapeutic efficacies for HSV-1 infection in mice, *Antiviral Res.*, 22, 1993, 175-188.
- [10] Das AK, Mandal SC, Banerjee SK, Sinha S, Das J, Saha BP and Pal M, Studies on antidiarrhoeal activity of *Punica granatum* seed extracts in rats, *J Ethnopharmacol.*, 68, 1999, 205-208
- [11] Satyanarayanan T, Rao DPC and Singh BS, Antibacterial activity of six medicinal plants extracts, *Indian Drugs*, 14, 1997, 209.
- [12] Siddiqui SA, A note on the isolation of three new bitter principles from neem (margosa) oil, *Curr. Sci.*, 11, 1942, 278-279.
- [13] Mitra CR, and Patel MS, *Indian Central Oilseeds Committee*, Hyderabad, 1963, 69-94.