

INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Life Sciences

Research Article.....!!!

Received: 17-10-2016; Revised: 31-10-2016; Accepted: 01-11-2016

ANTIBACTERIAL ACTIVITY OF THE EXTRACT OF SELECTIVE MEDICINAL PLANTS AGAINST *STAPHYLOCOCCUS AUREUS*

Padma Singh*, Alka Rani*, Rekha Negi, Parul, Pallavi Kumari and Bhavya Trivedi
Department of Microbiology, Kanya Gurukul Campus, Gurukul Kangri University, Haridwar,
(Uttarakhand)-249407, India

Keywords:

Antibacterial activity,
Plant extract,
Staphylococcus aureus,
Mulethi (*Glycyrrhiza
glabra*)

For Correspondence:

Dr. Padma Singh

Department of Microbiology,
Kanya Gurukul Campus,
Gurukul Kangri University,
Haridwar, (Uttarakhand)-
249407, India

E-mail:

drpadmasingh06@gmail.com

ABSTRACT

Due to the increase adverse effects of allopathic drugs and rapidly emerging antimicrobial resistant pathogens the search is going for new antimicrobial agent. Plants are rich source of antibacterial agents, which could exploited in human disease management. Medicinal plants are valuable natural sources effective against various infectious agents. Extracts of Amaltas (*Cassia fistula linn*), chitrak (*Plumbago roseus*), Garlic (*Allium sativum .linn*), Mulethi (*Glycyrrhiza glabra*), sadabahar (*Catharanthus roseus linn*), Ghikanwar (*Aloe barbadensis mill*) traditional medicinal plants were investigated for antibacterial activity. In this study, the potential antibacterial activity of extracts from different traditional medicinal plants against *Staphylococcus aureus* was investigated *in-vitro*. Susceptibility assays using disc diffusion and The minimum inhibitory concentration was also determined by using the disc diffusion method. The determination of minimum inhibitory concentration (MIC) were used to assess the antibacterial activity of alcoholic extracts from medicinal plants. Extracts from all medicinal plant the alcoholic extract of *Glycyrrhiza glabra* showed most potent antibacterial activity against *Staphylococcus aureus*.

INTRODUCTION

Medicinal plants are nature's gift to humans to make disease free healthy life. Infectious diseases continue to represent a significant challenge to human medicine. Clearly there is an urgent need for new and efficient drugs to treat the life threatening diseases. Large preparations of drugs currently used to treat infectious diseases are mostly natural products. Despite the progress in the field of Microbiology, incidence of epidemics due to drug resistant microbes and the emergence of unknown disease causing agents still occur. Herbal products remain highly effective instruments in the fight against microbial infections. Plant based antimicrobials represent a vast untapped source for medicine and they have enormous therapeutic potential. They are effective in treatment of infectious disease while simultaneously mitigating many of the side effects that are often associated with synthetic. The World Health Organization estimates that plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population (**Shaikh et al., 1994**).

Staphylococcus aureus is a Gram-positive bacterium responsible for morbidity and mortality. It is one of the leading causes of human infections of the skin, soft tissues, bones and joints, abscesses, and normal heart valves. *Staphylococcus aureus* flourishes in the hospital setting and is associated with blood stream and surgical wound infections and has become important nosocomial organism. *Staphylococcus aureus* is very common cause of infection in hospitals and in most liable to infect new born babies and patient, particularly surgical patients (**Seth et al., 1973**).

Antibiotic resistance has become a global concern now a days with the emergence of multidrug-resistant pathogens that has become a threat to the clinical efficacy of many existing antibiotics (**Westh et al., 2004**). Thus, there is a continuous and urgent need to find or to develop new antimicrobial compounds with novel mechanisms of action for new and re-emerging infectious diseases (**Rojas et al., 1992**). In recent years, it has been proposed that the herbal extracts may be used as natural antimicrobial agents to inhibit the growth of food borne pathogen (**Lee et al., 2007**) and as a source of various medicinal agents (**Krishnaraju et al., 2005**). Traditional medicine is an important source of potentially useful compounds for the development of phytotherapeutic agents. Antimicrobials of plant origin have enormous therapeutic potential in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials (**Lwu et al., 1999**).

Ayurveda is an ancient Indian system of medicinal plants are rich in secondary metabolites which are potential sources of drug and approximately 25% of prescribed medicines (Farnsworth and Bingel, 1977). It has been estimated that about 1000 ayurvedic remedies prepared from around 750 plants are being use at present. The present study is conducted to see the effect of different medicinal plants Amaltas (*Cassia fistula linn*), Chitrak (*Plumbago roseus*), Garlic (*Allium sativum .linn*), Mulethi (*Glycyrrhiza glabra*), Sadabahar (*Catharanthus roseus linn*), Ghikanwar (*Aloe barbadensis mill*) as well as the effect of antibiotic.

MATERIAL AND METHOD

Collection of plant material

All plants leaves and roots were collected in polythene bags. The leaves and roots were dried in the oven at 40°C for one week. The dried leaves were grinded to form fine powder by mean of a blender. The powder is placed in plastic bags and kept in a cool and dry place for further use.

Isolation and identification of test organism

Test microorganism i.e. *Staphylococcus aureus* was isolated from pus sample collected from pathological laboratory. Pus specimen is comparatively better than swabs. Test microorganism was identified by Gram staining and further identification was carried out by biochemical testing.

Extraction of active compounds using ethanol as solvent for extraction

Ten gram (10g) of the ground leaf and roots samples were separately soaked in 200 ml of ethanol and allowed to stand for about 72hr for extraction. After the 72 hr, it was then filtered using the whatman filter paper. The filtered samples were sterilized by passing through millipore filter and later evaporated to dryness.

Plant extracts sensitivity test

Disc diffusion method utilizes plant extract sensitivity discs. These discs were 5mm in diameter punched from whatman filter paper no.3 and sterilized by dry heat for one hour. A disc was impregnated with a 0.02 ml of plant extract dilution and this was placed on plate of sensitivity testing after inoculated with the test organism Ciprofloxacin disc was used as positive control (Peach and Tracey, 1955).

Antibiotic sensitivity test

The antibiotic sensitivity was investigated by disc diffusion method. Multidrug disc (Ciprofloxacin, Cefotaxime, Tetracycline, Cephalixin, Cotrimoxazole/Ampicilline, Salbactam,

Ofloxacin, Pefloxacin, Roxythromycin, Lincomycin, Gentamycine, Cloxacillin) was used for the *Staphylococcus aureus* (Kirby and bauer, 1966).

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration was determined using the disc diffusion method. The size of inhibition is inversely related to the minimum inhibitory concentration. The determination of the lowest concentration of the extracts capable of inhibiting the growth of *Staphylococcus aureus* (i.e. Minimum Inhibitory Concentration MIC) were carried out on square plates 10×10 cm containing 20 ml DST agar. For the test of MIC prepared the 10 different dilution(100%,84%,72%,60%,50%,36%,25%,18%,12%,6%)of plant extract of *Glycyrrhiza glabra*. Filter paper impregnated with plant extract were placed on the surface of the agar. (Rolinson and Russell, 1972) observed that many commonly used antibiotics required 1hour 30 minute to 4 hour for uniform diffusion of the drug from the paper into agar.

RESULT AND DICUSSION

The present study carried out determine the antimicrobial activity of Amaltas (*Cassia fistula linn*), chitrak (*Plumbago roseus*), Garlic (*Allium sativum.linn*), Mulethi (*Glycyrrhiza glabra*), sadabahar (*Catharanthus roseus linn*), Ghikanwar (*Aloe barbadensis mill*), against the *Staphylococcus aureus*. The *Staphylococcus aureus* was isolated from the pus sample after the Gram staining it was observed that isolated microorganism was Gram positive. on the basis of biochemical testing it was found that microorganism was *Staphylococcus aureus* and the results of biochemical test are tabulated in table 1. Commercially available plants were found effective against *Staphylococcus aureus* as evident by the size of inhibition zone. The different parts of plant like leaf and roots were taken in different dilution. Table.2 showing sensitivity at different dilution of plant extracts against *Staphylococcus aureus*. The increasing order of zone of inhibition of plant extract disc again *Staphylococcus aureus* was as follow Sadabahar>Amaltas>Garlic>Aloevera>Chitrak>Mulethi on the 50% concentration. Similar study was done by (Singh and Trivedi., 2015).

The findings of this research work have shown clearly that the sensitivity of the organisms to antibiotics was quite specific. The bacterial susceptibility to antibiotics was measured by utilizing agar diffusion technique that is disc method. Antibiotics susceptibility testing with discs is known for the simple and rapid method and therefore result were obtained within 24hrs. On the basis of zone of growth inhibition which indicate the sensitivity of microorganism with

corresponding antibiotics. *Staphylococcus aureus* is highly sensitive to Ciprofloxacin and Cefatoxime moderately sensitive to Pefloxacin and weakly sensitive to Tetracycline. The results have been shown in Table 3.

In the present study *Glycyrrhiza glabra* (Mulethi) showing maximum zone of inhibition in comparison to antibiotics and other plant extracts. Hence it is recommended for the MIC (Minimum Inhibitory Concentration) of plants extract. The MIC is defined as the lowest concentration of plant extract, which inhibit the growth of given strain of microorganism under controlled conditions .The result have been showing in Table 4 . Similar results were reported by **(Singh and Choudhary., 2015)**. A trial conducted in Japan demonstrated *Glycyrrhiza* roots to be effective against an original *Staphylococcus aureus* strain that had been made resistant to penicillin and streptomycin as well as against succeeding drug resistant culture or generation of that strain**(Mowrey et al., 1986)**. Antibacterial activity of extract may be due to the presence of phytochemical constituents present in the plant. Previous studies which have been done cleared that these plant contained bioactive compounds like alkaloid, glycosides, terpenoids, flavonoids and tannins **(Trivedi et al., 1969; Rami et al., 2013)**.

Table 1. Microbiological characteristics of *Staphylococcus aureus* isolated from pus sample.

S.No	Characteristics	Test	Observation
1	Colony Characteristics	NAM	Golden Yellowish and White Colonies
		Blood agar	Zone of β – heamolysis
2	Microscopic Characteristics	Gram Staining	Gram positive
		Motility	Non motile
		Shape	Ovoid and spherical
3	Biochemical Characteristics	Fermentation test	
		1-glucose	Acid
		2-sucrose	Acid
		3-lactose	Acid
		4-mannitol	Acid
		IMVIC test	
		1-Indole production	-
		2-Methyl Red(MR) test	-
		3-Voges-Prostauer(VP)	-
		4-Citric acid production	-
		Catalase activity	+
		Carbohydrates metabolism	-
		Starch hydrolysis	+
		Protein hydrolysis	+
Urease production	-		

(+) = Positive result, (-) = Negative result

Table 2. Antibacterial activity of different alcoholic dilution of plants extract against *Staphylococcus aureus*.

Extract dilution Concentration (%)	Zone of inhibition(mm)					
	Aloevera	Amaltas	Chitrak	Garlic	Mulethi	Sadabahar
100	11	9	13	13	15	8
50	9	8	12	9	12	7
25	7	7	10	8	11	8
12.5	6	7	10	7	10	6
Reference antibiotic (Ciproflaxacin)	28	27	25	25	20	25

Table 3. Multidisc for antimicrobial activity showing influence of effective antibiotics against *Staphylococcus aureus*.

Antimicrobial agents	Code	Content (μg)	Zone of inhibition (mm)
Ciprofloxacin	CP	5	25
Cefotaxime	CF	30	23
Tetracycline	TE	30	10
Cephalexin	PR	30	16
Co-trimoxazole /Ampicillin	BA	25	11
Salbactam	AS	20	21
Ofloxacin	OF	5	17
Pefloxacin	PF	10	20
Roxythromycin	RF	15	14
Lincomycin	LM	2	13
Gentamycine	GN	10	15
Cloxacillin	CX	1	18

Table 4: MIC (Minimum Inhibitory Concentration) of *Glycyrrhiza glabra* against *Staphylococcus aureus*.

Concentration of plant extract (%)	Zone of inhibition (mm)
	Mean \pm Std Error
6	8.6 \pm 0.66
12	10.3 \pm 0.33
18	11.6 \pm 0.66
25	12.3 \pm 0.66
36	12.6 \pm 0.33
50	14.0 \pm 0.0
60	14.0 \pm 0.0
72	16.3 \pm 0.33
84	17.6 \pm 0.33
100	21.3 \pm 0.66
Control Ethyl alcohol	nil

CONCLUSION

Due to indiscriminate use of antimicrobial drugs microorganism develop resistance to many antibiotics. In addition to this many of them are known to have side effects. Therefore is a need to screen local medicinal plants for the possible antibacterial properties. In present study aqueous extract of *Glycyrrhiza glabra* has high antibacterial activity against the *Staphylococcus aureus*. The all plant exreact are having great antimicrobial potential and can be used as raw materials for herbal therapy of infection.

REFERENCES

1. Bauer A.W., Kirby W.M.M., Sherris J.C., Truck M", Antibiotic susceptibility testing by a standardized single disk method,(1966).
2. Chaudhary P., Singh P", Antibacterial Potential Of Cinnamomum Tamala Extract And Its Chemical Analysis By GC-MS. International Journal of Pharmaceutical Research Scholars,(2014). V-3,1-2 2014.
3. Fransworth N. R., and Bingel,A.S", New natural products and plants drugs with pharmacological ,biological or therapeutical activity .Wanger and Wolff,springer, New York,(1977).pp-61-73.
4. Krishnaraju A.V., Rao T.V.N., Sundararaju D", Assessment of bioactivity of Indian medicinal plants using Brine shrimp (*Artemia salina*) lethality assay. Int. J. Appl. Sci. Eng., 2: 125-134. Lee S-H, Chang K-S, Su M-S, Huang Y-S, Jang H-D (2007). Effects of some Chinese medicinal plant extracts on five different fungi. Food Control,(2007). 18: 1547-1554.
5. Lee S.H, Chang K.S, Su M.S, Huang Y.S, Jang H.D", Effects of some Chinese medicinal plant extracts on five different fungi. Food Control,(2007) 18: 1547-1554.
6. Lwu M.W., Duncan A.R., Okunji O.C", New antimicrobial of plant origin. In: Perspective on new crops and new uses. (Eds),African Journal of Pure and Applied Chemistry,(1999). 5(5): 119-122.
7. Mowrey.D",the scientific validation of herbal medicine ,keak publishing USA,1986.
8. Peach K. and Tracey M. V., Triterpenes and Saponins. In; K. Peach and M. V. Tracey, Eds.Modern Methods of Plant analysis, Vol-III, I ed., Springer Verlag, Berlin, 64-65 (1955).
9. Rami E, Sipai S, Patel I. Studies on qualitative and quantitative phytochemical analysis of *Piper longum linn*. Int J Pharma Bio Sci,(2013).4(3):1381-1388.
10. Rolinson G.N., Russell, E.J", New method for antibiotic susceptibility testing.Antimicrob.Agents Chemother.(1972).2,51-56.
11. Rojas A., Hernandez L., Pereda-Miranda R., Mata R", (1992). Screening for antimicrobial activity of crude drug extracts and pure natural products from Mexican medicinal plants. J. Ethnopharmacol,(1992). 35: 275-283.
12. Seth Vimlesh., Ghosh Roy B.,Walia B.N.S and Ghai O.P",Indian J.Med.(1973).Res,61:910.
13. Shaik D., Malika FA., Rafi SM., Naqui B", Studies of antibacterial activity of ethanolic extract from *Nerium indiCum* and *Hibiscus rosasinensis*. J Islamic Acad Sci, 1994; 7: 167-8.
14. Singh P., Trivedi B., and Sood H", In vitro antibacterial activity of *Piper Longum L* And *Ficus Religiosa* Against *Escherichia Coli*. International Journal Of Institutional Pharamacy and Life Science,(2005). S((5):may -june2015.
15. Trivedi C., Shinde S.,Sharma RC", Preliminary phytochemical and pharmacological studies on *Ficus racemosa* (Gular). Indian J.Med . Res.1969;57:1070-1074.
16. Westh H., Zinn C.S., Rosdahl V.T", An international multicenter study of antimicrobial consumption and resistance in *Staphylococcus aureus* isolates from 15 hospitals in 14 countries. Microb. Drug Resist,(2004). 10: 169-176.