

## STUDIES ON *IN VITRO* SCREENING OF SOME MEDICINAL PLANT EXTRACTS FOR THEIR ANTIMICROBIAL ACTIVITIES.

S.S. PATOLE

DEPARTMENT OF ZOOLOGY, S.G. PATIL ASC COLLEGE,  
SAKRI-.

(e-mail : sspatole63@gmail.com)

The present study was conducted to evaluate the *in vitro* screening of antimicrobial activities of some medicinal plants against the four bacterial (*Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*) and two fungal (*Aspergillus niger* and *Penicillium chrysogenum*) species. The solvent extracts like aqueous, acetone, methanol, hexane and chloroform were tested for antimicrobial activities by disc diffusion method. The results showed that, among the plant tested, four medicinal plants i.e. *Adhatoda vasica*, *Alternanthera sessilis*, *Plumbago zelanica* and *Tridax procumbens* had strong inhibitory effect and inhibit the growth of microbes. Other plants like *Phyllanthus niruri* and *Withania somnifera* could inhibit moderate activity; whereas remaining plants had lowest activity. Further, among the extracts used, the antimicrobial activities were found to be better in polar solvents like aqueous extract and least in non-polar solvent extracts. Except *Adhatoda vasica* and *Tridax procumbens*, most of plants revealed antibacterial activity than antifungal.

**Key Words:** Antimicrobial, disc diffusion, *in vitro* screening, solvent extracts, medicinal plants, polar solvents.

### INTRODUCTION

India is profusely rich in the history of medicinal plants and its 75 % folk population is still using herbal preparations in the form of powder, extract and decoction, because they are easily available in nature and native have stronger faith on traditional plants (Rakesh *et al.*, 2009; Patole & Mahajan, 2009). In Indian medicinal system like ayurvedic, different parts of plants are employed as medicine to cure various diseases (Kumar *et al.*, 2007; Sharma *et al.*, 2009; Patil & Patole, 2010). These medicinal plants produce a number of secondary metabolites viz. glycosides, alkaloids and flavonoids, which are important for its medicinal values (Ravikumar *et al.*, 2005). Presently, many drugs from medicinal plants continue to occupy an important niche in modern medicine. On a global basis more than hundred drugs, all single chemical entities extracted from higher plants are currently in use, though some of these are now being made synthetically for economic reason (Karuppusamy & Karegam, 2005; Kumar *et al.*, 2007; Gaherwal *et al.*, 2010).

Khandesh region have a rich flora and broad tradition of medicinal plants (Patil, 2003; Mali *et al.*, 2006). We have (Patole & Mahajan, 2009) reported better antimicrobial potential of some medicinal plants from this region. In present investigation my aims is to prepare the solvent extracts of locally available 10 plants, which have medicinal properties and tested *in vitro* antimicrobial activities against two gram positive (*Bacillus subtilis* and *Staphylococcus aureus*), two gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacterial species and two fungal species (*Aspergillus niger* and *Penicillium chrysogenum*). Further, zone of inhibition of these plant extracts were compared with standard antibiotics like Oxytetracycline and Gentamicin.

## MATERIALS AND METHODS

**Collection of plant material :** Aerial part of fresh plant material was collected from near by places of Sakri (Dist- Dhule, M. S.). After confirmation of its identity and due identification, collected material in bulk, cleaned thoroughly with water and subsequently dried under shade. The dried materials were pulverized in a mechanical grinder to obtain coarse powder.

**Preparation of solvent extracts :** Powdered plant material (100 g) was successively extracted with 500 ml each of water, acetone, methanol, chloroform and hexane in a soxhlet apparatus for 72 h. The liquid extracts were concentrated separately under vacuum evaporator at 30 °C, the resulting dried extracts were preserved in a desiccator at 4 °C until further use. For antimicrobial screening, 1 % solvent extract was prepared freshly before use.

**Standard antibiotics :** The standard antibiotics such as Oxytetracycline (antibacterial) and Gentamicin (antifungal) were purchased from chemist shop and used to compare their zone of inhibition with various solvent extracts of experimental plants.

**Microorganisms and screening :** The microorganisms used include four bacterial species (*Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*) and two fungal species (*Aspergillus niger* and *Penicillium chrysogenum*). Strains of the microorganisms were obtained from Department of microbiology, Mooljee Jaitha College, Jalgaon (M.S.). The bacterial and fungal species were maintained on Nutrient agar medium (Himedia, M 012) and Potato Dextrose agar media (Himedia, M 096) respectively. *In vitro* screening of antimicrobial activities of plant extracts against bacterial and fungal species were employed by disc diffusion method (Bauer et al., 1996).

## RESULTS AND DISCUSSION

Table-1, depicts the *in vitro* screening of antimicrobial activities of medicinal plant extracts. The result of screening of plant extracts against the tested strains of bacteria and fungi revealed that, most of all solvent extracts of plants like *Adhatoda vasica*, *Alternanthera sessilis*, *Plumbago zelanica* and *Tridax procumbens* were inhibited higher zone of inhibition. Whereas, extracts of *Phyllanthus niruri* and *Withania somnifera* showed moderate activity. The least zone of inhibition was reported in plants viz. *Butea monosperma*, *Celosia argentea*, *Euphorbia hirta* and *Sphaeranthus indicus*. Among the solvent used, aqueous extract was most active; the order of activity were decreased from aqueous, methanolic, acetone, chloroform and hexane. This indicates that the majority of secondary metabolites could be extracted more in polar solvents. It was further reported that, the plants like *Adhatoda* and *Tridax*, showed both antibacterial and antifungal activities while remaining plants showed only antibacterial activities. The present finding is in agreement with earlier researchers (Aswal et al., 1996; Jeevan Ram et al., 2004; Ignacimuthu et al., 2006; Sharma et al., 2009; Hemashenpagam & Selvaraj, 2010). In comparison with standard antibiotics, they were showed 3 to 4 folds more zone of inhibition than plant extracts.

In nutshell, the investigation clearly revealed that, different solvent extracts of plants were tested against two gram positive and two gram negative species of bacteria and two species of fungal pathogenic strains of human being. The plant extracts showed species

**Table I :** *In vitro* antimicrobial screening some medicinal plants.

S. No.	Plant species	Nature of extract	Antimicrobial activity					
			Bacterial species				Fungal species	
			Gram positive		Gram negative		<i>A. niger</i>	<i>P. chrys.</i>
			<i>B. subtilis</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aerogi.</i>		
01	<i>Adhatoda vasica</i> Nees	Aq	++	++	+	+	+	+
		Ac	++	++	-	+	+	-
		Met	+	+	+	+	+	-
		Hex	+	+	+	+	-	-
		Chl	+	+	-	+	+	+
02	<i>Alternanthera sessilis</i> Linn	Aq	+	++	++	+	++	+
		Ac	+	+	+	+	-	-
		Met	-	+	+	+	-	-
		Hex	-	+	-	+	-	-
		Chl	+	-	-	+	-	-
03	<i>Butea monosperma</i> Taub	Aq	-	-	-	-	++	++
		Ac	-	-	-	-	+	-
		Met	-	-	-	-	-	+
		Hex	+	+	-	-	-	-
		Chl	-	-	-	-	-	-
04	<i>Celosia Argentea</i> Linn	Aq	+	-	++	-	+	+
		Ac	+	-	+	-	-	-
		Met	-	-	+	-	-	-
		Hex	-	-	-	-	-	-
		Chl	+	-	+	-	-	-
05	<i>Euphorbia Hirta</i> Linn	Aq	++	+	+	-	-	-
		Ac	-	-	-	+	-	-
		Met	-	-	-	+	-	-
		Hex	-	-	-	-	-	-
		Chl	-	-	-	-	-	-
06	<i>Phyllanthus Niruri</i> Linn	Aq	+++	++	+	++	+	++
		Ac	+++	-	-	+	-	-
		Met	+	-	-	-	++	+
		Hex	-	-	-	-	-	-
		Chl	-	-	-	-	-	-
07	<i>Plumbago Zelanica</i> Linn	Aq	++	+	++	+	+	+
		Ac	++	++	-	-	-	-
		Met	-	+	+	++	+	-
		Hex	-	+	-	+	-	-
		Chl	++	+++	++	+	+	-
08	<i>Sphaeranthus Indicus</i> Linn	Aq	+	+	+	-	+	-
		Ac	-	-	+	-	-	-
		Met	-	-	++	-	-	-
		Hex	-	-	-	-	-	-
		Chl	-	-	+	-	+	-

09	<i>Tridax procumbens</i> Linn.	Aq	+++	+	++	++	++	++
		Ac	+	-	-	+	-	+
		Met	+	-	+	+	+	+
		Hex	-	-	-	-	-	+
		Chl	-	-	-	-	+	+
10.	<i>Withania Somnifera</i> Dunal	Aq	++	++	++	++	-	-
		Ac	++	+++	+++	++	+	-
		Met	+	-	+++	+	++	-
		Hex	+	-	++	+	-	-
		Chl	+	+	++	+	-	-
11.	Oxytetracycline (antibacterial)	-	+++	+++	+++	+++	-	-
12.	Gentamicin (antifungal)	-	-	-	-	-	+++	+++
	Control	-	-	-	-	-	-	-

Antimicrobial activities (Zone of inhibition in mm, mean  $\pm$  SD (n = 3))

Nature of extracts : Aq : Aqueous; Ac : Acetone; Met : Methanol; Hex : Hexane; Chl : Chloroform.

Activities :

Nil activity =

Activity between 6.0 to 9.0 mm = +

Activity between 9.1 to 12.0 mm = ++

Activity between 12.1mm onwards = +++

Bacterial species: Gram positive i) *Bacillus subtilis* and ii) *Staphylococcus aureus*.

Gram negative i) *Escherichia coli* and ii) *Pseudomonas aeruginosa*

Fungal species: i) *Aspergillus niger* and ii) *Penicillium chrysogenum*.

specific and solvent specific susceptibility. These tested extracts were most active and their promising antimicrobial activities indicating the potential for discovery of antimicrobial principles. Therefore, further phytochemical studies are required to determine the types of compounds responsible for the antimicrobial effects of these species. The results also indicate that, scientific studies carried out on medicinal plants having traditional claims of effectiveness might warrant fruitful results. Several plant species used by local tribe's exhibit some degree of antimicrobial activity toward the pathogens which causes skin diseases such as eczema, scabies, itches etc. These plants could serve as useful sources for new antimicrobial agents.

### ACKNOWLEDGEMENT

Author is thankful to principal, S.G. Patil College, Sakri for providing necessary laboratory facilities. Thanks are due to the HOD, Microbiology, M.J. College, Jalgaon for providing microbial culture.

# REFERENCES

- ASWAL, B. S., GOEL, A. K., KULSHRESTHA, D. K., MEHROTRA, B. N. & PATNAIK, G. K. 1996. Screening of Indian plants for biological activity. *Ind. J. Exp. Biol.* **34**: 44-467.
- BAUER, R.W., KIRBY, M.D.K., SHERRIS, J.C. & TURCK, M. 1996. Antibiotic susceptibility testing by standard single disc diffusion method. *Am. J. Clin. Pathol.* **45** : 493-496.
- GAHERWAL, S., PRAKASH, M.M. & SHARMA, V. 2010. Anti-bacterial activity of *Eucalyptus* leaf extract against different bacteria. *Life Sci. Bull.* **7**(1) : 45-46.
- HEMASHENPAGAM, N. & SELVARAJ, T. 2010. Antimicrobial potential of *Solanum viarum* Dunal. *Geobios.* **37**(2-3) : 129-132.
- IGNACIMUTHU, S., DURAIPANDIYAN, V. & AYYANAR, M. 2006. Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamilnadu, India. *BMC Comple and Alternative medicine.* (<http://www.biomedcentral.com/1472-6882/6/35>).
- JEEVAN RAM, A., BHAKSHU, L.M. & VENKATA RAJU, R.R. 2004. *In Vitro* antimicrobial activity of certain medicinal plants from Eastern Ghats, India, used for skin diseases. *J. Ethno-pharmacology.* **90** : 353-357.
- KARUPPUSAMY, S. & KARMEGAM, N. 2005. Screening of ethno medicinal plants of Dindigul district (South India) for antimicrobial activity. *J. Ecobiol.* **17**(5) : 455-459.
- KUMAR, G.S., JAYAVEERA, K.N., ASHOK KUMAR, C.K., UMACHIGI, P. SANJAY., VRUSHABENDRA SWAMY, B.M. & KISHORE KUMAR, D.V. 2007. Antimicrobial effects of Indian medicinal plants against acne-inducing bacteria. *Trop. J. Pharm. Res.* **6** (2) : 717-723.
- MALI, R.G. HUNDIWALE, J.C. GAVIT, R.S. PATIL, D.A. & PATIL, K.S. 2006. Herbal abortifacients used in North Maharashtra. *Nat. Prod. Radiance.* **5**(4) : 315-318.
- PATIL, D.A. 2003. *Flora of Dhule and Nandurbar districts* (Maharashtra). Bishen Singh Mahendra Pal Singh, Publishers and Distributors, Dehra Dun, India.
- PATIL, S. K. & PATOLE, S. S. 2010. Ethno-medicinal studies from Charanmal ghat of Sakri tahsil. Dhulia (M.S.). *Life Sci. Bull.* Vol. **7**(1): 17-20.
- PATOLE, S.S. & MAHAJAN, R.T. 2009. Preliminary antimicrobial screening of sixty indigenous plants from Khandesh region, Maharashtra. *Uttar Pradesh J. Zool.* **30** (3) : 287-292.
- RAKESH, D., TRIPTI, T. & ANIL KUMAR. 2009. Study of antimicrobial effect of some medicinal plants. *Nat. J. Life Sci.* **6**(1) : 247-250.
- RAVIKUMAR, S., NAZAR, S., NURALSHIEFA, A. & ABIDEEN, S. 2005. Antibacterial activity of traditional therapeutic coastal medicinal plants against some pathogens. *J. Environ Biol.* **26** (2) : 383-386.
- SHARMA, D., ADITI, A.L. & SHARMA, A. 2009. *In vitro* comparative screening of antibacterial and antifungal activities of some common plants and weed extracts. *Asian. J. Exp. Sci.* **23** (1) :169-172.