RESEARCH PAPER *Asian Sciences* (June & December, 2009) Vol. 4 Issue 1 & 2 : 10-12

Antibacterial activity of *Anona squamosa and Tribulus terrestris* leaf extracts

P. GOPAL REDDY* , I. SAILAJA[1] AND I. ANAND SHAKER[2]
Department of Botany, Padmashri Vikhe Patil College, Loni, AHMEDNAGAR (M.S.) INDIA

ABSTRACT

The effect of ethanol and aqueous leaf extracts of *Anona squamosa* and *Tribulus terrestris* on the growth of Gram negative Pseudomonas aeruginosa , *Escherichia coli* and Gram positive Staphyloccous epidermidis, Streptococcus agalactiae bacterial strains was evaluated *in vitro* by agar well diffusion assay method. The extracts inhibited the growth of all test organisms. Extract of *A. squamosa* exhibited greater antibacterial activity than T.terrestris. Gram positive strains were found more sensitive than Gram negative strains. Ethanol extract showed comparatively more inhibitory effect than aqueous extract. Staphyloccous epidermidis was found more sensitive to the leaf extracts of *A.squamosa* while Streptococcus agalactiae to that of *T. terrestris*. Zone of inhibition increased with increase in concentration of the extract. The significance of these results is discussed. The results may be of importance in identification of new potential antibacterial compounds in plants.

Key words : *Anona squamosa*, *Tribulus terrestris*, Leaf extracts, Bacterial strains, Inhibition zone.

INTRODUCTION

Plants are rich in wide variety of secondary metabolites such as tannins, terpenoides, alkaloids, flavonides, phenols, essential oils which have antibacterial properties (Trease and Evans, 1972; Cowan, 1999). Many natural antimicrobial compounds can be derived from plants (Gordon and David, 2001). Plants based antimicrobials have enormous therapeutic potential and may become the base for the development of new medicines (Trease and Evans, 1972).

In recent years several workers (Ikram and Inamul, 1984; Naqvi *et al.*,1991; Samy *et al.*, 1998; Dorman and Deans, 2000; Samy and Ignacimuthu, 2000; Srinivasan *et al*., 2001; Kapoor *et al.*, 2007; Nair and Chanda, 2007a and 2007b; Seema *et al.*, 2007 and Sengottuvel *et al*., 2007) screened many plants for antibacterial properties.

In this study, leaf extract of two plants *A. squamosa* and *T. terrestris* were evaluated for potential antibacterial activity against the clinically significant two Gram negative bacterial strains, *Pseudomonas aeruginosa* ATCC27853, *Escherichia coli* ATCC25922, and two Gram positive bacterial strains *Staphylococcus epidermidis* ATCC12228, and *Streptococcus agalactiae* NCIM2401.

Anona squamosa belongs to Anonaceae family. It is cultivated mainly for the edible fruits. All parts of plant are used in natural medicine in the tropics. It is considered to be good source of natural antibacterial compounds (Oliver-Brever, 1986). *Tribulus terrestris* classified under the family Zygophyllaceae is a naturally grown wild plant native to tropical and sub tropical regions of the world. Different parts of *T. terrestris* are reported to posses antibacterial properties (Firas *et al.*, 2008).

MATERIALS AND METHODS

Fresh mature leaves of *Anona squamosa* and *Tribulus terrestris* were collected and washed in tap water. These leaves were then surface sterilised with 0.1% $HgCl_2$ for 1-2 minutes and again washed twice in sterile distilled water to ensure that the traces of $HgCl_2$ are removed and kept for drying in shade. The dried leaves were then ground into fine powder. 10 g of the powder was extracted in 100 ml ethanol and distilled water using soxhlet apparatus. Then the extracts were kept at room temperature for complete evaporation of solvent and water. The residue was mixed in appropriate amount of DMSO (Dimethyl Sulphoxide) to get the stock solutions of different concentrations *viz.*,100, 200, 300, 400, 500 µg / µl and used for determining the effect on the growth of Gram negative *Pseudomonas aeruginosa* and *Escherichia coli* and Gram positive *Staphyloccous epidermidis, Streptococcus agalactiae* bacterial strains.

The required bacterial strains procured from National Chemical Laboratory (NCL), Pune were maintained on nutrient agar slants for use as test organisms. A loopful of 24 hrs old test organisms was added in 30 ml sterile nutrient broth for activation and shaken thoroughly to obtain uniform suspension.

The antibacterial activity of the plant extract was determined following the agar well diffusion method

*** Author for correspondence.**
[1] Department of Molecular Biology and Biochemistry, College of Agricultural Biotechnology, Loni, AHMEDNAGAR (M.S.) INDIA
[2] Department of Biochemistry, Pravara Institute of Medical Sciences, Loni, AHMEDNAGAR (M.S.) INDIA

(Perez, 1990). 0.2ml of inoculum suspension containing 10^8cells / ml was inoculated in the molten Mueller-Hinton agar medium and kept on a rotary shaker. After proper homogenization, the medium was poured in sterile Petri plates and allowed to solidify. Then with a sterile cork borer a 6 mm well was made in the centre. 0.1ml of different concentrations (100, 200, 300, 400 and 500 µg/µl) of ethanol and aqueous plant extract was put in each well for bioassay. The test plates were incubated at 37^0C. Positive as well as negative control sets with Tetracycline and double distilled water in the wells, respectively were kept for each bacterial strain. After 24 hours, the diameter of zone of inhibition was measured.

RESULTS AND DISCUSSION

The ethanol and aqueous leaf extracts of *A. squamosa* and *T.terrestris* inhibited the growth of all test organisms thus demonstrating antibacterial properties of plants (Table 1 and 2). However the growth of Gram positive strains were found more inhibited than Gram negative strains. Nair and Chanda (2007b) also reported Gram positive strains were more sensitive. Ethanol extract showed more effect than aqueous extract in inhibiting the growth of the bacterial strains which is in confirmity with the investigations reported by Nair and Chanda (2007a) and Firas *et al.* (2008). At higher concentrations (400 and 500 ug/ul), *Staphyloccous epidermidis* was found more sensitive to the leaf extract of *A.squamosa* while *Streptococcus agalactiae* to that of *T.terrestris*. Diameter of the zone of inhibition was directly proportional to the concentration of the extract.

The drug resistant bacteria *E.coli* and *P. aeruginosa* were also inhibited by the extracts. But Gislene *et al.* (2000) also reported that *E.coli* did not show any sensitivity to the plant extracts.

Table 1: Effect of ethanol and aqueous leaf extracts of *A. squamosa* on the growth of bacteria

Sr. No.	Extract (µg/µl)	Zone of inhibition in (mm)							
		Pseudomonas aeruginosa		*Escherichia coli*		*Staphylococcus epidermidis*		*Streptococcus agalactiae*	
		A	B	A	B	A	B	A	B
1.	100	9	6	10	7	13	9	11	8
2.	200	10	7	12	8	14	11	13	10
3.	300	13	10	14	11	16	13	15	12
4.	400	16	14	15	13	19	16	18	15
5.	500	18	15	17	15	21	18	20	17
6.	+ve Ctrl tetracycline (500µg/µl)	12		11		17		16	
	-ve Ctrl Double distilled water	Nil		Nil		Nil		Nil	

A= Solvent extract. B= Aqueous extract

Table 2: Effect of ethanol and aqueous leaf extracts of *T. terrestris* on the growth of bacteria

Sr. No.	Extract (µg/µl)	Zone of inhibition in (mm)							
		Pseudomonas aeruginosa		*Escherichia coli*		*Staphylococcus epidermidis*		*Streptococcus agalactiae*	
		A	B	A	B	A	B	A	B
1.	100	7	6	8	6	9	7	9	8
2.	200	8	6	8	7	10	7	11	9
3.	300	10	7	9	7	11	8	12	10
4.	400	12	9	11	8	14	9	13	12
5.	500	13	9	12	9	15	11	17	13
6.	+ve Ctrl tetracycline (500µg/µl)	12		11		17		16	
	-ve Ctrl Double distilled water	Nil		Nil		Nil		Nil	

A= Solvent extract. B= Aqueous extract

Conclusion :

Investigations indicate that the higher plants are a vast untapped source for medicines and have an enormous therapeutic potential, they represent an alternative source for obtaining a great number of antimicrobial compounds. Continuous and further exploration of plant antimicrobials is thus needed.

Acknowledgement :

The authors are thankful to the Heads of the concerned institutions for encouragement and providing all the necessary facilities.

REFERENCES

Cowan Marjorie Murphy (1999). Plant products as antimicrobial agents.*Clinical Microbiol. Reviews,* **12**(4) : 564-582.

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

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.

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.

Gordon, M.C. and David, J. N. (2001). Natural product drug discovery in the next millennium. *Pharm Biol.,* **139** : 8-17.

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

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

Nair Ratish and Chanda Sumitra, V.(2007b). *In vitro* antimicrobial activiy of *Psidium guajava leaf* extracts against clinically important pathogenic microbial strains. *Brazilian J. Microbiol*., **38** : 452-458.

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.

Oliver-Brever (1986). *Medicinal plants in tropical West Africa*. Cambridge University press, Cambridge (U.K.).

Perez, C., Paul, M. and Bazerque, P.(1990). Antibiotic assay 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.

Srinivasan,D., Nathan,S., Suresh,T. and Lakshman Perumalswamy, D.(2001). Antimicrobial 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.

Accepted : September, 2009