Asian Journal of Bio Science, **Vol. 3 No. 2 : 333-336 (October, 2008)**

Antibacterial activity of *Aegle marmelos* correa leaves extract

C.C. GAVIMATH[1], Y.L. RAMACHANDRA[1]*, S. PADMALATHA RAI[2], H.V. SUDEEP[1], P.S. SUJAN GANAPATHY[1] **AND** B.T. KAVITHA[1]

[1]Department of Biotechnology and Bioinformatics, School of Biological Sciences, Kuvempu University, Jnana Sahyadri, SHIMOGA (KARNATAKA) INDIA

[2]Department of Biotechnology, Manipal Life Science Center, Manipal University, MANIPAL (KARNATAKA) INDIA.

(Accepted : September, 2008)

Many herbal remedies individually or in combination have been recommended in various medical treatises for the cure of different diseases. The therapeutic value of *Aegle marmelos* Correa (Rutaceae) commonly known as 'Bael' has been recognized in different system of traditional medication for the treatment of different diseases and ailments of human beings. The phytochemical and antibacterial studies of the leaf extracts of *A. marmelos* have been investigated. The antibacterial activity was investigated against multi resistant strains of *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Salmonella typhi, Proteus vulgaris, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* by disc diffusion method. The results showed that the antibacterial efficacy of petroleum ether extract of leaf was significant and nearer to the standard antibiotic Streptomycin tested. Further the activity was more pronounced in gram-negative strains and moderate in case of gram-positive strains studied. The study results suggest that this plant is promising in the development of phytomedicine for bacterial diseases.

Key words : Antibacterial activity, Bioactive components, Multi resistant strains, *Aegle marmelos*, Disk diffusion method.

INTRODUCTION

Finding healing powers in plants is an ancient idea. Since the advent of antibiotics in the 1950s the use of plant derivates as antimicrobials has been virtually non existent. World is endowed with rich wealth of medicinal plants. Plants have always been the principal form of medicine in India and presently they are becoming popular throughout the world, as people strive to stay health in the face of chronic stress and pollution and to treat illness with medicines that work in count with the body's own defense (Perumalsamy *et al*., 1998). Though the recovery is slow, the therapeutic use of medicinal plant is becoming popular because of its inability to cause the side effects and antibiotic resistant microorganisms (Rawat and Uniyal, 2003).

Antimicrobial properties of medicinal plants being increasingly reported from different parts of the world (David and Clark, 1998; Aswal *et al*., 1996; Ahmad *et al*., 1998). The traditional treatment approach is of much significance, especially in India due to the endemic presence of infective gastro intestinal diseases which are the major causes of infant and adult mortality (Miranda *et al*., 1993).

Aegle marmelos Correa belongs to the family Rutaceae. It is commonly called Bilwa or Bael and it is found throughout India. It is a medium to fairly large sized deciduous and glabrous tree bearing axillary spines and usually trifoliate leaves. Bael leaves are extremely useful for treating diabetes, jaundice, cholera, typhoid, asthma. The leaves are made into a poultice and used in the treatment of ophthalmia or severe inflammation of the eyes or conjunctiva with acute bronchitis and inflammation of the body. The decoction of leaves is useful for intermittent and is an expectorant or promotes the removal of mucous secretions from the bronchial tubes.

Considering the folkloric use of this species to treat infectious diseases stimulated the investigation of the antibacterial activity of the different polar solvent extracts from *A. marmelos* leaves against standard Gram-positive and Gram-negative human pathogenic bacteria including multi resistant strains.

MATERIALS AND METHODS

Collection and processing of plant materials:

The leaves of *A. marmelos* were collected from their natural habitat in the Western Ghats. The plant materials were thoroughly washed with distilled water and then dried under shade for about 10 days. The dried leaves were powdered and stored in air sealed plastic container at room temperature till the time of extraction. The dry leaf powder was subjected for soxhlet extraction using organic solvents *viz*. petroleum ether, Chloroform,

* Author for correspondence.

methanol, ethanol and water based on polarity. The extracts were evaporated to dryness under reduced pressure using Rotavapor.

Phytochemical screening:
The leaf extracts were subjected for preliminary phytochemical screening using standard methods of analysis for the presence of alkaloids, flavanoids, anthocyanin, carboxylic acid, coumarins, steroids, phytosterols and Xanthoproteins.

Determination of antibacterial activity:
The antibacterial tests were carried out against Gram-positive and Gram-negative bacteria. The pure cultures of bacteria were obtained from NCL Pune, Maratha Mandal Medical College, Belgaum. The antibacterial activity of leaf extracts were tested against each multi resistant bacterium by disc agar diffusion method (Berghe and Vlietinck, 1991; Cappuccino and Sherman, 1998). The nutrient broth was used to culture bacteria at 37°C for 24 hours. Sterilized nutrient agar medium was poured into the Petri plates to form a uniform depth of 5mm and allowed to solidify. Then 100µl of bacterial suspension was spread over the surface of each nutrient agar plate using a sterile cotton swab by swab culture technique. Then 20 µl of extract was loaded on each 6 mm diameter sterile Whatman No.1 filter paper disc, which was then aseptically placed on the surface of the nutrient agar at spaced intervals. The plates were incubated at 37°C for 24 hours. 20µl of sterile water was used as negative control and standard antibiotic Streptomycin (50mg/ml) as a positive control. Microbial growth inhibition was determined as the diameter of the inhibition zones around the discs. All tests were performed in triplicate. The resultant clear zones around the discs were measured in mms.

RESULTS AND DISCUSSION

The leaf extracts were subjected to preliminary phytochemical screening. Petroleum ether extract showed the presence of alkaloids, phenols, sterols and anthocyanins. Chloroform extract showed the presence of alkaloids, carboxylic acid, phenols and xanthoproteins. Methanol extract showed the presence of carboxylic acid, sterols and anthocyanins. Ethanol extract showed the presence of phenols, anthocyanins and xanthoproteins. Aqueous extract show the presence of phenols and anthocyanins (Table 1).

The antibacterial activity of the crude extracts of *A. marmelos* was determined against 7 strains which include Gram-negative and Gram-positive bacteria. The results revealed that the petroleum ether extract exhibited significant antibacterial activity against *Pseudomonas aeruginosa, Salmonella typhi, Klebsiella pneumoniae, Proteus vulgaris, Escherichia coli* and moderate activity against *Staphylococcus aureus* and *Bacillus subtilis*. The chloroform, methanol and ethanol extracts exhibited moderate antibacterial activity against all the seven types of bacteria. The aqueous extract exhibited least antibacterial activity against all the seven types of bacteria (Table 2).

Table 1: Preliminary phytochemical screening of Leaf extracts of *A. marmelos*

Phytochemicals	Petroleum ether	Chloroform	Methanol	Ethanol	Aqueous
Alkaloids	+	+	-	-	-
Carboxylic acid	-	+	+	-	-
Coumarins	-	-	-	-	-
Flavanoids	-	-	-	-	-
Phenols	+	+	-	+	+
Sterols	+	-	+	-	-
Anthocyanins	+	-	+	+	+
Xanthoproteins	-	+	-	+	-

+= Present, – = Not determined

Among the five leaf extracts used in the study, petroleum ether extract displayed maximum antibacterial activity against all the strains tested. Among the seven bacterial strains screened *Pseudomonas aeruginosa, Salmonella typhi* and *Klebsiella pneumoniae* were the most susceptible to this extract (30.00±0.61, 29.32±0.46 and 27.95±0.59, respectively). Most antimicrobial medicinal plants are more effective against Gram-positive than Gram-negative bacteria (Lin *et al*., 1999; Srinivasan *et al*., 2001). However, present current findings showed remarkable activity against Gram-negative bacteria including multi resistant Gram-negative strains. The results obtained suggested a potential application of A. marmelos for treatment and further investigations should be conducted in order to explore complete usage of this plant. Other medicinal plants containing phenolic compounds including tannins as major constituents are used topically for care and repair of skin wounds (Dweck, 2002). Tannins are considered nutritionally undesirable because they precipitate proteins, inhibit digestive enzymes and affect the absorption of vitamins and minerals. Further benefits include the absence of adverse effects and a low incidence of resistance (Spann *et al*., 2003). Antibiotic resistant bacteria continue to emerge rapidly, constituting a problem of increasing significance in common

Table: 2 Antibacterial activity of leaf extracts of *A. marmelos*

Bacterial strains tested	Leaf extracts used	Diameter of zone of inhibition in mm[(a)]	
		Leaf extract [(b)]	Positive control [(c)]
	Petroleum ether	13.33±0.33	
	Chloroform	8.29 ±0.15	
Staphylococcus aureus	Methanol	12.39±0.24	17.08±0.46
	Ethanol	10.01±0.18	
	Aqueous	7.57±0.67	
	Petroleum ether	16.67±0.33	
	Chloroform	14.41±0.48	
Bacillus subtilis	Methanol	13.22±0.56	25.12±0.88
	Ethanol	15.24±0.12	
	Aqueous	10.86+0.27	
	Petroleum ether	25.73±0.33	
	Chloroform	20.44±0.59	
Escherichia coli	Methanol	22.35±0.33	27.12±0.29
	Ethanol	24.68±0.60	
	Aqueous	19.00±0.24	
	Petroleum ether	29.32±0.46	
	Chloroform	18.45±0.17	31.07±0.33
Salmonella typhi.	Methanol	21.27±0.68	
	Ethanol	24.86±0.24	
	Aqueous	17.21±0.38	
	Petroleum ether	25.79±0.42	
	Chloroform	23.22±0.35	
Proteus vulgaris	Methanol	20.56±0.67	27.48±0.26
	Ethanol	24.76±0.59	
	Aqueous	20.80±0.41	
	Petroleum ether	30.00±0.61	
	Chloroform	24.65±0.35	
Pseudomonas aeruginosa	Methanol	24.11±0.29	31.97±0.68
	Ethanol	26.69±0.44	
	Aqueous	19.01±0.12	
	Petroleum ether	27.95±0.59	
	Chloroform	24.61±0.23	
Klebsiella pneumoniae	Methanol	26.54±0.81	29.06±0.37
	Ethanol	22.10±0.66	
	Aqueous	20.84±0.14	

(a)– Experiments were done in triplicates and the results are mean ± standard error values
(b) – 100mg/ml of leaf extract. (c) – Positive control (50mg/ml of Streptomycin)

pathogenic bacteria such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* which are predominant organisms, showed increased resistance to commonly used antibiotics (Valencia *et al*., 2004).

REFERENCES

Ahmad, I., Mehmood, Z. and Mehmood, I. (1998). Screening of some Indian medicinal plants for their antimicrobial properties. *J. Ethanopharmacol.*, **62** : 183-193.

Aswal, B.S. Goe, A.K. and Patneik, G.K. (1996). Screeing of Indian medicinal plants for biological activity. *Indian J. Exptal. Biol.*, **34** : 444- 467.

Berghe, D.A.V. and Vlietinck, A.J. (1991). Screening methods for antibacterial and antiviral agents from higher plants, in *Methods in plant Biochemistry* (Eds. P. M. Dey and J.B. Harborne), Vol. **6**, Academic Press, London, pp. 47-69.

Cappuccino, G. and Sherman, N. (1998). *Microbiology: A Laboratory Manual*, Benjamin Cumming Science Publishing, California, p. 254.

David, L. Lentz and Clark Alice, M. (1998). Antimicrobial properties of medicinal plants. *J. Ethnopharmacology,* **65** : 75-81.

Dweck, A.C. (2002). Herbal medicine for the skin. Their chemistry and effects on skin and mucous membranes. Personal care Mag., **3** : 19-21.

Lin, J., Opoku, A.R., Geheeb-Keller, M., Hutchings, A.D., Terblanche, S.E., Jager and A.K., Van Staden, J. (1999). Preliminary screening of some traditional zulu medicinal plants for anti-inflammatory and anti-microbial activities. *J. Ethnopharmacol,* **68** : 267-274.

Miranda, L., Peria Sathyavathi, M., Sirasat Antarkar, D.S. and Vaidya, A.B. (1993). *In vitro* action of selected medicinal plants against microorganisms involved in human gastro intestinal infections. *J. Res. Ayurveda sidha,* **8** : 149-153.

Perumalsamy, R., Ignacimuthu, S. and Sem, A. (1998). Screeing of 34 Indian medicinal plants for antibacterial properties. *J. Ethnopharmacol,* **62** : 173 – 182.

Rawat, R.B.S. and Uniyal, R.C. (2003). National Medicinal plants Board committed for overall development of the sector. *Agro Bios. Med. Plants,* **1** : 12 -16.

Spann, C.T., Tutrone, W.D. and Weinberg, J.M. (2003). Topical antibacterial agents for wound care: a primer. *Dermatol. Surg.,* **29** : 620-626.

Srinivasan, D., Nathan, S., Suresh, T., Perumalsamy, O. (2001). Anti-microbial activity of certain Indian medicinal plants used in folkloric medicine. *J. Ethnopharmacol.,* **74** : 217-220.

Valencia, I.C., Kirsner, R.S. and Kerdel, F.A. (2004). Microbiologic evaluation of skin wounds: alarming trend toward antibiotic resistance in an inpatient dermatology service during a 10-year period. *J. Am. Acad. Dermatol.,* **50** : 845-849.