SCREENING OF DIFFERENT INDIAN MEDICINAL PLANTS FOR ANTIBACTERIAL ACTIVITY AGAINST HUMAN AND ANIMAL PATHOGENS

SUJITH, S, SREEDEVI, R,SUJA R. S,ANUSREE G. K, JULIET,ANEESHA,V. A, RESHMA, R. SHAMEER, M

Abstract: The methanolic and aqueous extracts of different Indian medicinal plants were tested for antibacterial activity against various human and animal pathogens using agar gel diffusion test and the minimum inhibitory concentrations were assessed using broth dilution technique. The results showed the presence of antibacterial activity majorly against gram positive organisms, the most susceptible ones being *S. aureus* susceptible to 14 tested extracts and *S. pyogenes* susceptible to 12 extracts. The gram negative organisms were susceptible to the extracts of *Annona squamosa* only. The leaf extracts of *Mallotus philippensis* and *Allophyllus cobbe* and seed coat of *Tamarindus indica* were the most potent ones among the tested plants paving way for the identification of novel herbal antibacterial agents.

Key words: Antibacterial, Medicinal plants, Minimum inhibitory concentration, *S. aureus*.

Introduction: Nature has been a source of medicinal agents for thousands of years to combat various ailments of the world [1]. The medicinal values of these plants is attributed to the phytochemical active substances that produce a definite physiological action on human body viz alkaloids, tannins, flavonoids, phenolic compounds etc [2]. Use of local traditional herbs as primary health remedies is highly prevalent in Asia, Latin America and Africa and has been proclaimed as leads for new antimicrobial therapeutics. Moreover upsurge in the incidence of antimicrobial resistance to many of the commercial antibiotics also triggered an elaborate research on plant based antimicrobial agents [3]. About 80% of individuals from developed countries use traditional herbal medicine, a source of novel drug compounds for the welfare of human health and should be hence investigated to understand their properties, safety and efficacy in order to identify and develop new potent antimicrobial compounds and fractions [4].

Materials and Methods: Plant materials Leaves of *Azadirachta indica, Annona squamosa, Senna alata, Allophyllus cobbe, Mallotus philippensis, Murraya paniculata, Vitex negundo, Chromoleana odorata*, whole plant of *Smithia sensitiva* and seed coat of *Tamarindus indica* were collected from different localities of the district of Wayanad (Kerala) during December 2012 to April 2013 and identified at MS Swaminathan Research Foundation, Kalpetta.

Extraction of plant material: For the preparation of methanolic extract, the air dried and powdered plant materials (100 g) were extracted using methanol in a soxhlet and evaporated to dryness using rotary vacuum evaporator and stored under refrigeration where as 250 g of each plant material were mixed with 5 times water and a decoction was prepared by boiling to produce the aqueous extract. The extract was dried using rotary vacuum evaporator and stored under refrigeration.

Antimicrobial assay: Bacterial strains Tests were performed against various bacterial strains listed in table 1.

Table 1: List of microorganisms used for antibacterial assay

Sl No.	Name of the organism	MTCC no
1	*Escherichia coli*	40
2	*Salmonella typhimurium*	3224
3	*Pasteurella multocida*	1148
4	*Pseudomonas aeruginosa*	4999
5	*Staphylococcus aureus*	3160
6	*Streptococcus pyogenes*	1928
7	*Listeria monocytogenes*	657
8	*Enterococcus faecalis*	9845
9	*Klebsiella pneumonia*	9828

.

Antibacterial screening: The extracts were dissolved in 10% DMSO/ tween 80 solutions to get concentrations of 500, 250, 100, 50, 25 and 12.5 mg/ml of the solution. In all the tests 10% DMSO/ tween 80 solution was kept as negative control. Octadisc (Himedia) containing amoxicillin 10 mcg, tetracycline 30 mcg, co- trimoxazole 25 mcg, ciprofloxacin 5 mcg, gentamycin 10 mcg, erythromycin 15 mcg, chloramphenicol 30 mcg and cefalexin 30 mcg were used as positive control.

Antimicrobial assay was performed in Muller Hinton (MH) agar plates. Microbial cultures with 0.5 McFarland Standard turbidity equivalents were prepared by inoculating different cultures in nutrient broth and were further diluted to 1:10 to get a concentration of 106 CFU/ml. About 0.2 ml of the diluted inoculums was applied directly to the plate and spread using a sterile L- shaped spreader. Wells were bored into the agar using a sterile 6 mm well borer and the wells were filled with 25 µl of the DMSO/ tween 80 diluted extracts in different concentrations and incubated at 370 C for 24 hrs. Inhibition zones were measured and recorded as the mean diameter (mm) of complete growth inhibition control [5]. The minimum inhibitory concentration of different test extracts was assessed in 96 well microtitre plates. 100 µl of MH broth was mixed with equal volume of the extract. The dose range was selected based upon the last concentration that showed antibacterial property and the first concentration that did not show inhibition and consequently five equal dilutions in the same range was prepared to calculate the MIC. 100 µl of the inoculums specimens were added to each well and incubated for 24 hrs at 37°C. Microbial growth was observed by the presence of turbidity. The lowest concentration that showed no growth was selected as the MIC [6]

.

Results:

Table 2: Percent yield of extract from different plants

Plant	Part tested	Type of extract	% yield
Azadirachta indica	Leaf	Alcoholic	15.17
		Aqueous	13.12
Tamarindus indicus	Seed coat	Alcoholic	32.98
		Aqueous	16.30
Mallotus philippensis	Leaf	Alcoholic	12.53
		Aqueous	10.49
Allophyllus cobbe	Leaf	Alcoholic	17.08
		Aqueous	10.00
Vitex negundo	Leaf	Alcoholic	44.10
		Aqueous	14.08
Smithia sensitiva	Whole plant	Alcoholic	12.12
		Aqueous	8.34
Annona squamosa	Leaf	Alcoholic	10.11
		Aqueous	14.70
Murraya paniculata	Leaf	Alcoholic	14.69
		Aqueous	13.01
Chromoleana odorata	Leaf	Alcoholic	13.20
		Aqueous	13.88
Senna alata	Leaf	Alcoholic	16.89
		Aqueous	14.78

In the present study the invitro antibacterial activity of 20 different extracts was qualitatively and quantitatively assessed against nine different pathogens of veterinary and human importance by the presence or absence of inhibition zones and MIC values. Extracts showing an inhibition zone of more than 11 mm were selected and the MIC values were used for comparison between extracts. According to the results presented in table 2, the extracts of the plants showed antibacterial activities against one or more bacterial strains. The data indicated variation in sensitivity of the different bacterial strains to the different extracts. The gram negative organisms were sensitive to the aqueous extract of *A. squamosa* where as *S. aureus* and *S. pyogenes* were sensitive to 14 and 12 extracts respectively. The inhibitory activity was

seen from a range of 500 mg/ml to 12.5 mg/ml.

Discussion: Emergence of multidrug resistant bacteria as well as undesirable side effects of various drugs has triggered immense interest in development of novel antimicrobial drugs from plant origin. In the present study ten different plants commonly used by traditional healers as well as in Ayurvedic medicine were tested for their antibacterial activity invitro against different human as well as animal pathogens (Table 1). The methanolic and aqueous extracts of the tested plants were active mainly against G+ve pathogens, predominantly *S. aureus* and *S. pyogenes*. *S. pyogenes* is a common pathogenic bacterium responsible for a variety of cutaneous and systemic infections. The growth of *S. aureus, S. pyogenes, L. Monocytogenes and E. faecalis* were inhibited by the methanolic extracts of *M. Philipinensis, A. cobbe* and *T. indica* seed coat at 50- 12.5 mg/ml. The aqueous extracts were not as potent as the methanolic extracts whereas G-ve organisms were resistant to the herbal extracts even at doses of 500 mg/ml.

Table 3: Minimum Inhibitory Concentration of different methanolic extracts (mg/ml) against different pathogens

		E.coli	*S. typhimurium*	*P. multocida*	*P. aeruginosa*	*S. aureus*	*S. pyogenes*	*L. monocytogenes*	*E. faecalis*	*K. pneumoniae*
A. indica	Methanolic			250		50		100		
	Aqueous	-	-	-	-	250	500	100	-	-
T. indica	Methanolic	-	-	50	-	12.5	12.5	12.5	12.5	-
	Aqueous	-	-	25	-	25	25	-	-	-
M. Philippinensis	Methanolic	-	-	100	-	12.5	100	12.5	50	-
	Aqueous	-	-	-	250	12.5	-	250	-	-
A. cobbe	Methanolic	-	-	100	-	50	12.5	100	12.5	-
	Aqueous	-	-	-	-	25	12.5	-	-	-
V. Negundo	Methanolic	-	-	-	-	500	12.5	-	-	-
	Aqueous	-	-	-	-	100	-	100	-	-
S. sensitiva	Methanolic	-	-	-	-	-	500	-	-	-
	Aqueous	-	-	-	-	-	12.5	25	-	-
A. squamosa	Methanolic	-	-	-	-	12.5	-	12.5	-	-
	Aqueous	250	250	-	500	100	-	-	-	250
M. paniculata	Methanolic	-	-	-	-	-	100	-	-	-
	Aqueous	-	-	-	-	-	-	-	-	-
C. odorata	Methanolic	-	-	-	250	-	25	12.5	-	-
	Aqueous	-	-	-	500	12.5	-	250	-	-
S. alata	Methanolic	-	-	-	250	-	250	50	50	-
	Aqueous	-	-	-	-	50	-	-	-	-

.

The differences in the susceptibility of the G-ve and G+ve organisms can be attributed to the differences in cell wall composition of the bacterium [7]. The methanolic extracts were found to be more effective antibacterial agent as compared to the respective aqueous extracts which could be correlated with the better solubility of antimicrobially active substances in the alcohol solvent as compared to water and hexane [8]. The agar diffusion assay is a qualitative method used for the screening of large numbers of antibacterial samples and the activities identified are to be confirmed using the micro broth dilution method in which antimicrobial activity is expressed as MIC of the extracts [9]. Although scientific reports are available on antibacterial activity of A. indica, V. negundo and A. squamosa, studies on the antibacterial activity of M. philipinensis, A. cobbe, S. sensitiva and T. indica seed coat are limited. Although there are reports of antibacterial activity of methanolic and ethanolic extract of neem leaves on P. aeuroginosa [10]-[11] and E. coli [11], the present study could not demonstrate the effect probably due to difference in strain of the organism and the site of plant collection. The methanolic fraction of flower extract of Senna alata produced an inhibition zone of 10 mm in the growth of E. coli [12], we could obtain only 8 mm and was considered non significant and the reason could be the limited concentration of active principles in the leaf as compared to the flower. The difference in potencies may also be due to the difference in the stage of collection of the plant and sensitivity of the strains tested [13]-[14]. The antimicrobial activity might be due to the presence of active components like iso-flavonoids that complex with the bacterial cell wall [15] or terpenoids that have the capacity to disrupt the cell wall and inhibit the growth of bacterium [16]. Detailed investigations pertinent to the active molecules present in these extracts are yet to be under taken. Hence the study indicated the identification of novel herbal antimicrobial agents which can be further explored so as to develop into a new effective antimicrobial agent

.

References:

1. R. Nair, T. Kalariya, and S. Chanda, “Antibacterial activity of some selected Indian medicinal plants”, Turkish Journal of Biology, 2005, pp 2941-47.
2. M. S. H. Shihabuden, H. D. Priscilla, and K. Thirumurugan, “Antimicrobial activity and phytochemical analysis of selected Indian folk medicinal plants”, International Journal of Pharmaceutical Science and Research, 1 (10), 2010, pp 430-434.
3. D. Dhanalakshmi, R. Dhivya, and K. Manimegalai, “Antibacterial activity of selected medicinal plants from south India”. Hygeia:Journal of drugs medicine, 5 (1), 2013, pp 63-68.
4. M. Khond, J. D. Bhosle, T. Arif, T. K. Mandal, M. M. Padhi, and R. Dabur, “Screening of some medicinal plant extracts for Invitro antimicrobial activity”, Middle East Journal of Scientific Research, 4 (4), 2009, pp 271-278.
5. N. Chelli-Chentouf, A. T. Meddah, C. Mullie, A. Aoues, and B. Meddah, “In vitro and in vivo antimicrobial activity of Algerian HoggarSalvadorapersica L. extracts against microbial strains from children’s oral cavity”, Journal of Ethno pharmacology, 144, 2012, pp 57–66.
6. G. F. Moura- Costa, S. A. Nocchi, L. Ceole, J. Carlos P deMello, C. V. Nakamura, B. P. Dias Filho, L. G. Temponi, and J. G. Nakamura, “Antimicrobial activity of plants used as medicinal on an indigenous reserve in Riodas Cobras, Parana´ , Brazil”, Journal of Ethno pharmacology, 143, 2012, pp 631–638.
7. J. D. Tamokoua, M. F. Tala, H. W. Wabo, J. R. Kuiatea, and P. Taneb, “Antimicrobial activities of methanol extract and compounds from stem bark of *Vismia rubescens”,* Journal of Ethno pharmacology, 124, 2009, pp 571-575.

I. Ahmad, Z. Mehmood, and F. Mohammad, “Screening of some Indian medicinal plants for their antimicrobial properties”. Journal of Ethno pharmacology, 62, 1998, pp 183–193.

8. E. M. Tekwu, A. C. Pieme, and V. P. Beng, “Investigations of antimicrobial activity of some Cameroonian medicinal plant extracts against bacteria and yeast with gastrointestinal relevance”, Journal of Ethno pharmacology, 142, 2012, pp 265-273.
9. S. Maragathavalli, S. Brindha, N. S. Kaviyarasi, B. Annadurai, and S. K. Gangwar, “Antimicrobial activity in leaf extract of neem (*Azadirachta indica* linn.)”, International Journal of Science and Nature, 3(1), 2012, pp 110-113.

10. P. Chaturvedi, A. Bag, V. Rawat, N. S. Jyala, V. Satyavali, and P. K. Jha, "Antibacterial effects of *Azadirachta indica* leaf and bark extracts in clinical isolates of diabetic patients". National Journal of Integrated Research in Medicine 2(1), 2011, pp 5-10.
11. M. Idu, S. E. Omonigho, and C. L. Igeleke, "Preliminary investigation into the Phytochemistry and Antimicrobial activity of *senna alata* L. flower". Pakistan Journal of Biological Sciences, 10 (5), 2007, pp 806-809.
12. L. F. Nimri, M. M. Meqdam, and A. Alkofahi, "Antibacterial activity of Jordanian medicinal plants", Pharmaceutical Biology, 37 (3), 1999, pp 196-201.
13. Ahmad, and A. Z. Beg, "Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens". Journal of Ethno pharmacology, 74, 2001, pp 113-123.
14. M. M .Cowan, "Plant products as antimicrobial agents", Clinical Microbiology Review, 12, 1999, pp 564–582.
15. S. Arvind, F. C. Reg, and A. P. Enzo, "Identification of antimicrobial components of an ethanolic extract of the Australian medicinal plant, *Eremophila duttonii*". Phytotherapy Research, 18, 2004, pp 615–618.

***

Dept of Pharmacology & Toxicology, e-mail: sujith@kvasu.ac.in.
Department of Pharmacology & Toxicology, e-mail: sreedeviramachandran2007@gmail.com.
Department of Pharmacology & Toxicology, e-mail: sujavet@gmail.com
Research Assistant, Department of Pharmacology & Toxicology, e-mail: anusaaranga@gmail.com,
Assistant Professor and head, Department of Pharmacology & Toxicology, e-mail: sanis@kvasu.ac.in
Teaching Assistant, Department of Veterinary Physiology, e-mail: muhammeds01@hotmail.com
Department of Pharmacology and Toxicology,
College of Veterinary and Animal Sciences, Pookode, Wayanad, Kerala- 673576.
Post Graduate Scholar, Indian Veterinary Research Institute, U.P. e-mail: rearres@gmail.com.
Post Graduate Scholar, Indian Veterinary Research Institute, U.P. e-mail: aneesha221@gmail.com