Nature of Pharmaceutical Technology

Antibacterial Activity of Herbal Plant Extracts against Human Pathogen

M.I.Zahir Hussain[a*], P.Vasantha Kumar[a], A.Syed Mohamed[b] and G.Subash Chandran[c]

[a]Department of Advanced Zoology and Biotechnology, Sadakathullah Appa College,Tirunelveli-627 011; [b]Department of Chemistry, Sadakathullah Appa College, Tirunelveli- 627 011; [c]Government Siddha Medical College, Palayamkottai – 627 011.

Published: 15 December, 2011; Vol. No.1(4): 4-8; Online: www.nptjournal.com/documents/nptdec0002

Abstract
The usage of herbal plants as medicine contains a wide range of ingredients that can be used to treat chronic as well as infectious diseases. The herbal plant extract of Keelanelli (*Phyllanthus* niruri), Adathoda (*Justicia* adhatoda), Kuppaimeni (*Acalypha indica)*, Nandhiyavattum (Ervatamia coronoriae) and Aavaram (*Cassia auriculata*) were tested against various human pathogens. The extracts of these plants were prepared and screened against different microorganisms responsible for various infections. The alcoholic, acetone and chloroform extract of these plants were screened against three human pathogenic bacterial of *Escherichia coli, Salmonella typhi* and *Enterobacter aerogens*. The results of the present study clearly showed that water extract *J.* adhatoda plant extracts showed significant antibacterial activity against *E.coli*. The overall antibacterial activity of various herbal extract was observed against *S.typhi*.

Keywords: Antibacterial Activity,Medicinal Plants, Human pathogen

The usage of herbal medicines in Asia represents a long history of human interactions with the environment. Herbal plants used in traditional medicine contain a wide range of ingredients that can be used to treat chronic as well as infectious diseases (Higa *et al.*, 1994). A vast knowledge of how to use the plants against different illnesses may be expected to have accumulated in areas where the use of herbal plants is still of great importance (Diallo *et al.*, 1999). The medicinal value of the herbal plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds (Edeoga *et al.*, 2005). The use of medicinal plants as traditional medicines is well known in rural areas of many developing countries (Sandhu and Heinrich, 2005; Gupta *et al.*, 2005). Traditional healers claim that their medicine is cheaper, more effective and impart least side effects as compared to synthetic medicines. In developing countries, low-income people such as farmers, people of small isolate villages and native communities use folk medicine for the treatment of common infections (Rojas *et al*., 2006).

The authors declare no conflict of interest.
This article is NPT direct Email Submission.
Freely available on online through the NPT open access www.nptjournal.com.
Received: Nov5, 2011
Accepted: November, 29, 2011

*Author to whom correspondence should be addressed.
Email:mizahirhussain@gmail.com

This article contains supporting information online at www.nptjournal.com/documents/ nptdec0002

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents (Sandhu and Heinrich, 2005). Such a fact is cause for concern, because of the number of patients in hospitals who have suppressed immunity, and due to new bacterial strains, which are multi-resistant. Consequently, new infections can occur in hospitals resulting in high mortality. The development of drug resistance in human pathogens against commonly used antibiotics

has necessitated a search for new antimicrobial substances from other sources including plants (Erdogrul, 2002). Herbal plants have become the focus of intense study in terms of validation of their traditional uses through the determination of their actual pharmacological effects. Synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often with adulterations and side effects. Screening of herbal plants for antimicrobial and elementological activities are important for finding potential new compounds for therapeutic use. Therefore, there is need to search new infection fighting strategies to control microbial infections.

Materials Methods

The hebal plant of Keelanelli (*Phyllanthus* niruri), Adathoda (*Justicia* adhatoda), Kuppaimeni (*Acalypha indica)*, Nandhiyavattum (Ervatamia coronoriae) and Aavaram (*Cassia auriculata*) were selected for present study. The plants were collected from Tirunelveli District, South India and shadow dried. The dried plant materials was powedered and stored in air tight container. 20g of each plant materils was soaked in 100 ml of distilled water, butanol, alcohol, acetone and chloroform for 24 hours with intermittent shaking. The plant extract were filtered through whatman No. 1 filter paper. The filtrate was collected and stored at 4ºC for futher use.

Antimicrobial Screening

The alcoholic, acetone and chloroform extract of four herbal plants were screened against three human pathogenic bacteria of *Escherichia coli, Salmonella typhi* and *Enterobacter aerogens.* The cultures of these microorganisms were maintained at 4ºC on slopes of nutrient agar medium. Active cultures of microorganisms were prepared by transferring a loopful of cells from the stock culture to the test tubes which containing broth was incubated without agitation for 24 hours at 37ºC. This was used for study of antibacterial activity of herbal plant extract.

Antimicrobial Susceptibilty Test

The antimicrobial activity was determined by the disc diffusion method (Farag *et al.*, 1989). In vitro antibacterial activity by different herbal plant extracts was screened by using muller hinton agar medium. The agar plates were prepared in sterile condition. The muller hinton agar plates were each seeded with 0.1 ml of an overnight culture of each bacterial (equivalent to 10^{-7} to 10^{-8} CFU/ml) and spread uniformly over the nutient agar plate by using L-rod techmique method. The inoculum was allowed to dry for 5 minutes. Sterile disc of 6mm diameter was prepeared by using filter paper of Whatman No.1. Filter paper discs which contained 10µl of plant extracts were placed on the inoculated plates. The petri dishes were subsequently incubated at 37ºC for 24 hour. After incubation the growth inhibition rings were quantified by measuring the diameter of the zone of inhibition in mm using transparent ruler in millimeter (including the diameter of the disc) from the lower surface of the petri dishes (Lopez- Garcia *et al*., 1992). The control is a paper disc soaked with appropriate solvent. All the assays were carried out in triplicate.

Results

Antibacterial effect of Butanolic plant extract

The Microorganism of *S. typhi, E. coli* and *E. aerogens* showed 0.5, 0.4 and 0.5mm zone of inhibition for the extract of *J. adhatoda* respectively. The *P. niruri* plant extract showed zone of inhibition of 0.7, 0.3 and 0.6mm respectively against *S. typhi, E. coli* and *E. aerogens* respectively. *A. indica* plant extract exhibited zone of inhibition of 0.7, 0.5 and 0.4mm against *S. typhi, E. coli* and *E. aerogens* respectively. The Butanolic extract of *E. coronoriae* showed zone of inhibition in the order of *E. aerogens>E. coli>S. typhi* (Table-1)

Antibacterial effect of Acetone plant extract

The *J. adhatoda* plant extract showed zone of inhibition of 0.7, 0.6 and 0.3mm respectively against *S. typhi, E. coli* and *E. aerogens* respectively. The extract of *P. niruri* showed 0.7 to 0.8 mm zone of inhibition against *E.coli, S.typhi* and *E.aerogens*. the extract of *A. indica* showed 0.4, 0.7 and 0.4mm of zone of inhibition against *S. typhi, E. coli* and *E. aerogens* respectively. The acetone extract of *E. coronoriae* showed no zone of inhibition against E.coli. *E. coronoriae* acetone extract showed 0.6 and 0.5 mm against *S. typhi* and *E. aerogens* respectively (Table -2).

Antimicrobial effect of chloroform plant extract

Extract of *J. adhatoda* showed zone of inhibition of 0.7, 0.5 and 0.8mm respectively against *S. typhi, E. coli* and *E. aerogens*

respectively. The extract of *P. niruri* showed 0.4mm zone of inhibition against all tested microorganisms. *A. indica* plant extract showed 0.5 and 0.7mm of zone of inhibition against *S. typhi* and *E. aerogens* respectively. *E. coronoriae* acetone extract showed 0.4, 0.6 and 0.4mm against *S. typhi, E. coli and E. aerogens* respectively (Table -3).

Table -1: Antibacterial activity of butanolic plant extracts against different microorganisms

Sl. No	Organisms	Zone of Inhibition (mm)				
		Ceftriaxone (Antibiotic)	*Justicia adhatoda*	*Phyllanthus niruri*	*Acalypha indica*	*Ervatamia coronoriae*
1	*Salmonella typhi*	0.5±0.09	0.5±0.11	0.7±0.22	0.7±0.14	0.5±0.18
2	*Escherichia coli*	0.4±0.17	0.4±0.12	0.3±0.14	0.5±0.12	0.6±0.12
3	*Enterobacter aerogens*	0.6 ±0.11	0.5±0.17	0.6±0.16	0.4±0.16	0.8±0.19

Table- 2: Antibacterial activity of acetone extract against different microorganism

Sl. No	Organisms	Zone of inhibition (mm)				
		Ceftriaxone (Antibiotic)	*Justicia adhatoda*	*Phyllanthus niruri*	*Acalypha indica*	*Ervatamia coronoriae*
1.	*Salmonella typhi*	0.6±0.17	0.7±0.12	0.8±0.14	0.4±0.16	0.6±0.11
2.	*Escherichia coli*	0.7±0.16	0.6±0.16	0.8±0.22	0.7±0.38	0
3.	*Enterobacter aerogens*	0.6±0.14	0.3±0.19	0.7±0.14	0.4±0.16	0.5±0.34

Table -3: Antibacterial activity of chloroform plant extract against different microorganisms

Sl. No	Organisms	Ceftriaxone (Antibiotic)	Zone of inhibition (mm)			
			Justicia adhatoda	*Phyllanthus niruri*	*Acalypha indica*	*Ervatamia coronoriae*
1.	*Salmonella typhi*	0.4±0.16	0.7±0.18	0.4±0.17	0.5±0.6	0.4±0.28
2.	*Escherichia coli*	0.5±0.21	0.5±0.16	0.4±0.18	0	0.6±0.28
3.	*Enterobacter aerogens*	0.4±0.28	0.8±0.18	0.4±0.19	0.7±0.17	0.4±0.14

Table- 4: Antibacterial activity of plant extract of water against different microorganisms

Sl. No	Organisms	Ceftriaxone (Antibiotic)	zone of inhibition (mm)			
			Justicia adhatoda	*Phyllanthus niruri*	*Acalypha indica*	*Ervatamia coronoriae*
1.	*Salmonella typhi*	0.4±0.16	0.7±0.16	0.3±0.17	0.2±0.09	0.8±0.28
2.	*Escherichia coli*	0.5±0.18	1.1±0.36	0.8±0.28	0.7±0.19	0.9±0.22
3.	*Enterobacter aerogens*	0.6±0.17	0.6±0.18	0	0.3±0.26	0.5±0.18

Antimicrobial effect of water extract of plant

The *J. adhatoda* plant extract showed zone of inhibition of 0.7, 1.1 and 0.6mm respectively against *S. typhi, E. coli* and *E. aerogens* respectively. The microorganism of *S. typhi* and *E. coli* showed 0.3, and 0.8mm zone of inhibition respectively for the extract of *P. niruri*. The water extract of *P. niruri* showed no zone of inhibition against *E. aerogens.* The water extract of *A. indica* showed 0.2, 0.7 and 0.3mm of zone of inhibition against *S. typhi, E. coli* and *E. aerogens* respectively. *E. coronoriae* plant extract of water showed 0.8, 0.9 and 0.5mm against *S. typhi, E. coli and E. aerogens* respectively.

The use of higher plants and preparations made from them to treat infections is a longstanding practice in a large part of the world population, especially in developing countries, where there is dependence on traditional medicine for a variety of ailments Interest in plants with

antimicrobial properties has revived as a consequence of current problems associated with the use of antibiotics (Erdogrul, 2006). The present studies aimed at the investigation of herbal plant with antimicrobial activity against pathogenic microorganisms. The results obtained from the diffusion method showed that the water, acetone, butanol and chloroform of extract of various plants had antibacterial effects. The preliminary studies have been demonstrated that the various herbal plant extracts have antibacterial activity against pathogenic bacterial strains. The results of the present study clearly showed that acetonic extract of *J. adhatoda* and *A. indica* plant extracts showed antibacterial activity against tested pathogenic bacterial strains. The effectiveness of the active compounds present in the herbal plant extracts cause the production of growth inhibition zones that appear as clear areas surrounding the wells. Antibacterial activity may be due to active components which are present in plant extracts. However, some herbal plant extracts was unable to exhibit antibacterial activity against tested bacterial strains. Acetonic extract of *E. coronoriae*, chloroformic extract of *A. indicas* showed no zone of inhibition against *E.coli*. These bacterial strains may have some kind of resistance mechanisms e.g. enzymatic inactivation, target sites modification and decrease intracellular drug accumulation (Rojas *et al.*, 2006) or the concentration of the compound used may not be sufficient. No inhibition was observed with controls, which proves that solvents could not act as antibacterial agents. In almost all tests, acetone extracts showed better inhibition against all tested bacterial strains, indicating that active ingredients in plant materials could be extracted into acetone (Gislene *et al*., 2000 and Aboaba, *et al*.,2001). However, highest antibacterial activity was observed against *E. aerogens*. Plant extracts in different solvents seems to have toxic effect against tested bacterial stains or might have increased the survival ability of pathogenic bacteria. Phytochemical screening of herbal plants revealed the presence of biologically active substances such as alkaloids, steroids, triterpenoids and flavonoids. The results obtained from preliminary phytochemical screening are comparable with the results reported earlier (Bandaranayake, 1995). The secondary metabolites may exert antibacterial activity against tested bacterial strains (Subash Chandran *et al.*, 2009). In addition to above phytochemical groups tannins, anthocyanins, polyphenols, coumarins and essential oils may have some antibacterial activity subjected to phytochemical screening (Johnson *et al*., 2010). It is promising that the tested plant species could be used to synthesis novel antibiotics for bacterial infections, especially for antibiotic resistant bacterial infections. Further research is necessary for successful separation, purification and characterization of biologically active compounds using chromatographic methods and spectroscopic techniques. Further studies are being carried out in order to separate the individual components that are present in plant extracts.

References

Aboaba, O.O. and Efuwape, B.M.2001. Antibacterial properties of some Nigerian spices. *Bio. Res. Comm*., 13: 183 - 188.

Bandaranayake, W.M. 1995. Survey of mangrove plants from Northern Australia for phytochemical constituents and UV-absorbing compounds. *Current Topics in Phytochemistry* (Life Science Advances), 14:69-78

Diallo, D., Hveem, B., Mahmoud, M.A,, Betge, G., Paulsen, B.S. and Maiga, A.1999. An ethnobotanical survey of herbal drugs of Gourma district, Mali. *Pharmaceutical Biology*, 37:80-91.

Edeoga, H.O, Okwu, D.E. and Mbaebie, B.O. 2005. Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology*, 4:685-688.

Erdogrul, O.T. 2002. Antibacterial activities of some plant extracts used in folk medicine. *Pharmaceutical Biology*, 40:269-273.

Erturk, O., Kati, H., Yayli, N. and Demirbag, Z. 2006. Antimicrobial properties of *Silene multifida* (Adams) Rohrb. plant extract. *Turk. J. Biol.*, 17-21.

Farag, R.S., Daw, Z.Y., Hevedi F.M. and Elbaroty, G.S.A.1989. Antimicrobial activity of some Egyptan specie essential oils. *J. Food Protection*,52 (9): 665-667.

Gislene G. F., Nascimento, Juliana Locatelli, Freitas,P.C. and Silva,G.L.2000. Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria. *Brazilian Journal of Microbiology*, 31:247-256

Gupta, M.P., Solis, P.N., Calderon, A.I., Guionneau-Sinclair, F., Correa, M., Galdames, C., Guerra, C., Espinosa, A., Alvenda, G.I.,

Robles, G. and Ocampo, R. 2005. Medical ethnobotany of the Teribes of Bocas del Toro, Panama. *Journal of Ethnopharmacology*, 96:389-401

Higa, T., Anka, J., Kitamara, A., Koyama, T., Akshashi, M. and Uchida, T. 1994. Bioactive compounds from marine sponges. *Pure and Appl. Hem*., 66: 2227-2236.

Johnson, M., Wesely, E.G., Zahir Hussain, M.I. and Selvan, N.2010. In *vivo* and *in vitro* phytochemical and antibacterial efficacy of *Baliospermum montanum* (Willd.) Muell. Arg. *Asian Pacific Journal of Tropical Medicine*, 412-420.

Lopez-Garcia, R. E., Hernandez- Perez, M., Rabanal, R.M., Darias, V., Arias, A. and Sanz, J. 1992. Essential oils and antimicrobial activity of two varieties of *Cedronella canariensis*. *J. Ethnopharmacology*, 36: 207-211.

Rojas, J.J., Ochoa, V.J., Ocampo, S.A. and Munoz, J.F. 2006. Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: A possible alternative in the treatment of non-nosocomial infections. *BMC Compelmentary and Alternative Medicine*, 6:2

Sandhu, D.S. and Heinrich, M. 2005. The use of health foods, spices and other botanicals in the Sikh community in London. *Phytotherapy Research*,19:633-642.

Subash Chandran, G., Zahir Hussain, M.I. and Murugesan, A.G. 2009. Evaluation of antibacterial activity of *Andrographis paniculata*, Nees by disk diffusion method. *Siddha Papers*, 02 (06):1-9.