Available Online at http://www.recentscientific.com

ISSN: 0976-3031

International Journal of Recent Scientific Research
Vol. 6, Issue, 7, pp.4942-4945, July, 2015

International Journal of Recent Scientific Research

RESEARCH ARTICLE

COMPARATIVE ASSESSMENT OF ANTIBACTERIAL EFFICACY OF CRUDE LEAF EXTRACTS OF *MILLETTIA (L.)* PANIGRAHI AGAINST ENTERIC PATHOGENS

Anandhan Anuswedha*[1] and N.Rashitha Begum[2]

*[1,2]Department of Applied Microbiology, Justice Basheer Ahmed Sayeed College for Women, Chennai, Tamil Nadu, India

ARTICLE INFO

Article History:

Received 5th, June, 2015
Received in revised form 12th, June, 2015
Accepted 6th, July, 2015
Published online 28th, July, 2015

Key words:

drug resistance, crude extracts, antibacterial activity, enteric pathogens

ABSTRACT

Drug resistance is one of the most serious global threats to the treatment of infectious diseases. Currently, there is a growing interest in using natural antibacterial compounds from plants, herbs and spices for the treatment of these bacterial infections. The study was designed to study and screen the antibacterial activity of *Millettia pinnata Linn.*, *Panigrahi* crude extracts against multidrug resistant enteric pathogens. The crude extracts were prepared with different solvents and then screened using the agar disc diffusion assay. The crude extracts which showed maximum activity were subjected to MIC/MBC assay to determine the minimum concentration of the crude extract which would inhibit/kill the clinical pathogens. The concentration of the crude extract was 100mg/ml. MIC results corresponded with that of the disc diffusion assay. The ethanol extracts showed higher antibacterial activity which suggests the potential use of ethanol extracts of *Millettia pinnata Linn.*, *Panigrahi* in the treatment of infectious diseases caused by enteric pathogens.

INTRODUCTION

Folklore medicinal plants have been used in many countries including India and China to cure various ailments. The developing countries are endowed with rich flora as they are mostly situated on the tropical belt and between them, cover a wide range of geographical and climatic conditions. Of about 15,000 species of higher plants in India, medicinal uses are attributed to at least 1500 species (Hussain *et al.*, 1992). The indiscriminate use of commercially available antibiotics for the treatment of infectious diseases developed multiple drug resistance in the microorganisms, putting new challenge before the drug industries for identification of new efficient antimicrobial compounds. Herbal drug therapy is regarded as an important alternate, leading the researchers to focus and evaluate the traditionally recommended medicinal plants for their efficacy in various disease conditions. *Millettia pinnata Linn. Panigrahi* is a medium sized glabrous tree belonging to the family Fabaceae .It is popularly known as Karanja in Hindi, Indian Beech in English and Pongam in Tamil The medicinal tree is native to Western Ghats and chiefly found in tidal forests of India (Krishnamurthi, 1969). Historically, *Pongamia* has been used as folk medicinal plant, particularly in Ayurveda and Siddha systems of Indian medicine (Meera *et al.*, 2003). All parts of the plant have been used as a crude drug for the treatment of tumours, piles, skin diseases, itches, abscess, painful rheumatic joints wounds, ulcers, diarrhea etc., (Shoba and Thomas, 2001). Extract of the plant possess significant anti-diarrhoeal, anti-fungal, anti-plasmodial, anti-ulcerogenic, anti-inflammatory and analgesic activities (Sangwan. S *et al.*, 2010) More recently, the effectiveness of *M. pinnata* as a source of biomedicines has been reported (Brijesh *et al.*, 2006), specifically as antimicrobial and therapeutic agents. Leaves are antihelminthitic, digestive and laxative used for inflammations, piles and wounds. Juice of leaves is used for cold, cough, diarrhea, dyspepsia, flatulence, gonorrhea, leprosy (Ambasta *et al.*, 1992; Bhattacharjee, 2001). Worldwide, diarrhoeal diseases are the leading cause of death in children, the second leading cause of death for all ages and are probably responsible for more years of potential life lost than all other diaseases combined (Richard, 1993). In the present study, the antibacterial ability of the leaf extracts of *Millettia pinnata Linn. Panigrahi* have been scrutinized against multi-drug resistant enteric pathogens.

MATERIALS AND METHODS

Preparation of plant powder

The leaves of *Millettia pinnata Linn. Panigrahi* were obtained from the medicinal farm of Arignar Anna Government Siddha

*Corresponding author: **Anandhan Anuswedha**
Department of Applied Microbiology, Justice Basheer Ahmed Sayeed College for Women, Chennai, Tamil Nadu, India

College and Hospital, Arumbakkam, Chennai. The leaves were separated and washed twice with double distilled water and then surface sterilised using 70% ethanol. The leaves were shade dried for 1-2 weeks. The leaves were then ground into a coarse powder form using a mixer.

Preparation of crude extract

The crude extracts were prepared by hot and cold method of extraction. In the hot method of extraction, about 1 gram of the powdered plant material was mixed with 10 ml of the solvent, incubated in a shaker at 37ºC for 4 hours at 250 rpm after which it was placed in a water bath at 60ºC for 2 hours. The supernatant was filtered and dried in air at room temperature. For the cold method of extraction, about 1 gram of the of the powdered plant material was mixed with 10 ml of the solvent, incubated in a shaker at 37ºC for 4 hours at 250 rpm. The supernatant filtered and then dried in air at room temperature. The solvents used were water, ether, ethanol, methanol, chloroform, acetone and dichloromethane. The residue obtained after drying was dissolved in the appropriate solvent and used for antibacterial screening.

Microbial cultures used

The test organisms used for screening were *Staphylococcus aureus, Salmonella typhi, Salmonella paratyphi A, Salmonella paratyphi B, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei, E.coli, Klebsiella pneumoniae, Proteus mirabilis and Proteus vulgaris.* All the strains were confirmed laboratory isolates of the Department of Microbiology, J.B.A.S College for Women, Chennai.

Preliminary Screening using Disc Diffusion Assay

Discs(6mm) prepared from Whatmann No.1 filter paper was sterilised and impregnated with 20µl of various crude solvent extracts (concentration:100mg/ml). Broth cultures of the microorganisms were prepared by transferring 2-3 isolated colonies to Nutrient Broth and incubating the culture at 37C for 4 hours in the incubator. The culture was checked for turbidity by comparing with the McFarland Standard (0.5).

A lawn culture of the organisms to be tested was made on the Mueller Hinton agar media. The prepared discs were placed on the plate in a way such that each disc was at least 20mm from one another. The plates were then incubated at 37°C for 24 hours. The inhibition zone around each disc both in the experiment and the control were measured. Standard antibiotics as per the organisms tested were included as positive control and respective solvents without the plant extracts were used as the negative control.

Table I *Preliminary Screening of Crude Solvent Extracts Of Millettia pinnata Linn. Panigrahi.:*

Organisms Tested	***Inhibition Zone Diameter in mm***													
	Ethanol		Methanol		Ether		Aqu Eous		chloroform		Dichloro methanol		Acetone	
	H	C	H	C	H	C	H	C	H	C	H	C	H	C
Staphylococcus aureus	-	19	18	13	-	-	-	-	11	11	-	-	10	11
E.coli	12	10	8	8	-	-	-	-	9	9	-	-	10	9
Klebsiella pneumoniae	7	15	-	-	-	-	-	-	10	10	-	-	10	10
Salmonella typhi	14	14	10	13	-	-	-	-	11	9	-	-	12	9
Salmonella paratyphi A	-	-	-	-	-	-	-	-	11	7	-	-	9	-
Salmonella paratyphi B	15	18	7	12	-	-	-	-	9	9	-	-	9	-
Shigella dysenteriae	-	-	-	-	-	-	-	-	11	10	-	-	11	9
Shigella boydii	8	8	-	-	-	-	-	-	-	-	-	-	12	9
Shigella flexneri	-	-	-	-	-	-	-	-	-	-	-	-	13	9
Shigella sonnei	10	10	-	-	-	-	-	-	-	-	-	-	10	10
Proteus mirabilis	-	-	9	9	-	-	-	-	11	12	-	-	11	-
Proteus vulgaris	-	-	9	10	-	-	-	-	9	10	-	-	11	-

H- Hot method, C-cold method

Table II Determination of Minimum inhibitory concentration/Minimum bactericidal concentration

Organisms tested	**Crude Extracts**	**Concentration of the extracts in mg/ml**						
		100	**50**	**25**	**12.5**	**6.25**	**3.125**	**1.56**
Staphylococcus aureus	EC	+	+	+	+	+	-	-
	MH	+	+	+	+	+	-	-
	MC	+	+	+	-	-	-	-
Escherichia coli	EH	+	+	+	+	-	-	-
Klebsiella pneumonaiae	EC	+	+	+	+	-	-	-
	EH	+	+	+	+	+	-	-
Salmonella typhi	EC	+	+	+	+	-	-	-
	MC	+	+	+	+	-	-	-
Salmonella paratyphi B	EC	+	+	+	+	-	-	-
Shigella boydii	AH	+	+	+	+	+	-	-
Shigella flexneri	AH	+	+	+	+	+	-	-
Proteus mirabilis	CC	+	+	+	+	-	-	-

Determination of Minimum Inhibition Concentration/Minimum Bactericidal Concentration

The Microbroth dilution was performed on a microtitre plate. Doubling dilutions of the crude extract were prepared in Mueller Hinton broth. Bacterial cultures of 10^6 cfu/ml dilution were prepared with McFarland standard (0.5) and 10µl were added to each well of the microtitre plate and mixed well. The microtitre plates were incubated at 37ºC overnight and a loopful of the culture was streaked on to nutrient agar plates. The plates were incubated at 37ºC overnight. The growth/no growth pattern of the organisms corresponded to the MIC /MBC of the crude extract.

RESULTS

In the disc diffusion assay, ethanolic extract showed inhibition zone ranging from 19-10mm, followed by the methanolic extract inhibition zone ranging from 18-8mm, acetone extract inhibition zone ranging from 13-9mm and chloroform extracts inhibition zone ranging from 12-9mm.

The crude extracts which showed a zone diameter of 12mm or more, were chosen for the MIC assay

EC- Ethanolic cold extract, MH-methanolic hot extract, EH-ethanolic hot extract, MC- methanolic cold extract, AH-acetone hot extract, CC- chloroform cold extract

In the MIC assay for *Staphylococcus aureus*, a MIC of 6.25mg/ml was shown by ethanolic cold and methanolic hot extract, and a MIC of 12.5 mg/ml was shown by methanolic cold extract.

In the MIC assay for *E.coli*, a MIC of 6.25mg/ml was shown by ethanolic hot extract. For *Klebsiella pneumoniae*, a MIC of 6.25mg/ml was shown by ethanolic cold extract. For *Salmonella typhi*, a MIC of 3.125mg/ml was shown by ethanolic hot extract, a MIC of 6.25mg/ml was shown by ethanolic and methanolic cold extract. For *Salmonella paratyphi B*, ethanol cold extract shown an MIC value of 6.25mg/ml. For *Shigella boydii* and *Shigella flexneri*, acetone hot extract showed an MIC of 3.125mg/ml. For *Proteus mirabilis*, the chloroform cold extract showed an MIC value of 6.25mg/ml.

DISCUSSION

Plant sources have been widely investigated for the search of new and innovative antibacterial agents to be used for the treatment of infectious diseases. One of the most important infectious disease burdens in India are diarrheal diseases. Diarrhea is the third leading cause of childhood mortality in India, and is responsible for 13% of all deaths/year in children under 5 years of age.(ref) Diarrhoeal diseases are caused by enteric bacterial pathogens such as *E.coli, Salmonella ,Shigella* and *Campylobacter* that contaminate food and water.(ref) Bacterial antibiotic resistance is an emerging and serious public health concern due to the compromised efficacy of antimicrobial agents used in the treatment of infectious diseases.

Under the current circumstance, in our study the crude leaf extracts of *Millettia pinnata* had shown considerable antibacterial activity against the multi-drug resistant enteric pathogens. The ethanol, methanol, acetone and chloroform extracts have shown maximum activity whereas the ether, aqueous and dichloromethanol extract has shown no activity against the pathogens tested at a starting concentration of 100 mg/ml. In the study done by Vivek. K. Bajpai *et al.*, the various organic extracts of chloroform, ethyl acetate and methanol, derived from the leaves of Pongamia pinnata (L.) Pierre was evaluated for antibacterial potential against some representative food spoilage and food-borne pathogenic bacteria like Bacillus subtilis ATCC6633, Staphylococcus aureus ATCC6538, Listeria monocytogenes ATCC19118, L. monocytogenes ATCC19166, Pseudomonas aeruginosa ATCC6432 and Salmonella typhimurium ATCC2512 at a concentration of 2500 µg/mL. The chloroform, ethyl acetate and methanol extracts displayed significantly higher antibacterial activity as compared to streptomycin which is similar to our studies. Brijesh *et al*.,2006 *et al*., evaluated the anti-microbial effect of crude leaf extract of *P.pinnata* on production and action of enterotoxins. Its extraction has no anti-bacterial, anti-giardial, and anti-rotaviral activities but reduce the production of cholera toxin and bacterial invasion to epithelial cells. This indicates that the extraction of *P. pinnata* has selective anti-diarrhoeal Action which is also evident from our studies.

CONCLUSION

To conclude, the various solvent extracts of *Millettia pinnata Linn. Panigrahi* has shown potential antibacterial activity especially against multi-drug resistant enteric pathogens at a concentration of 100mg/ml.. This medicinal tree which is endemic to the Indian subcontinent is of immense pharmacological, medicinal and economic value. The crude extracts can further be investigated using advanced phytoanalytical techniques to identify the active principle which is responsible for eliciting the antibacterial effect. The leaf extracts of this medicinal tree can be explored as a valuable source of antibacterial agent specifically against multi-drug resistant enteric pathogens.

References

Ambasta, S.P., Ramchandran, K., Kashyapa, K., Chand R., 1992. The Useful Plants of India. Council of Science and Industrial Research (CSIR), New Delhi, India.

Bajpai,V.K., Rahman,A., Shukla,S., Mehta, A., Shukla, S., Arafat, S.M.Y., Rahman,M.M., Ferdousi,Z., 2009.Antibacterial activity of leaf extracts of Pongamia pinnata from India.Pharma. Biol.,47(12):1162-1167

Bauer A.W, Kirby W.M.M ,Sherris J.C and Turck M.1966. Antibiotic suspectibility testing by a standardised single disk method. Am. J. of Clin. Pathol.,; 45:493-6

Bhattacharjee, S.K., 2001. Handbook of Medicinal Plants. 3rd Edn., Pointer Publishers, Jaipur, India. 478p.

Brijesh, S., Daswani, P.G., Tetali, P., 2006. Studies on *Pongamia pinnata* (L.) Pierre leaves: understanding the mechanism(s) of action in infectious diarrhea. J Zhejiang Univ. Sci. B. 7(8), 665 674.

Eloff. J.N.1998. Which extractant should be used for the screening and isolation of antimicrobial components from plants?. J. Ethnopharmacol.,;60(1); 1-8

Husain A. Status Report on Medicinal Plants for NAM Countries, Dec. 1992, Centre for Science and Technology of NAM and other Developing Countries, New Delhi ; 78-80.

Krishnamurthi A. 1969. The Wealth of India, vol. VIII. Publication and Information Directorate

Meera B., Kumar S., Kalidhar S.B., 2003. A review of the chemistry and biological activity of *Pongamia pinnata*. J. Med. Arom. Plant Sci. 25, 441- 65.

Navarro V., Villarreal M.L, Rojas G. and Lozoya X.1996. Antimicrobial evaluation of some plants used in

Mexican traditional medicine for the treatment of infectious diseases. J. Ethnopharmacol.53:143-147

Nayan, R. B and Shukla,V.J .2011.Antibacterial and antifungal activities from leaf extracts of *Cassia fistula* l.: An ethnomedicinal plant.J.Adv.Pharm.Technol.Res.,2(2):104-109

Nostro, A., M.P. Germano, V. D'Angelo, A. Marino and M.A. Cannatelli.2000. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Lett. Applied Microbial.,; 30: 379-384.

Richard.G.Diarrhoeal diseases: current concepts and future challenges.Trans.Roy.Soc,Trop.Med.Hyg.,87:S31-S32

Sangwan, S., Rao, D.V., Sharma, R.A., 2010. A review on *Pongamia Pinnata* (L.) Pierre: A great versatile Leguminous plant. Nat. Sci. 8(11), 130-139.

Shoba G.F. and Thomas M., 2001. Study of antidiarrhoeal activity of four medicinal plants in castor-oil induced diarrhoea. J. Ethnopharmacol. ,76(1), 73- 76.

How to cite this article:

Anandhan Anuswedha and N.Rashitha Begum., Comparative Assessment of Antibacterial Efficacy of Crude Leaf Extracts of Millettia (l.) Panigrahi Against enteric Pathogens. *International Journal of Recent Scientific Research Vol. 6, Issue, 7, pp.4942-4945, July, 2015*

****