

ANTIMICROBIAL ACTIVITY OF LEAF EXTRACT OF TWO MEDICINAL PLANTS OF BOGHORA HILL (MORIGAON) ASSAM AGAINST HUMAN PATHOGENS

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ABSTRACT

The present study deals with the antibacterial activity of leaf extracts of the some medicinal plants against human pathogens. The leaf extracts of *Paederia foetida* and *Hibiscus esculentus* showed high antibacterial activity against *E. coli* and *Staphylococcus aureus* which are usually considered as facultative human pathogens. The *P. foetida* leaf extract showed very effective degree of Zone of Inhibition (ZOI) against growth of *E. coli*. Likewise *H. esculentus* was found fruitful against the *S. aureus*. Both plant leaf extracts showed very effective degree of Zone of Inhibition (ZOI) against *E. coli* and *S. aureus*.

INTRODUCTION

Emergence of antibiotic resistance by bacteria commonly used for treatment of a variety of infectious diseases has alarmed the medical field. Infections with the multiple drug resistant (MDR) *Staphylococci*, *Enterococci*, *Mycobacteria* and *Salmonella typhi* are become major threat for health sector. Because of this, the development of new and effective drug is necessitated from various sources. Plants are the major sources of therapeutic compounds that have been used in a variety of human ailments. A huge number of plants species of north – eastern region and Assam of India is known to have medicinal properties and used by tribes. The medicinal values of the most of these are not yet evaluated scientifically for assessment of their potential as useful drugs. Destruction of forest and lack of proper documentation have made some of these species rare, threatened or endangered. Developing of modern biotechnology based conservation methodology like tissue culture, germ plasm culture and screening of plant metabolites for discovering of potential drugs of pharmaceutical industry, can adopted for scientific exploration of these valuable bioresources.

Symptoms related to gastro – intestinal disorders include diarrhoea, dysentery, intestinal inflammation and abdominal pains or cramps with or without vomiting. Diarrhoeal disease has been recognized as the greatest killer of infants and young children in the developing world. Well over 500 million episodes of diarrhea in children under five are estimated to occur annually in Asia, Africa and Latin America. At least five million children die due to such disorders (WHO, 1996;

Barbara and Bennett, 1998). Causal bacterial pathogens related to the pathogenesis associated with ailments related to the gastro- intestinal tract include *Campylobacter* species, *Bacillus subtilis*, *Vibrio cholerae*, *E. coli*, *Clostridium difficile*, *Salmonella species* and *Staphylococcus aureus*. Natural products from plants may offer new agents for antibacterial use. A special feature of higher plants is their capacity to produce a large number of organic chemicals of high structural diversity, the so called secondary metabolites (Evans *et al.*, 1986). In Africa, traditional health practices involving the use of remedies derived from plants are common and widespread (Rukangira, 2001). In the late 1980s, an estimated 65% of the world's population depended on or at least used medicines derived from plants in health care (Farnsworth, 1988). Many traditional used medicinal plants in South Africa are sold in marketplaces due to increased demands for cheap medicines, high unemployment rate and increased in HIV infections (Fyhrquist *et al.*, 2002). Approximately 65% of the medicinal plants used worldwide are tree species, most of which are becoming endangered, rare or threatened due to unsustainable harvesting methods (Gates, 2000). Antibiotic resistance is a major problem in hospitals as well as in community settings. Gram – positive pathogens such as *Staphylococcus aureus*, *Enterococcus faecalis*, *Enterococcus faecium* and *Streptococcus pneumonia* are becoming resistant to most of the existing antibiotics (Tally and De Bruin 2000). Many plants used in Tunisian traditional medicine have the potential to provide pharmacologically active natural products. Interest in ethnopharmacy as a source of these compounds has increased worldwide, particularly in the search for drugs to

counter multi-resistant microorganisms. Plants with antimicrobial activities have become more interesting because many people are aware of problems associated with the over-prescription and misuse of traditional antibiotics (Mothana and Lindequest, 2005). *Paederia foetida* and *Hibiscus esculentus* are most popular medicinal plants and are usually used against stomach troubles, diarrhoea, hypertension, skin diseases and in urinary troubles and dental-carries in Assam. The hidden chemical compounds may deteriorate the physiological systems if taken in exceed doses which is a usually being seen among the common people (Shivakumar and Alagesaboothi, 2006). Ailments and their treatment with medicinal plants is an old age practice. Use of medicinal plants extract which are rich in antibacterial compounds could be an alternate way to eliminate these bacteria from pelatable items (Santos, 1985) which has already proved in vitro by Kaushik and Dhiman (2000). Antimicrobial drugs are used in medicinal practices for treating food borne diseases (Abramowics, 1990). Chemical present in plants, uses by tribes of Assam as folk medicine, to treat various disease caused by bacteria, fungus, protozoa. Sometimes tribal people uses medicinal plants to cure some metabolic disorders also. They administered this plants extract oral drop, paste and juice by preparing decoction.

India profusely rich in the history of medicinal plants and is 75% folk population is still using herbal preparations in the form of powder, Extract and decoction because these are easily available in nature and the natives have stronger faith on traditional plants. These drugs of medicinal value are competing today in markets. Various plants exhibit various types of antimicrobial activities. Even parasitic plants and Orchids also are of great's medicinal value, which are found to be antimicrobial (Kaushik and Dhiman, 2000).

The present investigations were under taken to test antibacterial activities of ethanolic extract of *Paederia foetida* and *Hibiscus esculentus* against to bacteria *E.coli* MTCC723 and *S. aureus* MTCC96.

MATERIALS AND METHODS

Collection and identification of plant material

The plants were collected in May 2011 from Boghora hill of Morigaon District, Assam during their full growing season and were identified through literature available in the Department of Botany, Morigaon College as well as internet based information. The voucher specimen numbers of the plants were preserved.

Preparation of plant material

Leaves of the plants were collected and washed with tap water twice and again washed with distilled water. Then the leaves were cut into small pieces (2-3 cm), dried under shed, for 2-3 weeks to make it suitable for grinding. Shed drying is done, because volatile constituents may be lost due to the evaporation and degradation of constituents mainly the ones like glycosides and amino acids which are essential of medicinal use. The samples were ground by using mixer grinder mechanically. Then dried leaf powdered is stored in closed vessels for further use.

Preparation of leaf extracts

10g of the powdered plant leaf extract was subjected to the soxhlet successive extraction method (60-80°C) using 100mL ethanol in the order of increasing polarity of solvent for a period of 10h. The extracts obtained were dried at 40°C (Pratima *et al.*, 2011) and centrifuged it 5000 rpm for 10 min. and filtered with Whatman filter paper. The ethanol was evaporated through Rotary evaporator. The standard solution of the extracts were prepared in 1% DMSO (Merck).

Preparation of cultures

E. coli MTCC723 and *Staphylococcus aureus* MTCC96 are collected from Institute of Microbial Technology (IMTECH), Chandigarh, India. Fresh bacterial cultures were prepared by adding a loopful of old culture of bacteria to the sterilized nutrient broth. The medium was shaken well and incubated at 37°C for 18 – 28h. The incubated culture used for checking antibacterial activity.

Antimicrobial assay

Disc diffusion method

To carry out antibacterial activity of plant, disc diffusion method as described by Robert *et al.* (2009) was performed. In this, Muller Hinton agar medium was prepared and sterilized. Then media were poured into plate and kept for solidification. 0.1mL of bacterial culture was inoculated through spread plate technique. Whatman's filter paper no.1 discs (5mm diameter) were soaked in 1% DMSO extracts. The soaked discs were placed on the surface of inoculated plate and allowed to dry in laminar air flow. These plates were incubated at 37°C for 24h in inverted position. The streptomycin (20µg/mL) and 1% DMSO were taken as positive and negative control respectively. The experiment was carried out in triplicates and average ZOI (zone of inhibition) was recorded. The ZOI of plants extract were compared with ZOI showed by streptomycin (20µg/mL) in same bacteria.

Agar well diffusion method

The antibacterial assay was done by using the agar well diffusion method as described by (Ahmed *et al.*, 2008). The assay was done to find out if the plant extracts had any antibacterial activity. Bacterial cultural was adjusted to 0.5 McFarland standards before the tests. The media used for the assay was Muller Hinton Agar. The standard solutions of the extracts were prepared in 1% DMSO (Merck). The sterile liquid culture medium (20mL) was poured into each petriplate. The solidified plates were inoculated by spread plate method with an inoculums corresponding to 0.5 McFarland standards. Four well of 5mm diameter were punched into the agar with the sterilized well puncture with 5mm diameter size and 50µL of plant extract were added into two of the wells. Streptomycin (20µg/mL) and 1% DMSO was taken as the as positive control and negative control respectively. The plates were then sealed with paraffin and kept for incubation at 37°C for 18h. The antibacterial activity was evaluated by measuring the ZOI diameter observed using zone scale (mention Hi Antibiotic Zone Scale). The test was conducted in triplicates.

Determination of minimum inhibitory concentration (MIC)

The determination of MIC was done following the protocol of Wang *et al.*, 2010. With little modification. MIC activity was

determined using 96 well micro plates. Stock solution of the extracts of *Paederia foetida* and *Hibiscus esculentus* was prepared at a concentration of 10mg/mL. various concentrations of the extract were prepared by serial dilution. 100 μ L of extract of each concentration was added in each of the wells. After this 100 μ L of the bacterial inoculums corresponding to 0.5McFarland Standard was added into each well. Streptomycin was taken as the positive control while DMSO 1% was the negative control. The plates were then incubated at 37°C for 16h in an incubator. After the incubation period was over, 40 μ L of MTT solution (0.2 mg/mL) was added into each well and then further incubated at 37°C for 30 – 45 minutes. The bacteria – formazan complexes were collected using a pipette. The complexes were transferred into glass test tubes added 1mL of 1% DMSO and 1mL of distilled water. The tubes were mixed manually to accelerate the dissolution process. The absorbance of the mixtures were recorded at 550nm.

RESULTS

The results of antibacterial activity in both Disc diffusion method and agar well method by measuring the diameter of inhibition zone are shown in the Table 1. In both the method, the ZOI was same (). The leaf extract of *Paederia foetida* showed maximum inhibition zone against *E. coli* (40mm) and moderate against *Staphylococcus aureus* (13mm). In case of *E. coli*, ZOI was found efficient when it was compared to ZOI of standard antibiotic Streptomycin but ZOI of *Staphylococcus aureus* was temperate compared to antibiotic. The leaf extract of *Hibiscus esculentus* showed inhibition zone 18mm and 33mm for *E. coli* and *Staphylococcus aureus* respectively. In case of *Staphylococcus aureus*, ZOI was found effective when it was compared to ZOI of standard antibiotic Streptomycin but ZOI of *E. coli* was temperate compared to standard

antibiotic streptomycin.

Of the two medicinal plants, *Paederia foetida* was found to be most active against the growth of *E. coli* and showed maximum ZOI (40mm) in ethanol extracts. Likewise *Hibiscus esculentus* was found fruitful against the growth of *Staphylococcus aureus* and showed maximum ZOI (33mm) in ethanol extracts. *P. foetida* was found to be less active against the growth of *S. aureus* and showed minimum ZOI (13mm). The *H. esculentus* was found to be less active against the growth of *E. coli* and showed ZOI (18mm). During the experiment it was clearly observed that both the leaf extracts possessed the potentiality against the growth of *E.coli* but the *Staphylococcus aureus* showed moderate resistance to these leaf extracts.

The MIC of antibiotics has been determined in resistant bacteria. The MIC assay for each antibiotic was then performed in the extract of *Paederia foetida* and *Hibiscus esculentus* at final concentrations of 25 μ g/mL X MIC.

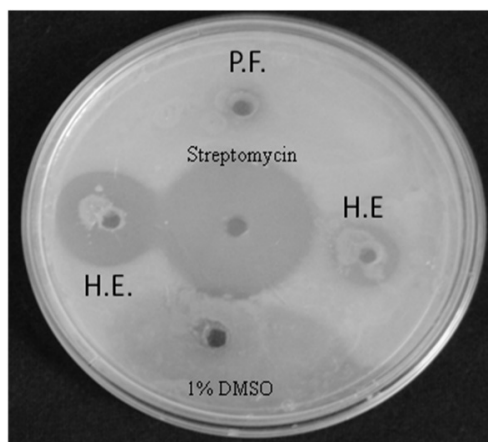
Minimum Inhibitory Concentration (MIC) of streptomycin (standard) against *E. coli* and *S. aureus* in this study was 1 μ g/mL and 31 μ g/mL respectively.

DISCUSSION

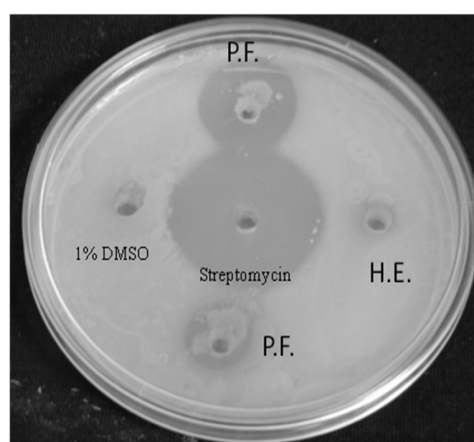
Popular knowledge of plants used by human is based on thousands of years of experience. By “trial and error”, people learnt how to recognize and use plants, including those with a magic – religious function (Camejo - Rodrigues *et al.*, 2003). Medicinal plants are the base for the development of new drug and the survival of human kind as well as other livestock. Even though the traditional medicinal practitioners are the best sources of information about the knowledge of the medicinal information as they considered their indigenous knowledge as a professional secret, only to be passed orally to their older son, at their oldest age (Jensen, 1981). In vitro pharmacological

Table 1: Zone of Inhibition and MIC values against human pathogens by plant extract

Plant species	Part used	Test organisms	Extract	ZOI values (mm)	MIC values (mg/mL)
<i>Paederia foetida</i>	Leaf	<i>E. coli</i> MTCC723	Ethanol	40	5.13
		<i>S. aureus</i> MTCC96	Ethanol	13	3.12
<i>Hibiscus esculentus</i>	Leaf	<i>E. coli</i> MTCC723	Ethanol	18	2.39
		<i>S. aureus</i> MTCC96	Ethanol	33	4.34



S. aureus



E.coli

Figure 1: Antibacterial activity by disc method

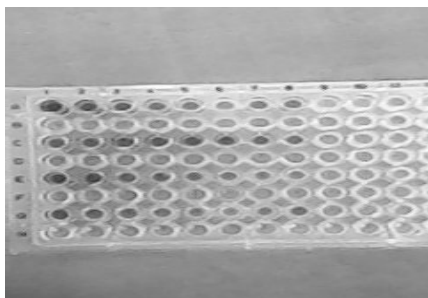


Figure 2: MIC test in microtitre plate

investigations of traditionally used medicinal plants offers an incredible opportunity to explore and investigate a wide range of plant-based drugs as a potential source of novel biologically active agents to validate claims made on their safety and efficacy (Gautam et al., 2007). Tuberculosis continues to be an enormous global concern; it is spreading and infects millions of people annually (Janin, 2007). The mode of antimicrobial action of tannins may be also related to their ability to inactivate microbial adhesions, enzymes and cell (Cowan, 1999).

From the above study it can be concluded that *Paederia foetida* was found to be most active against the growth of *E. coli*. Likewise *Hibiscus esculentus* was found fruitful against the growth of *Staphylococcus aureus*. It was clearly observed that all the two leaf extracts possessed the potentiality against the growth of *E. coli* but the *Staphylococcus aureus* strain showed moderate activity to these leaf extracts. These medicinal plants can be explored in the production of the useful drugs as they are commonly available.

Antibacterial activity observed by some of the tested plant extracts in this study indicates the presence of bioactive agents, which required further investigation. Ongoing research is currently focused on the isolation, identification and molecular characterization of bioactive compounds by using NMR, XRD, UPLC and GC-MS.

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REFERENCES

Abramowics, M. 1990. The choice of antimicrobial drugs. *Medical letter on Drugs and Therapeutics*. **32**: 41-48.

Ahmed, N., Uzir, B., Ayaz, S. and Ahmed, V. U. 2008. Antibacterial activity of marine bacteria from Arabian Sea of Pakistan. *J. Microbiol.* **4(2)**: 1 – 11.

Barbara, B. and Bennett, M. D. 1998. Gastroenteritis. *Medical Update for Psychiatrists*. **3**: 95-98.

Camejo-Rodrigues, J., Ascensao, L., Bonet, M. A. and Valles, J. 2003. An ethnobotanical study of medicinal and aromatic plants in the Natural Park of Serra de Sao Mamede (Portugal). *J. Ethnopharmacol.* **89**: 199 -209.

Cowan, M. M. 1999. Plant products as antimicrobial agents. *Clinical microbiology Reviews*. **12**: 564 – 582.

Evans, J. S. Pattison, E. and Morris, P. 1986. Antimicrobial agents from cell culture, in secondary metabolites in plants cell culture, Eds. P. Morris, A. Stafford and M. Fowler (Cambridge Univ., London).

Farnsworth, N. R. 1988. Screening plants for new medicines. In: Wilson, E.O.(Ed.), Biodiversity. National Academic Press, Washington DC, pp. 83-97.

Fyhrquist, P., Mwasumbi, L., Haeggstrom, C. A., Vuorela, H., Hiltunen, R., Vuorela, P., 2002. Ethnobotanical and antimicrobial investigation on some species of *Terminalia* and *Combretum* (Combretaceae) growing in Tanzania, *J. Ethnopharmacology*. **79**: 169-177.

Tally, F.P. and DeBruin, M. F. 2000. *J. Antimicrobial Chemother.* **46**: 523-526.

Gates, P. 2000. Herbal warning: medicinal trees – a resource for the future being squandered in the present. Trees in trouble: a new campaign to save the world's rarest species. Supplement to BBC wildlife Magazine, October, pp.15.

Gautam, R., Saklani, A., Jachak, S. M. 2007. Indian medicinal plants as a source of antimicrobial agents. *J. Ethnopharmacol.* **110**: 200 -234.

Janin, Y. L. 2007. Antituberculosis drugs: ten years of research. *Bioorganic and Medicinal Chemistry*. **15**: 2479 -2513.

Jensen, P. C. M. 1981. Species, Condiments and Medicinal plants in Ethiopia: Their taxonomic and agricultural significance, centre for agricultural publishing and documentation. Wageningen, the Netherlands.

Kaushik, P. and Dhiman, A. K. 2000. Medicinal plants and raw drugs of India. Bishen Singh mahendra pal singh, Pos Box no.137, Dehradun, pp. X 11+623.

Mothana R. A. and Lindequist, U. 2005. Antimicrobial activity of some medicinal plants of the island Soqotra. *J. Ethnopharmacol.* **96**: 177-181.

Robert, A. P., Lorraine, F., Walter, M. and Ronald, M. R. 2009. Laboratory exercises in Microbiology (3rd Ed.). U.S: John Wiley & Sons, Inc.

Rukangira, E. 2001. The African herbal industry: constraints and challenges. *Conserve Africa International* 126, 1-23.

Santos, R. B. 1985. Phytochemical investigation of seven medicinal plants containing Alkaloids and antimicrobials, M: NRCP Annual Reports, pp. 19.

Sivakumar, R. and Alagesaboothi, C. 2006. Antibacterial activity of two different forms of *Abrus precatorius* L, *J. of Adv. Plant Sciences*, pp. 409-413.

World Health Organization (WHO), 1996. Water and Sanitation. *Fact sheet*. **112**: Geneva.

Wang, H., Cheng, H. Wang, F., Wei, D. and Wang, X. 2010. An improved 3-(4, 5- dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) reduction assay for evaluating the viability of *Escherichia coli* cells. *J. of Microbiol. Methods*. **82(2010)**: 330 – 333.