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Antibacterial activity of some selected plants traditionally used as medicine in Manipur

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Methanolic leaf extracts of the plant species *Elsholtzia blanda* Benth., *Elsholtzia communis* (Collett & Hemsl) Diels., *Polygonum posumbu* Buchanam-Hamilton ex D. Don and *Zanthoxylum acanthopodium* DC. using methanol as a solvent were tested against 10 human pathogenic bacteria for potential antibacterial activity. The study revealed that all extracts show varied degree of antibacterial activity against the tested bacterial pathogens. The antibacterial activity was determined using agar well diffusion method. Methanolic extract of the leaf *Zanthoxylum acanthopodium* DC. showed antibacterial activity against five bacterial strains from among the ten bacteria tested followed by *Polygonum posumbu*, *Elsholtzia communis* and *Elsholtzia blanda*. *Clostridium sporogenes* was found to be susceptible to all the plants tested. Minimum inhibitory concentration of the plants against the tested organism ranged between 3.125-12.5 mg/ml. Hence these plants can be used to discover bioactive natural products that may serve as leads in the development of the new pharmaceuticals.

Key words: Antibacterial, human pathogens, methanolic extract, traditional medicine.

INTRODUCTION

The use of plants as therapeutic agents in addition to being used as food is an age long practice (Motley, 1994). Over the ages humans have relied on nature for their basic needs for the production of food stuffs, shelters, clothings, means of transport, fertilizers, flavours and fragrances and not least medicines (Chhetri et al., 2008). Medicinal plants are considerably useful and economically essential. They contain active constituents that are used in the treatment of many human diseases (Menghani et al., 2011). Medicinal plants are cheap and handy to most of the population on the globe (Oluma et al., 2004). Of the 250,000 higher plant species on the earth, more than 80,000 are medicinal (Egwaikhide et al., 2007). Over three quarters of the world population relies mainly on plants and plant extract for health care. More than 30% of the entire plants species at one time or the other was used for medicinal purposes. However, the therapeutic uses of plants by the primitive people lack scientific explanation (Dutta, 1994).

Infectious diseases account for high proportion of health problems in the developing countries including India. Antibacterial resistance among bacterial pathogens in recent time is a critical area of public health concern (Hart and Kariuki, 1998). There has been an increased

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bacterial resistance to antimicrobial agents over the past decades and the outlook for the use of antimicrobial drugs in the future is still uncertain. Moreover, antibiotics are sometimes associated with adverse effects on the host, including hypersensitivity, immune-suppression and allergic reaction (McDonnell and Russell, 1999). This situation has forced scientist to search for new antimicrobial substances from various sources as novel antimicrobial chemotherapeutic agents, but the cost of production of synthetic drugs is high and they produce adverse side effects compared to plant derived drugs (Abiramasundari et al., 2011). Biomolecules of plant origin appear to be one of the alternatives for the control of these antibiotic resistant human pathogens (Raghavendra et al., 2006). Plants have provided a good source of anti-infective agents; emetine, quinine, berberine, tannins, terpenoids, alkaloids and flavonoids continue to be highly efficient instruments in the fight against microbial infections (Cowan, 1999).

Therefore, the increasing prevalence of multidrug resistant strains of microorganisms and the recent appearance of strains with reduce susceptibility to antibiotics put forward an urgent need to search for new sources of antimicrobial agent (Sieradzki et al., 1999). Hence, in the present study an attempt has been made to evaluate antibacterial potential of four medicinal plants namey: *Elsholtzia blanda* Benth., *Elsholtzia communis* (Collett & Hemsl) Diels., *Polygonum posumbu* Buchanam-Hamilton ex D. Don and *Zanthoxylum acanthopodium* DC. which are used as traditional remedies of cough, throat infection, gastric problems and bronchitis respectively in Manipur, India.

MATERIALS AND METHODS

Plant material

Four medicinal plants viz *Elsholtzia blanda* Benth. (leaf), *Elsholtzia communis* (Collett & Hemsl) Diels. (leaf), *Polygonum posumbu* Buchanam-Hamilton ex D. Don (leaf) and *Zanthoxylum acanthopodium* DC. (leaf) were screened.

Preparation of the plant extract

Plant parts were cleaned, air dried in the shade and powdered into fine powder. 10 g of the powdered plant material was soaked in 100 ml of 80% methanol and extracted for 24 h at room temperature with shaking at 150 rpm and further centrifuged at 15000 × *g* for 10 min to pellet solids. The mixture was then filtered and evaporated using the Buchi rotavapor. The dried extracts were resuspended in 5% dimethyl sulphoxide to a final concentration of 100 mg/ml.

Microorganisms used

All bacteria selected are ATCC cultures which include *Bacillus cereus* (10876), *Clostridium perfringens* (13124) *Clostridium sporogenes* (11437), *Klebsiella pneumoniae* (10031), *Niserria gonorrhoea* (19424), *Staphylococcus aureus* (11632), *Pseudomonas aeruginosa* (15442), *Shigella boydii* (9207), *Shigella flexineri* (9199), *Shigella sonnie* (25931).

Preparation of inoculums

Stock cultures were maintained at 4°C on nutrient agar. Fresh cultures are prepared by transferring a loopful of cells from the stock cultures to Mueller Hinton Broth.

Antimicrobial susceptibility testing

The antimicrobial activity of the methanolic plant extracts were screened against the pathogens by using the agar well diffusion assay (Bauer et al., 1966). An inoculum suspension was swabbed uniformly to solidified 20 ml Mueller Hinton agar and the inoculums were allowed to dry for 5 min. Holes of 6 mm in diameter were made in the seeded agar using sterile cork borer. Aliquot of 50 µl from each plant crude extract (100 mg/ml) was added into each hole on the seeded medium and allowed to stand for 1 h for proper diffusion and further incubated at 37°C for 24 h. The antimicrobial activity was evaluated by measuring the inhibition zone diameter in millimetres (mm) around the wells. Aliquots of phosphate buffer saline were used as negative control. These studies were accomplished in triplicates.

Determination of minimum inhibitory concentration (MIC)

The MIC is determined for the highly active plant that showed significant antibacterial activity against the test bacteria according to the methods of Nakamura et al. (1999) and Dulgar and Aki (2009) with some modifications. Serial dilution of each of the extracts were prepared using the 5% DMSO (dimethyl sulphoxide) to produce the final concentrations of 100, 50, 25,12.5, 6.25 and 3.125%. This dilution represents concentrations of 100, 50, 25, 12.5, 6.25 and 3.125 mg/ml, respectively. 100 µl from each dilution is transferred to 96 well microplate. The bacterial suspension is adjusted to 1×10^8 CFU/ml in Mueller Hinton broth and 100 µl of it is transferred to the respective wells. The microplates were incubated for 24 h at 37°C. MIC values were determined by plating 50 µl from clear wells onto Mueller Hinton Agar. The MIC was considered the lowest concentration of the sample that prevented visible growth. The samples were examined in triplicates.

RESULTS AND DISCUSSION

The antibacterial activity of plant parts used in folk medicines in Manipur from four medicinal plant species has been evaluated *in-vitro* against ten human pathogens (Figure 1). All the four methanolic extracts resulted in consistent inhibition zones against one or more of the bacteria tested in well diffusion assays. The tested plants showed negative as well as positive activities against tested bacteria. *Z. acanthopodium* DC. shows moderate to good (11-15 mm diameter zone of inhibition). The largest zone of inhibition 18 mm was recorded against *Shigella sonnie* with the leaf of *Elsholtzia communis* (Collett & Hemsl) Diels. All the four plant extract namely: *Elsholtzia blanda* Benth., *Elsholtzia communis* (Collett & Hemsl) Diels, *Polygonum posumbu* Buchanam-Hamilton ex D. Don and *Z. acanthopodium* DC. were found active against *Clostridium sporogenes*, 3 extracts active against

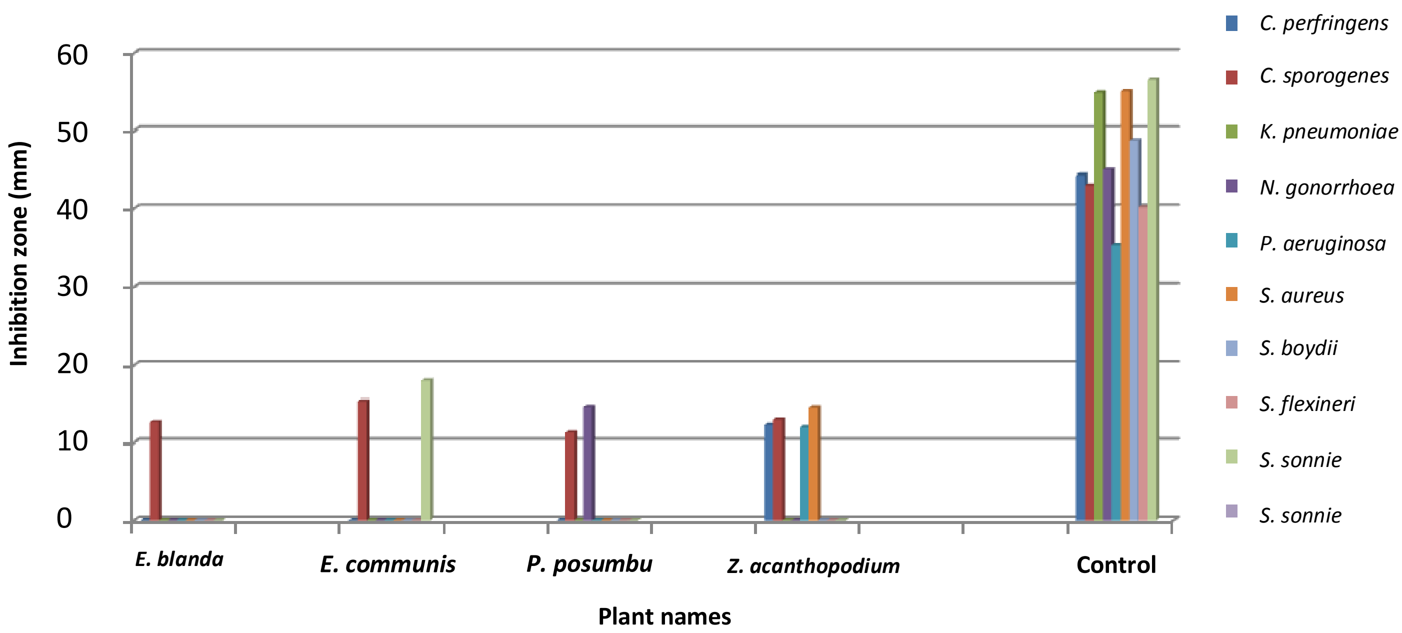

Figure 1. Antibacterial activity of four plant extract.

Table 1. Ethnobotanical information on the plant used as medicine in Manipur (Singh et al., 2003).

Scientific name (family)	Local name in Manipuri	Plant part used	Traditional used
Elsholtzia blanda Benth.	Kanghuman	Leaf	Cough, dyspepsia. Leaf paste applied on forehead in dizziness
Elsholtzia communis (Collett & Hemsl) Diels. (Lamiaceae)	Lomba	Leaf	Fever, cough, high blood pressure, nose bleeding and menstrual disorder
Polygonum posumbu Buchanam-Hamilton ex D Don.	Phakpai	Leaf	Fever and dyspepsia
Zanthoxylum acanthopodium DC. (Rutaceae)	Mukthrubi	Leaf	Fever, dyspepsia, cough, bronchitis. Seed oil applied on rheumatism

Bacillus cereus. The extract of *Elsholtzia communis* (Collett & Hemsl) Diels and *Polygonum posumbu* Buchanam-Hamilton ex D. Don showed active against only for *Shigella sonnie*, *Niserria gonorrhoea,* respectively (Table 1). On the basis of the result obtained in this present investigation it can be concluded that methanolic extract of *Z. acanthopodium* DC. leaves have significant antibacterial activity (Table 2). The highest inhibition was shown by *Elsholtzia communis* (Collett & Hemsl) Diels followed by *Polygonum posumbu* Buchanam-Hamilton ex D. Don, *Z. acanthopodium* DC. and *E. blanda* Benth. respectively. *Z. acanthopodium* DC. shows inhibition to five different bacterial strains from among the ten bacteria tested (Table 3). *C. sporogenes* is susceptible to all the plant extract tested whereas *Klebsiella pneumoniae* does not show susceptibility to all the four plants tested.

Minimum inhibitory concentrations (MIC) were established for all the active plant extracts. The MIC values range from 3.125-12.5 mg/ml. *E. blanda* Benth. and *Polygonum posumbu* Buchanam-Hamilton ex D Don. shows the highest antibacterial efficacy against *B. cereus* at 3.125 mg/ml and *Elsholtzia communis* (Collett & Hemsl) Diels against *C. sporogenes* and *S. sonnie* at 3.125 mg/ml. Also *Z. acanthopodium* DC also proved to possess the highest antibacterial activity against *P. aeruginosa* and *S. aureus* at an MIC value of 3.125 mg/ml. The least inhibition was shown by *Polygonum posumbu* Buchanam-Hamilton ex D. Don against *C. sporogenes* (MIC value of 12.5 mg/ml). In classifying the

Table 2. Antibacterial activity of methanolic extract of selected plant leaves against ten human pathogenic bacteria.

Human pathogenic bacteria	Zone of inhibition (mm)				
	Elsholtzia blanda	***E. communis***	***Polygonum posumbu***	***Z. acanthopodium***	***Control**
Bacillus cereus	12 ± 0.00	0.00 ± 0.00	12.33 ± 0.58	10.33 ± 0.57	30 ± 0.50
Clostridium perfringens	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	12.30 ± 0.49	44.33 ± 0.58
Clostridium sporogenes	12.67 ± 0.57	15.33 ± 0.57	11.33 ± 0.58	13.00 ± 1.00	43 ± 00.00
Klebsiella pneumonia	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	00.00 ± 00.0	55 ± 00.00
Niserria gonorrhea	0.00 ± 0.00	0.00 ± 0.00	14.67 ± 0.70	00.00 ± 00.00	45 ± 0.50
Pseudomonas aeruginosa	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	12 ± 1.00	35.33 ± 0.29
Staphylococus aureus	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	14.60 ± 0.58	55.16 ± 0.28
Shigella boydii	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	00.00 ± 00.00	48.83 ± 0.29
Shigella flexineri	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	00.00 ± 00.00	40.17 ± 0.29
Shigella sonnie	0.00 ± 0.00	18.00 ± 0.00	0.00 ± 0.00	00.00 ± 00.00	56.5 ± 0.5

*Imepenem. Each value represented by Mean±Standard Deviation.

Table 3. Minimum inhibitory concentration.

Plant	Bacteria	MIC (mg/ml)
E.blanda Benth.	*B. cereus*	3.125
E.blanda Benth.	*C. perfringens*	6.25
Elsholtzia communis (Collett & Hemsl) Diels	*C. sporogenes*	3.125
Elsholtzia communis (Collett & Hemsl) Diels	*S.sonnie*	3.125
Polygonum posumbu Buchanam-Hamilton ex D Don.	*B. cereus*	3.125
Polygonum posumbu Buchanam-Hamilton ex D Don.	*C. sporogenes*	12.5
Polygonum posumbu Buchanam-Hamilton ex D Don.	*N. gonorrhoea*	3.125
Z. acanthopodium DC.	*B. cereus*	6.25
Z. acanthopodium DC.	*C. sporogenes*	6.25
Z. acanthopodium DC.	*C. perfringens*	6.25
Z. acanthopodium DC.	*P. aeruginosa*	3.125
Z. acanthopodium DC.	*S. aureus*	3.125

antibacterial activity as Gram positive or Gram negative, it would generally be expected that a much greater number would be active against Gram positive than Gram negative bacteria (McCutcheon et al., 1992). The present finding also revealed that from among the ten human pathogens investigated, seven Gram positive bacteria showed sensitivity against the plant extracts supporting the above views. Hence from the above observation it can be concluded that Gram positive bacteria are more susceptible than the Gram negative bacteria.

The present study justify the use of *Z. acanthopodium* DC., *Elsholtzia communis* (Collett & Hemsl) Diels*, E. blanda* Benth. *and Polygonum posumbu* Buchanam-Hamilton ex D Don. in the traditional system of medicine to treat various infectious diseases. The investigation also supports the used of tested plants in traditional medicine. The used of these plants in folk medicine suggest that they represent an economic safe alternative to treat infectious diseases. Based on this, further pharmacological investigations need to be undergone in order to isolate and identify active compounds.

Conflict of interests

The author(s) have not declared any conflict of interests.

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