In vitro antibacterial activity of the ethanol extract of Paederia foetida L. (Rubiaceae) leaves

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The plant *Paederia foetida* Linn., locally known as "Gondhabadali", has traditionally been used for medicinal purposes, though its mechanisms of beneficial effects have remained largely unknown. We investigated antibacterial activity of the plant to justify the traditional claims that "Gondhabadali" plants are effective in killing a host of bacteria with a concurrent healing from various infectious diseases including shigellosis, diarrhea etc. The test microorganisms included two Gram-positive (*Staphylococcus aureus, Enterococcus faecalis*) and three Gram-negative (*Escherichia coli, Salmonella typhimurium, Shigella flexneri*) bacteria. The extract showed significant antibacterial activities against *S. flexneri, S. aureus, E. coli* and *E. faecalis*. The preliminary screening experiment revealed that the most susceptible bacterium was *S. flexneri* while the most resistant was *S. typhimurium*. The results of the present experiment clearly indicate that *Paederia foetida* plant could be used for its antibacterial activity.

The history of disease and medication is as old as that of human being. Microorganisms remain one of the most potent reasons for human diseases since the dawn of history. The range of microorganisms is large and so is the variety of diseases that they cause. Despite the existence of potent antimicrobial agents, resistant or multi-resistant strains are continuously appearing, imposing the need for a continuous search and development of new drugs. Hence many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as soil, microorganisms, animals and plants. One such resource is herbal medicines, and their systematic screening may result in the discovery of novel effective compounds. The microorganisms used in the present antibacterial study are highly pathogenic towards human beings.

Paederia foetida Linn. is a perennial climbing shrub belonging to the family Rubiaceae. The plant releases a strong fetid odor when bruised. A variety of therapeutic properties have been attributed to Paederia foetida plant in folk medicine. It is used to treat enteritis, flatulence and stomachache (Johnson T., 1999). Paederia foetida is a popular shrub used as a remedy for diarrhea and dysentery in Bangladesh (Ghani A., 2003). Considering the therapeutic potential of the plant, the present study was aimed to evaluate the antibacterial activity of the plant against five clinically very important bacteria. The antimicrobial assessment was performed by simple agar well diffusion method.

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Collection of plant materials: The leaves of *Paederia foetida* used in this study were collected from the "Botanical Garden" of Jahangirnagar University, during the month of February 2007.

Preparation of the plant extracts: The *Paederia foetida* leaves were first cut and grinded. The pasted leaves were packed in a soxhlet apparatus and subjected to mild hot continuous percolation for 12 hour using absolute ethanol as solvent. The ethanol extract was concentrated under reduced pressure at 50°C using rotary evaporator. The concentrated extract was further dried by placing within a desiccator (approximate yield 10%, w/w). Dried ethanol-free extract of *Paederia foetida* was dissolved in physiological saline (0.9% NaCl) and sonicated within a bath sonicator to make a homogenous solution of 500 mg/ml.

Antibacterial Assay: The bacterial strains were collected from International Centre for Diarrheal Diseases Research, Bangladesh (ICDDR, B). The bacteria were grown in nutrient agar plates at 37° C and pure colonies were maintained in nutrient agar slants at 4°C. Susceptibility tests were performed by a modified agar-well diffusion method (Perez, et al., 1990) using nutrient agar as the culture media. A 100 μl volume of the standard suspension of test bacterial strain was spread evenly on a previously bored (using a 7 mm cork borer) plate using a sterile glass rod spreader. The plates were allowed to dry at room temperature. Subsequently 150, 100 and 50 μl of Paederia foetida extract (500 mg/ml) were introduced into the wells. After holding the plates at 2-8° C for 2 hours to allow diffusion of the extract into the agar, the plates were incubated at 37° C for 24 hours. The diameter of zone of inhibition was measured to the nearest millimeter (Khalil, et al., 2001, Okeke, et al. 2001). Physiological saline was used as control. The tests were performed in duplicate for the microorganisms evaluated and the final results were presented as the mean±standard error of mean. The whole experiment was performed under strict aseptic conditions.

Amongst all the test bacterial strains, *S. flexneri* showed maximum zone of inhibition $(27\pm1 \text{ mm})$ at the highest amount of extract (75 mg). This was followed by *E. faecalis* $(24\pm1 \text{ mm})$ and *E. coli* $(22\pm0.5 \text{ mm})$. *S. aureus* displayed the lowest diameter of zone of inhibition (19 ± 1) at that amount of extract. All the test bacteria responded to the extract in a dose-dependent way. However *S. typhimurium* showed no zone of inhibition (Table 1). Thus it proved to be resistant towards the ethanol extract of the *Paederia foetida* at the applied amounts (75, 50 and 25 mg). Thus the ethanol extract of *Paederia foetida* showed strong antibacterial activity against *S. flexneri* and *E. faecalis* and moderate activity against *E. coli* and *S. aureus*. The results are expressed as the radial extent of the annular zone of inhibition including the 7 mm diameter of the well.

Diameter of Zone of Inhibition (mm) Name of bacteria Amount of extract per well Gram positive 75 mg 25 mg 50 mg 17 ± 0.5 24 ±1 Enterococcus faecalis 20 ± 0.5 Staphylococcus aureus 19 ± 1 17 ± 2 15 ± 2 Gram negative Shigella flexneri 27 ± 1 21 ± 0.5 18 ± 0.5 Escherichia coli 22 ± 0.5 19 ± 0.5 17 ± 0.5 0 0 Salmonella typhimurium 0

Table 1. Antibacterial effects of Paederia foetida extract.

Results are presented as mean \pm SEM (n = 2). Zero sign (0) denotes no zone of inhibition.

As evident from the above discussion, *Paederia foetida* contains important chemical substances that is responsible for its antibacterial activity. Thus the results indicate that there is some rationale behind the ethnomedicinal use of the plant for treating various infectious diseases. However, a more extensive biological and chemical investigation is necessary to characterize the active antibacterial principle(s).

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