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Screening of antibacterial, antifungal and cytotoxic activities of *Polygonum hydropiper* L. stem extracts

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Abstract

The aim of the study was to investigate the antibacterial, antifungal and cytotoxic activities of ethanol extract of *Polygonum hydropiper* stem. Disc diffusion method measuring minimum inhibitory concentration (MIC) was used to demonstrate antibacterial and antifungal activities. Stem extract showed significant antibacterial activities against three gram-positive (*Bacillus subtilis*, *Bacillus megaterium* and *Staphylococcus aureus*) and four gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Shigella sonnei*) bacteria resulting 16 to 64 μg/ml MIC values against these bacteria. Further, the antifungal activities of stem extract were not highly remarked but still to be considered as inhibitory to tested fungi. LC_{50} of the extract against brine shrimp nauplii was 35.46μg/ml and indicates the cytotoxic potentiality of *Polygonum hydropiper* stem. Results obtained in this study suggest that *Polygonum hydropiper* stem can also be used as a source of antimicrobial and cytotoxic substances for possible for plant protections.

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Introduction

Nature has been a source of medicinal agents for thousands of years. Impressive number of modern drugs has been isolated from natural sources and is being as traditional medicine (Doughari *et al.,* 2008). As a result, plant based traditional medicine continues to play an essential role in health care, with 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care (Owolabi *et al.,* 2007). Therefore, plants are being investigated to better understand their properties, safety and efficacy (Nascimento *et al.,* 2000). Many studies revealed the medicinal value of different plant parts, such as peptide, alkaloid, essential oils, phenols, ethanol, primary and secondary metabolites (Doughari *et al.,* 2008). In addition, plants also produce some therapeutic agents which act against human pathogens, including bacteria, fungi or virus (El astal *et al.,* 2005).

Polygonum hydropiper L. is an important medicinal plant belongs to the family of Polygonaceae. The whole plant has been found to contain flavones and flavonoid glycosides, such as quercetin galactosides, a sesquiterpene acid, viscosumic acid, oxymethyl-anthraquinones and polygonic acid (Furukawa *et al.,* 2002). It also possesses bitter, stimulant, tonic, diuretic, carminative, anthelmintic, emmeragogue, haemostatic and lithotripter properties (Sharma 2003). Presence of some insecticidal properties has also been reported in *Polygonum hydropiper* (Mollah and Islam 2005; Kundu *et al.,* 2007). Moreover, *Polygonum hydropiper* is used for the treatment of wide range diseases including diarrhea, dyspepsia, itching skin, excessive menstrual bleeding, etc. (Chevallier, 1996). This plant is also of economic interest of its wide ranging pharmacological activity. That is why investigation of its antibacterial and antifungal properties is of great demand. Furthermore, there are few reports on the cytotoxic activity of *Polygonum* species which deserved to be extensively investigated. The aims of this study were to determine antibacterial, antifungal and cytotoxic activities of ethanol extract of *Polygonum hydropiper* stem against some pathogenic bacteria, fungi and brine shrimp nauplii.

Materials and methods

Plant materials

The stems of *Polygonum hydropiper* were collected from the Rajshahi University Campus, Rajshahi, Bangladesh. The stems were cut, air-dried and powdered in a grinding machine and stored in an airtight container until further analyzed.

Preparation of extract

Powdered dried stems (300g) of the plant were extracted (cold) with ethanol (1.25L) in flat bottom glass container, through occasional shaking and stirring for 15 days. The whole mixture was then filtered and the filtrate was dried in vacuo using a rotatory evaporator (Wei *et al.* 2006) to afford a blackish mass.

Organisms

Antibacterial activity and minimum inhibitory concentration (MIC) were determined against three gram-positive bacteria (*Bacillus subtilis, ATCC no. 465; Bacillus megaterium, ATCC no. 11562; Staphylococcus aureus, ATCC no. 39344*) and four gram-negative bacteria (*Escherichia coli, ATCC no. 11775; Pseudomonas aeruginosa, ATCC no. 87414; Salmonella typhi, ATCC no. 19214*; *Shigella sonnei, ATCC no. 11126*). Antifungal screening was carried out against five fungi (*Aspergillus fumigatus, ATCC no. T33439; Aspergillus niger, ATCC no. 53346; Aspergillus flavus, ATCC no. 12693; Candida albicans, ATCC no. 10231; Rizopus oryzae, ATCC no. 20344*). Cytotoxicity was determined against brine shrimp nauplii. Brine shrimp nauplii were obtained by hatching brine shrimp eggs (Carolina Biological Supply Company, Burlington, NC, USA) in artificial sea water (3.8% sodium chloride solution) for 48h. These organisms were collected from the Microbiology

Research Laboratory at Department of Pharmacy, Rajshahi University, Bangladesh.

Growth media

Nutrient agar media (pH 7.2), nutrient broth media (pH 6.8) and Sabouraud dextrose agar media (pH 5.6), artificial sea water (3.8% sodium chloride solution) having pH 8.4 were used for antibacterial screening, MIC determination, antifungal screening and cytotoxicity determination, respectively.

Antibacterial screening

Antibacterial screening is generally performed by disc diffusion method (Dash *et al.* 2005). Briefly, 20 ml of nutrient agar were plated in petri dish with 0.1 ml of each bacterial culture. Filter paper discs (6 mm in diameter) impregnated with plant extracts were placed on test organism-seeded plates. Ethanol was used to dissolve the extract and was completely evaporated before application on test organism seeded plates. Blank disc impregnated with solvent ethanol followed by during off was used as negative control. The activity was determined after 18 hours of incubation at 37°C. The diameters of zone of inhibition produced by the extract were then compared with the standard antibiotic kanamycin 30μm/disc. Each sample was used in triplicate for the determination of antibacterial activity.

Minimum inhibitory concentration (MIC) determination

Serial dilution technique (Iwaki *et al.* 2006) was applied for the determination of minimum inhibitory concentration of the extracts against these bacteria. The plant extract (0.512 mg) was dissolved in 2 ml distilled water to obtain stock solution having concentration of 256 μg/ml. The serial dilution technique, 1ml prepared stock solution was transferred to test tube containing 1ml nutrient broth medium to give concentration of 128 μm/ml, from which 1ml was transferred to another test tube containing 1ml nutrient broth medium. After 18 hours of incubation at 37°C, the tubes were then examined for the growth. The MIC of the extract was taken as the lowest concentration that showed no growth. Growth was observed in those tubes where the concentration of the extract was below the inhibitory level and the broth medium was observed turbid (cloudy). Kanamycin was used as negative and positive control, respectively.

Antifungal screening

The antifungal activity of the extract was tested by disc diffusion method (Dash *et al.* 2005) against the five pathogenic fungi at the concentrations of 150 μg/disc and 300 μg/disc for each. Here 20ml quantities of Sabouraud dextrose were plated in petri dish. Blank disc impregnated with solvent ethanol followed by drying off was used as negative control. The activity was determined after 72 hours of incubation at 32°C. The diameter of zone of inhibition produced by the extract was then compared with the standard antibiotic kanamycin 30 μg/disc. Each sample was used in triplicate for the determination of antifungal activity.

Cytotoxicity assay

The cytotoxicity assay was performed on brine shrimp nauplii using Mayer method (Hossain *et al.* 2004). Brine shrimp nauplii were obtained by hatching brine shrimp eggs (Carolina Biological Supply Company, Burlington, NC, USA) in artificial sea water (3.8% sodium chloride solution) for 48 h. Dissolution of extract was performed in artificial sea water using DMSO. Each 5 ml solution of different concentrations (0.5, 1, 2, 5, 10, 20 and 40μg/ml) of the extract was taken in different vials where brine shrimp nauplii were given and observed for mortality for 24 h. The resulting data were transformed to probit analysis (Al-Bari *et al.* 2007) for the determination of LC_{50} values of the extract. Each sample was used in triplicate for the determination of cytotoxicity.

Results

Antibacterial activity

The results representing antibacterial activity of ethanol extract of stem of *P. hydropiper* presented in Table 1. The highest activity of plant extract was 26.3 mm diameter of zone inhibition found against *Bacillus subtilis* followed by 26.0 mm diameter of zone inhibition against *Eschirichia coli* at the concentration of 300 µg/disc. In contrast, the lowest activity of plant extract was 13.6 mm diameter of zone inhibition observed against *Salmonella typhi* at the concentration of 150 µg/disc.

Minimum Inhibitory Concentration (MIC)

The MIC values of the extract against tested bacteria were shown in Table 2. The MIC values were 16, 32, 32, 16, 16, 32 and 64 µg/ml respectively, against the tested organisms. The MIC values against the tested gram-positive bacteria ranged from 16 to 32 µg/ml and against gram-negative bacteria from 16 to 64 µg/ml.

Table 1. Antibacterial activity of ethanol extract of *Polygonum hydropiper* stems. Values are the mean with standard error.

Test organisms	Diameter of zone of inhibition (mm)			ANOVA
	Ethanol ext. 150µg/disc (Mean±SE)	Ethanol ext. 300µg/disc (Mean±SE)	Kanamycin 30µg/disc (Mean±SE)	
Gram-positive				
Bacillus subtilis	16.6±0.61	26.3±0.49	36.0±0.01	P<0.05
Bacillus megaterium	15.0±0.05	24.0±1.07	35.6±0.22	P<0.05
Stapphylococcus aureus	15.6±0.43	25.3±0.46	34.0±0.53	P<0.05
Gram-negative				
Escherichia coli	16.3±0.70	26.0±1.03	35.0±0.51	P<0.05
Pseudomonas aeruginosa	14.0±1.04	25.3±0.43	32.7±0.66	P<0.05
Salmonella typhi	13.6±0.43	25.0±0.07	33.6±0.90	P<0.05
Shigella sonnei	15.6±0.70	23.0±0.52	34.3±0.83	P<0.05

Note: The control disc used for solvent had no zone of inhibition; therefore, it has not been presented.

Table 2. The MIC values of ethanol extract of *Polygonum hydropiper* stems. Values are the mean with standard error.

Test organisms	MIC values of ethanol extract (µg/ml)	MIC values of kanamycin (µg/ml)	t-test
Gram-positive			
Bacillus subtilis	16±0.20	2±0.01	P<0.05
Bacillus megaterium	32±2.02	8±0.03	P<0.05
Stapphylococcus aureus	32±0.12	8±1.10	P<0.05
Gram-negative			
Escherichia coli	16±0.97	8±0.08	P<0.05
Pseudomonas aeruginosa	16±1.24	4±0.11	P<0.05
Salmonella typhi	32±1.04	4±0.53	P<0.05
Shigella sonnei	64±2.5	8±0.87	P<0.05

Antifungal activity

The antifungal activities of ethanol extract of *Polygonum hydropiper* stems and standard kanamycin (30 µg/disc) were determined at the concentrations of 150 µg/disc and 300 µg/disc against five pathogenic fungi (Table 3). The highest activity was 20.7 mm diameter of zone inhibition observed against *Aspergillus fumigatas* followed by 20.3 mm diameter of zone inhibition against *Aspergillus flavus* at the concentration of 300 µg/disc. The lowest activity

was 11.0 mm diameter of zone inhibition found against *Candida albicaus* at the concentration of 150 µg/disc.

Cytotoxicity assay

In cytotoxicity assay with brine shrimp nauplii, the LC_{50} value of the ethanol extract of stems of the plant was 35.46 µg/ml. The cytotoxicity of the plant extract was compared with cytotoxicity of standard ampicillin. LC_{50} value of ampicillin was 16.18 (Table 4). No mortality was found in the control group.

Table 3. Antifungal activity of ethanol extract of *Polygonum hydropiper* stems. Values are the mean with standard error.

Test organisms	Diameter of zone of inhibition (mm)			ANOVA
	Ethanol ext. 150µg/disc (Mean±SE)	Ethanol ext. 300µg/disc (Mean±SE)	Kanamycin 30µg/disc (Mean±SE)	
Aspergillus fumigatus	13.7±1.2	20.7±1.2	24.7±0.9	P<0.05
Aspergillus niger	13.0±1.0	20.0±0.6	23.7±0.7	P<0.05
Aspergillus flavus	13.3±0.3	20.3±0.3	24.0±0.6	P<0.05
Candida albicans	11.0±1.2	19.0±1.2	22.3±0.9	P<0.05
Rizopus oryzae	12.0±1.2	20.0±1.7	21.3±0.9	P<0.05

Note: The control disc used for solvent had no zone of inhibition; therefore, it has not been presented.

Table 4. Cytotoxicity of ethanol extract of *Polygonum hydropiper* stems.

Sample	LC_{50} (µg/ml)	95% confidence limits (µg/ml)	Regeneration equation	X2 value
Plant extract	35.46	23.63-53.20	Y=3.13+1.20	0.54
Ampicillin	16.18	7.16-36.53	Y=4.05+0.77	0.04

Discussion

As per data recorded, in comparison to reference standard kanamycin 30µg/disc, the ethanol extract of *Polygonum hydropiper* stem showed significant antibacterial activity at 150 µg/disc and 300 µg/disc. In the present investigation, we found that the ethanol extract showed comparatively better antibacterial activity against the gram-positive bacteria than the gram-negative bacteria. Many authors reported antibacterial activity of different medicinal plant extracts (Khan *et al.* 2007; Jain *et al.* 2008) and our present finding supported the previous investigations. Antibacterial potency of plant extract against these bacteria expressed in MIC as presented in Table 2 indicated the plant extract is more effective against gram-positive at lower concentration than that against gram-negative bacteria. The ethanol extract of stem was shown strong activity against all of the pathogenic fungi. Different plant extract have been reported for their antifungal properties (Asghari *et al.* 2006; Akinpelu *et al.* 2006; Khan *et al.* 2007; Jain *et al.* 2008), which supports our present investigation. It is evident that the plant extract was found to be lethal to brine shrimps nauplii indicating that the extract is biologically active. Many scientists have been reported a correlation between cytotoxicity and activity against the brine shrimps nauplii using extracts or isolated compounds from various plants (McLaughlin 1992; Hossain *et al.* 2004; Khan *et al.* 2007; Al-Bari *et al.* 2007).

It is probably the first report on the antibacterial, antifungal and cytotoxic studies on stem extracts of *Polygonum hydropiper*. Further, remarkable antimicrobial and cytotoxic activities found by the experiment support the claims of traditional medicine. It was concluded that this finding can be source of antibiotic substances for possible treatment of bacterial and fungal infections. Moreover, it explores

the potential use as plant protection resource. However, to know the extract mechanism of action of *Polygonum hydropiper* stem extract, further studies with purified fractions/bioactive compounds are warranted.

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