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The Determination of Antibacterial and Antifungal Activities of *Polygonum hydropiper* (L.) Root Extract

[1]*M. F. Hasan, R. Das,* [2]*Alam Khan,* [1]*M.S. Hossain and* [1]*M. Rahman*

[1]Department of Genetic Engineering and Biotechnology,
[2]Department of Pharmacy, University of Rajshahi, Rajshahi-6205, Bangladesh

Abstract: A study was conducted to determine the antibacterial and antifungal activities of *Polygonum hydropiper* (L.) root extract on chloroform against both bacteria and fungi using the disc diffusion method. The extract showed significant antibacterial activities against four gram-positive (*Bacillus subtilis, Bacillus megaterium*, *Stapphylococcus aureus* and *Enterobacter aerogenes*) and four gram-negative (*Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi* and *Shigella sonnei*) bacteria. The minimum inhibitory concentration (MIC) values against these bacteria ranged from 16 to 64 µg/ml. The antifungal activities were found strong against six fungi (A*spergillus fumigatus, Aspergillus niger, Aspergillus flavus, Candida albicans*, *Rizopus oryzae* and *Tricophyton rubrum*). It can be used in the folk medicine at different parts of the world to treat many diseases including bacterial and fungal infections.

Key words: *Polygonum hydropiper* · Chloroform extract of roots · Antibacterial activity · MIC · Antifungal activity

INTRODUCTION

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 [1]. The plant also has some insecticidal properties [2, 3]. The plant also possesses bitter, stimulant, tonic, diuretic, carminative, anthelmintic, emmeragogue, haemostatic and lithotripter properties [4]. The whole plant, either on its own or mixed with other herbs, is decocted and used in the treatment of a wide range of ailments including diarrhoea, dyspepsia, itching skin, excessive menstrual bleeding and hemorrhoids [5]. This species is of economic interest of its wide ranging pharmacological activity and one of the major constraints in utilizing natural populations is the existence of plant to plant chemovariability. That is why, there is an increasing awareness in people for the use of this herbal medicinal plant day by day.

The frequency of life-threatening infections caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality in immunocompromised patients in developing countries [6]. To overcome this problem many works have been done which aim at knowing the different antimicrobial and phytochemical constituents of medicinal plants and using them for the treatment of microbial infections as possible alternatives to chemically synthetic drugs [7]. There are many reports on antimicrobial activities of several medicinal plant species including, *Polygonum multiflorum* [8], *Polygonum tinctorium* [9] and *Amorphophallus campanulatus* [10]. But there are no reports on antimicrobial activities on this valuable plant. Hence, the present study was undertaken to determine the antibacterial and antifungal activities of chloroform extract of *Polygonum hydropiper* roots.

MATERIALS AND METHODS

Plant Materials Collection: The roots of *Polygonum hydropiper* were collected during January, 2007 from the Rajshahi University Campus, Rajshahi, Bangladesh and were identified by Md. Shahed Alam, Senior technical officer, Herbarium Museum, Department of Botany, University of Rajshahi, Bangladesh, where its voucher specimen (No. 10203) was deposited, for reference.

Corresponding Author: M. Rahman, Department of Genetic Engineering and Biotechnology, University of Rajshahi, Rajshahi-6205, Bangladesh

Plant Material Extraction: The roots were cut, air-dried powdered in a grinding machine and stored in an airtight container. Powdered dried roots (300 g) of the plant were extracted (cold) with chloroform (1.25 L) 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 [11] to afford a blackish mass.

Organisms Collection: Antibacterial activity and minimum inhibitory concentration (MIC) were determined against four gram-positive bacteria (*Bacillus subtilis, Bacillus megaterium*, *Stapphylococcus aureus* and *Enterobacter aerogenes*) and four gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi* and *Shigella sonnei*). All the organisms were collected from the Microbiology Research Laboratory of the pharmacy Department, Rajshahi University, Bangladesh. Antifungal screening was carried out against six fungi (*Aspergillus fumigatus, Aspergillus niger, Aspergillus flavus, Candida albicans*, *Rizopus oryzae* and *Tricophyton rubrum*). These organisms were collected from the Microbiology Laboratory, Institute of Biological Sciences, Rajshahi University, Bangladesh.

Growth Media and Conditions: Nutrient agar media (Difco laboratories) pH 7.2, nutrient broth media (Difco laboratories) pH 6.8 and Sabouraud dextrose agar media (Biolife Vole Monza) pH 5.6 were used for antibacterial screening, MIC determination and antifungal screening determination, respectively.

Antibacterial Screening: Antibacterial screening is generally performed by disc diffusion method [10,12], which is a qualitative to semi quantitative test. Briefly 20 ml quantities of nutrient agar were plated in petri dish with 0.1 ml of a 10^{-2} dilution of each bacterial culture. Filter paper discs (6 mm in diameter) impregnated with various concentrations of plant extracts were placed on test organism-seeded plates. Chloroform was used to dissolve the extract and was completely evaporated before application on test organism seeded plates. Blank disc impregnated with solvent chloroform followed by during off was used as negative control. The activity was determined after 18 h of incubation at 37°C. The diameters of zone of inhibition produced by the extract were then compared with the standard antibiotic kanamycin 30 µm/disc. Each sample was used in triplicate for the determination of antibacterial activity.

Minimum Inhibitory Concentration (MIC) Measurements: A current definition of the minimum inhibitory concentration (MIC) is the lowest concentration which resulted in maintenance or reduction of inoculums viability [13]. Serial tube dilution technique [9, 10] was used to determine of MIC of the extracts against four gram-positive and four gram-negative bacteria. The plant extract (0.512 mg) was dissolved in 2 ml distilled water (2 drops tween-80 was added to facilitate dissolution) to obtain stock solution. After preparation of suspensions of test organisms (10^7 organism per ml), 1 drop of suspension (0.02 ml) was added to each broth dilution. After 18 h 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). Distilled water with 2 drops of tween-80 and kanamycin were used as negative and positive control, respectively.

Antifungal Screening: The antifungal activity of the extract was tested by disc diffusion method [10,12] against the five pathogenic fungi at the concentrations of 150 µg/disc and 300 µg/disc for each. Here 20 ml quantities of Sabouraud dextrose were plated in petri dish. Blank disc impregnated with solvent chloroform followed by drying off was used as negative control. The activity was determined after 72 h of incubation at room temperature (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.

RESULTS AND DISCUSSION

Antibacterial Activity: The results representing antibacterial activity of chloroform extract of roots of *P. hydropiper* presented in Table 1. The highest activity of plant extract was 25.6 mm diameter of zone inhibition found against *Bacillus subtilis* followed by 25.3 mm diameter of zone inhibition against *Enterbacter aerogenes* at the concentration of 300 µg/disc. On the left hand, the lowest activity of plant extract was 13.0 mm diameter of zone inhibition observed against *Salmonella typhi* at the concentration of 150 µg/disc. In the comparison to reference standard kanamycin 30 µg/disc, the chloroform extract of *Polygonum hydropiper* root showed significant

Table 1: Antibacterial activity of chloroform extract of *Polygonum hydropiper* roots

Test organisms	Diameter of zone of inhibition (mm) Chloroform ext. 150 µg/disc (M±SE)	Chloroform ext. 300 µg/disc (M±SE)	Kanamycin 30 µg/disc (M±SE)
Gram-positive			
Bacillus subtilis	15.6±0.4	25.6±0.3	35.0±0.0
Bacillus megaterium	15.3±0.7	24.3±0.4	34.0±0.5
Stapphylococcus aureus	16.0±0.0	25.0±0.0	34.6±0.2
Enterobacter aerogenes	15.0±0.0	25.3±0.4	34.0±0.0
Gram-negative			
Escherichia coli	14.0±0.0	25.0±0.0	35.0±0.0
Pseudomonas aeruginosa	15.3±0.7	23.0±0.5	34.3±0.8
Salmonella typhi	13.0±0.0	24.0±0.0	32.7±0.6
Shigella sonnei	13.3±0.7	22.0±0.0	33.6±0.9

Note: The control disc used for solvent had no zone of inhibition, so there data was omitted from the above data. Data are represented in the form of mean of three tests ± SE of the standard group

Table 2: Minimum inhibitory concentration (MIC) of chloroform extract of *Polygonum hydropiper* roots

Test organisms	MIC values of chloroform extract (µg/ml)	MIC values of kanamycin (µg/ml)
Gram-positive		
Bacillus subtilis	16	2
Bacillus megaterium	32	2
Stapphylococcus aureus	16	4
Enterbacter aerogenes	16	4
Gram-negative		
Escherichia coli	16	4
Pseudomonas aeruginosa	32	8
Salmonella typhi	32	4
Shigella sonnei	64	8

Table 3: Antifungal activity of chloroform extract of *Polygonum hydropiper* roots

Test organisms	Diameter of zone of inhibition (mm) Chloroform ext. 150 µg/disc (M±SE)	Chloroform ext. 300 µg/disc (M±SE)	Kanamycin 30 µg/disc (M±SE)
Aspergillus fumigatus	14.7±0.6	20.0±0.0	25.0±0.6
Aspergillus niger	13.0±0.3	19.0±0.6	25.0±0.0
Aspergillus flavus	13.0±0.6	17.0±1.2	22.0±0.0
Candida albicans	13.0±0.0	16.3±0.9	20.3±0.9
Rizopus oryzae	12.7±0.9	16.7±1.2	20.7±1.8
Tricophyton rubrum	14.0±1.0	18.0±0.0	24.0±0.0

Note: The control disc used for solvent had no zone of inhibition, so there data was omitted from the above data. Data are represented in the form of mean of three tests ± SE of the standard group

antibacterial activity at 300 µg/disc. In the present experiment, we found that the chloroform 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 [10, 14-16] and our present investigation supported the previous findings.

Minimum Inhibitory Concentration (MIC) Measurement: The Minimum inhibitory concentration (MIC) values of the extract against tested bacteria were shown in Table 2. The MIC values were 16, 32, 16, 16,16, 32, 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. Antibacterial potency of plant extract against these bacteria expressed in MIC indicated the plant extract is more effective against gram-positive at lower concentration than that against gram-negative bacteria.

Antifungal Activity: The antifungal activities of chloroform extract of *Polygonum hydropiper* roots and standard kanamycin (30 µg/disc) were determined at the concentrations of 150 µg/disc and 300 µg/disc against six pathogenic fungi (Table 3). The highest activity was 20.0 mm diameter of zone inhibition observed against *Aspergillus fumigatus* followed by 19.0 mm diameter of zone inhibition against *Aspergillus niger* at the concentration of 300 µg/disc. On the left hand, the lowest activity was 12.7 mm diameter of zone inhibition found against *Rhizopus oryzae* at the concentration of 150 µg/disc. Different plant extracts have been reported for their antifungal properties [7, 10, 14, 17, 18], which supports our present findings. Overall, the chloroform extract of *Polygonum hydropiper* root showed significant activity against al the tested pathogenic fungi.

CONCLUSION

It was concluded that the chloroform extract of *P. hydropiper* roots demonstrated a strong activity against both gram-positive and gram-negative bacteria and fungi. This investigation can be used in the folk medicine and source of antibacterial substances for possible treatment of many diseases including bacterial and fungal infections. However, to know the extract mechanism of action of *P. hydropiper* root extract, further studies with purified fractions/ bioactive compounds are warranted.

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