Research Article

Antimicrobial activity of *Polygonium hydropiper* leaves against plant and human pathogenic fungal and bacterial strains

Muhammad Waqas^{1*}, Anwar Ali Shad¹, Humaira Wasila², Ejaz Ahmad Khan³, Omaira Bashir⁴, Mamoon Ur Rashid³ and Imran Ahmad⁵

1. Department of Agricultural Chemistry, The University of Agriculture, Peshawar-Pakistan

2. Department of Human Nutrition, The University of Agriculture Peshawar-Pakistan

3. Faculty of Agriculture, Gomal University, D.I. Khan-Pakistan

4. Institute of Chemical Sciences, Gomal University, D.I. Khan-Pakistan

5. Department of Horticulture, The University of Agriculture Peshawar-Pakistan

*Corresponding author's email: waqasgandapur404@gmail.com

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Abstract

The present study was conducted to investigate the antimicrobial activities of *Polygonium hydropiper* L. leaves extract against bacteria and fungi using the disc diffusion method. The extract showed significant antibacterial activities against four strains of gram positive bacteria namely *Clavibacter*, *Staphylococcus aureus*, *Lactococcus lactis* and *Bacillus clausii* species and four strains of gram negative bacteria namely *Escherichia coli*, *Citrobacter*, *Proteus* and *Xanthomonas* species. The minimum inhibitory concentration (MIC) values against the bacterial culture were ranged from 16 to 64 $\mu\text{g mL}^{-1}$. The antifungal activities were found against six toxic fungal strains namely *Penicillium*, *Fusarium*, *Trichoderma*, *Aspergillus niger*, *Pythium*, and *Acremonium*. It was concluded that *Polygonium hydropiper* methanolic leaves extract acted as an antibacterial and antifungal agent and can be used in wide range of pharmaceuticals industries. Such as Quinine was the first natural product to be produced on industrial scale for its use against malaria.

Keywords: *Polygonium Hydropiper*. L; Methanol Extract; Leaves; Antimicrobial; Minimum Inhibitory Concentration

Introduction

Polygonium Hydropiper .L. belongs to the family “Polygonaceae” having high medicinal value. Whole *Polygonium* plant contain large quantity of secondary metabolites and bioactive compound such as 4-Isobutyl-6-methyl-5-oxo-3a,4,5,7a-

tetrahydro-1H-inden-13-oic acid (named viscosumic acid) and quercetin 3-O-(6"-feruloyl)-beta-D-galactopyranoside, and the known 3',5-dihydroxy-3,4',5',7-tetramethoxyflavone have been isolated from *Polygonum viscosum* and polygonic acid [1]. The plant also possesses some

natural compound having insecticidal characteristic. [2]. Whole plant itself or in combination with some other medicinal herbs are used as remedies against various infectious diseases such as diarrhea, itching skin, dyspepsia, excessive menstrual bleeding and hemorrhoid [3]. This species has high pharmacological characteristic and having economical value. Therefore utilization of these herbal plants is increasing day by day among the people. The rate of the pathogenic infectious diseases is increasing day by day worldwide because of exposure to the adverse atmosphere in developing countries and therefore mortality rate is also high in these countries. Thus it is need of the day to make remedies against these pathogenic diseases [4]. In order to get rid from this infectious disease many discoveries have been made by using plants against microbial activities on local level as an alternate of synthetic medicines [5]. The present study was conducted with the aims to investigate the antimicrobial activities of methanol extract of *Polygonium hydropiper* leaves.

Material and Methods

Plant material collection

The leaves of the *Polygonium hydropiper* (L) were collected from Tehsil Muree, District Rawalpindi, Punjab, Pakistan. The leaves of the plant were identified at Department of Agronomy Faculty of Agriculture Gomal University Dera Ismail Khan.

Plant material extraction

The leaves were washed with tap water and then were cut with manual cutter and dried at 50 °C [6]. After drying they were ground with help of grinding machine and powdered sample was stored in an air tight container. The powder of the leaves sample (250 g) was extracted through cold extraction method by using methanol (1 L) in flat bottom flask. When the mixture was completely dissolved, it was filtrated using

Watsmann filter paper and the filtrate was separated from solvent using rotary evaporator to get the crude extract [7].

Organism collection

Antimicrobial activities and minimum inhibitory concentration were investigated against different strain of bacterial and fungal cultural. The antibacterial and minimum inhibitory concentration was determined against four gram-positive bacteria *Clavibacter*, *Staphylococcus aureus*, *Lactococcus lactis* and *Bacillus Clausii* and four gram negative bacteria *Escherichia coli*, *Citrobacter*, *Proteus* and *Xanthomonas*. The antifungal screening was conducted against four strain of the fungus *Penicillium*, *Fusarium oxysporum*, *Trichoderma*, *Aspergillus Niger*, *Pythium*, and *Acremonium*. The entire organisms were collected from the Toxicology Laboratory, Department of Agricultural Chemistry, Faculty of Nutrition Sciences, The University of Agriculture Peshawar, Pakistan.

Media for the growth of fungal and bacterial cultural

Nutrient Broth media (Difco laboratories) having pH 6.8, nutrient agar media having pH 7.2 and Potato dextrose agar (PDA) media having pH 5.6 were used for antibacterial minimum inhibitory concentration and antifungal screening.

Antibacterial screening

Antibacterial activity of the plant extract was tested by agar disc diffusion method [8]. Filter disc (6mm) were placed on the petri dishes pre-inoculated with bacterial pathogen. The plant methanol extract were poured onto the filter disc. Dimethyl sulphoxide (DMSO) was used (10µg/µL) as negative control against the tested bacterial strain while the Vibromycin (30µg/disc) was used as positive control. The experiment was repeated three times. All the Petri plates were held for 20 min at room temperature for the diffusion of the sample extract and

then kept into an incubator for 24 hours at 37 °C for bacterial growth. The zone of inhibition was calculated after 24 hrs [9].

Antifungal screening

The antifungal screening was performed using disc diffusion method. [10]. The antifungal activity was carried out against four pathogenic strains of the fungi at the concentration of 150 µg/disc and 300 µg/disc. Dimethyl sulphoxide (DMSO) 10 µg/mL was used as negative control while acrobate 20 µg/disc was used as positive control against four strains of the fungus. All the petri dishes were held for 20 min at room temperature till to complete diffusion of the extract into the disc and then incubated at 27 °C for 72 hours for fungal growth. The antifungal effect was determined by zone of inhibition and was measured with the help of ruler.

Minimum inhibitory concentration

The minimum inhibitory concentration of the plant extract was determined by the method described [11]. In order to calculate the MIC the extract of the sample was diluted with nutrient broth of the concentration 50, 25, 12.5, 6.75, 3.12 and 1.56 mg/mL prepared from stock solution (500 mg/mL) in test tubes and the control tubes were also prepared. The tubes were incubated at 37 °C for 24 hours. The minimum concentration of the leaves extract

where no bacterial growth was seen was considered as MIC.

Results and discussion

Antibacterial screening

The antibacterial screening of methanol extract were presented in Table 1. The maximum MIC (24.2 mm) of plant methanol extract was recorded against *Calvibacter* while lowest MIC (21.1 mm) was observed against *Proteus* at 300 µg/disc. The concentration 150 µg/disc showed maximum activity (16.3 mm) against *Bacillus clausii* while minimum zone of inhibition (12.3 mm) was found against *Citrobacter*. When compared with standard (Vibromycin) at concentration of 30 µg/disc. It was noticed that the standard (Vibromycin) showed maximum zone of inhibition (4 µg/mL) against gram positive bacteria *Bacillus clausii* and *Lactococcus* followed by *Styphylococcus aureus* and *Clavibacter* (2 µg/mL). Similarly it was also found that Standard (Vibromycin) showed maximum inhibitory zone against gram negative bacteria *Citrobacter* and *Proteus* (8 µg/mL) followed by *Xanthomonas sp* and *Escherichia Coli* at 4 µg/mL. In the present research it was also observed that methanol extract showed good result against positive and negative bacterial strains and confirmed the investigation of the previous findings. The figure one shows the zone of inhibition of antibacterial activities. (Figure 1) [12].

Table 1. Results of antibacterial activity of *Polygonium hydropiper* (L.) leaves Diameter of zone of inhibition (nm)

Type	Species	MeOH Extract Conc.	MeOH Extract Conc.	Vibromycin (STD)
		300 µg/disc (M±SE)	150 µg/disc (M±SE)	30 µg/disc (M±SE)
Gram Positive	<i>Clavibacter</i>	24.2 ±0.6	14.6 ±0.4	34.0 ±0.0
	<i>Staphylococcus aureus</i>	23.1 ±0.7	14.5 ±0.3	33.0 ±0.4
	<i>Lactococcus lactis</i>	22.2 ±0.3	14.4 ±0.2	33.0 ±0.3
	<i>Bacillus clausii</i>	16.3 ±0.2	16.3 ±0.1	33.0 ±0.0
Gram –negative	<i>Xanthomonas sp.</i>	24.0 ±0.0	13.2 ±0.7	34.1 ±0.2
	<i>Citrobacter</i>	23.1 ±0.1	12.3 ±0.1	33.2 ±0.1
	<i>Escherichia coli</i>	23.0 ±0.3	13.1 ±0.1	33.4 ±0.0
	<i>Proteus</i>	21.1 ±0.4	14.2 ±0.0	36.0 ±0.1

The Dimethylsulphoxide was used negative control having no zone of inhibition. All the data are as mean of three test ± SE of the standard group

Antifungal screening

The antifungal screening of the methanolic extract of *Polygonum hydropiper* L leaves were investigated and results are presented in Table 2. Two different concentrations 150µg/disc and 300µg/disc were tested against six fungal strains i.e. *Penicillium*, *Fusarium oxysporum*, *Trichoderma*, *Aspergillus niger*, *Pythium*, and *Acremonium*. The highest activity (18.8 mm) was observed against *Fusarium oxysporum* followed by *Pythium* (18.3 mm) and *Penicillium* while the minimum zone of inhibition (15.2 ± 0.4) was showed by *Acremonium* at 300ug/disc. The antifungal activity was decreased by decreasing the amount of concentration for all the six

fungal species. It was observed that the extract showed maximum activity (13.3 ± 0.0) against *Aspergillus niger* while it showed minimum activity (11.0 ± 0.3) against *Pythium* at 150µg/disc. The standard Vibromycin (20µg/disc) significantly inhibited the growth of all the six different fungi. Previously literature showed that activities of the various extract have been carried out against various strains of pathogenic fungus [13]. Overall result showed that leaves extract of *Polygonum hydropiper* has significant effect against tested fungal strains. The figure one shows the zone of inhibition of antifungal activities.(Fig.2).

Table 2. Results of antifungal activity of *Polygonum hydropiper* leaves Diameter of zone of inhibition (nm)

Tested fungal sp	MeOH Extract Conc	MeOH Extract Conc.	Acrobate (STD)
	300µg/disc (M±SE)	150µg/disc (M±SE)	20µg/disc (M±SE)
<i>Penicillium</i>	18.0±0.0	12.1±0.3	24.0±01
<i>Fusarium oxysporum</i>	18.8±0.0	12.0±0.5	24.3±04
<i>Trichoderma</i>	18.3±0.2	11.6±0.6	23.4±03
<i>Aspergillus niger</i>	17.0±0.3	13.3±0.0	24.1±05
<i>Pythium</i>	18.5±0.7	11.0±0.3	22.4±07
<i>Acremonium</i>	15.2±0.4	12.7±0.3	23.1±0.0

The Dimethylsulphoxide was used Positive control having no zone of inhibition. All the data are represented as mean of three test± SE of the standard group

Minimum Inhibitory Concentration Calculation

Data in Table 3 represent the Minimum Inhibitory Concentration (MIC) value of the sample plant extract against selected strains of bacteria which were 16, 32, 16, 16, 32, 32 and 64µg/ml against bacterial culture. The minimum inhibitory concentration against four gram positive tested bacterial strains were ranged from 16µg/ml to 32µg/ml and

then (MIC) value against the four strains of gram negative bacteria were ranged from 16µg/ml to 64µg/ml. The (MIC) value of the extract showed that methanol extract of leaves of *Polygonum hydropiper* L is more effective against positive bacterial cultural as compared to the gram negative bacteria at minimum concentration. The figure two shows MIC value of MeOH extract against bacterial isolates. (Figure 2). [14].

Table 3. Minimum Inhibitory Concentration (MIC) of methanolic extract of *Polygonum hydropiper*

Type	Species	(MIC) value of(MeOH) µg/mL	(MIC)value of Vibromycin µg/mL
Gram Positive	<i>Clavibacter</i>	16	2
	<i>Staphylococcus aureus</i>	32	2
	<i>Lactococcus</i>	16	4
	<i>Bacillus Clausii</i>	16	4
Gram Negative	<i>Xanthomonas</i>	16	4
	<i>Citrobacter</i>	32	8
	<i>Escherichia coli</i>	32	4
	<i>Proteus</i>	64	8

Figure 1. Antimicrobial activities of MeOH extract at two different concentrations

Figure 2. Minimum inhibitory concentration of MeOH extract against bacterial isolates

Conclusion and recommendation

It is concluded from the present research that methanol extract of leaves of *Polygonum hydropiper* has showed significant antibacterial potential against both gram positive and gram negative bacteria. Moreover the results also showed that extract has strong antifungal activity. Further work is needed to isolate and purify compounds using HPLC. These bioactive compounds can be used against bacterial and fungal strains in most proper way in Pharmaceutical industry.

Authors' contributions

Conceived and designed the experiments: AA Shad & M Waqas, Performed the experiments: M Waqas, Analyzed the data: MU Rashid, Provided reagents: O Bashir, Wrote the paper: M Waqas.

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