Antimicrobial Potential and Physio-Chemical Analysis of *Polygonum barbatum* L

[1] Sara Khan, [1] Umar Farooq*, [1] Afsar Khan**, [1] Sadia Naz, [1] Rizwana Sarwar, [2] Nosheen Mirza
[1]*Department of Chemistry, COMSATS Institute of Information Technology, Abbottabad-22060, Pakistan.*
[2]*Department of Environmental Sciences, COMSATS Institute of Information Technology, Abbottabad-22060, Pakistan*
umarf@ciit.net.pk*,afsarhej@yahoo.com**

(Received on 18th March 2013, accepted in revised form 4th November 2013)

Summary: The present study deals with the investigation of antimicrobial activity by AWD method and DD method against Gram-positive (*B subtilis, M luteus, S aureus*) and Gram-negative (*S setubal, P picketti*) bacteria, and two fungal strains (*Aspergillus fumigatus, Aspergillus flavus*). It was observed that *Polygonum barbatum* showed significant antimicrobial activity. Among all fractions the *n*-hexane fraction showed significant activity against *S setubal* (ZOI 18mm). The *n*-BuOH fraction of *Polygonum barbatum* was active against both fungal strains (ZOI 27mm).

Keywords: *Polygonum barbatum*, Antimicrobial activity, Physio-chemical analysis.

Introduction

The genus *Polygonum* comprises of weedy species, found in temperate region of the world [1]. The genus *Polygonum* holds tremendous importance due to certain magical healing powers embedded in them responsible for treatment of various illnesses [2]. Phytochemical studies on this genus showed the presence of derivatives of cinnamic acid and flavonoids [3]. Similarly, sitosterone and sesquiterpene viscozulenic acid with analgesic, anti-inflammatory, CNS depressant and anti HIV-1 activities have been reported [4]. Acetophenone has been isolated from *Polygonum barbatum* [5], quercetin and its derivatives isolated from *Polygonum equisetiforme*, have been analyzed for possible antimicrobial activity [6]. Flavonoids and flavonoid glucosides were isolated from *Polygonum hydropiper* with antioxidative activity [7]. Anthraquinone emodin has been isolated from *Polygonum hypoleucum* with regulation of cell proliferation [8]. Antioxidant [9], antitumor, and free radical scavenging activities have also been reported in few species of the genus *Polygonum* [10, 11].

Polygonum barbatum is perennial herb adequately found in marshy areas, river banks, ponds, small ditches etc. in South and South East Asia [12]. It is used as vegetable [13]. Plant has been employed in treatment of oral cancer in indigenous system [14]. *Polygonum barbatum* has been used in folk medicine as astringent, in colic pain, scabies, pain, fever, inflammation, as diuretic agent, and as antivenom [15-17]. Previously, *Polygonum barbatum* has shown brine shrimp toxicity, spasmolytic and cholinergic activities [18].

Polygonum barbatum (whole plant) was selected for the evaluation of possible antimicrobial activity as previous data on antimicrobial activity of the leaf extract of this plant [19] presented to be a suitable candidate for analysis of antimicrobial activity against different bacteria and fungi and physiochemical test.

Results and Discussion

Antibacterial Activity

Antibacterial activity of fractions was analyzed against Gram-positive and Gram-negative bacteria. The *n*-BuOH fraction showed enhanced activity against *S. aureus* (Fig. 1), but it was moderately active against all other bacterial strains. Similarly, *n*-hexane fraction (Fig. 2) showed good activity against *B. subtilus* and *S. setubal* compared to the ciprofloxacin used as standard. Likewise, EtOAc fraction (Fig. 3) illustrated activity against *P. picketti*. Chloroform fraction (Fig. 4) renders plant's activity against all bacterial strains but it was more active against *S. setubal* and *P. picketti.*

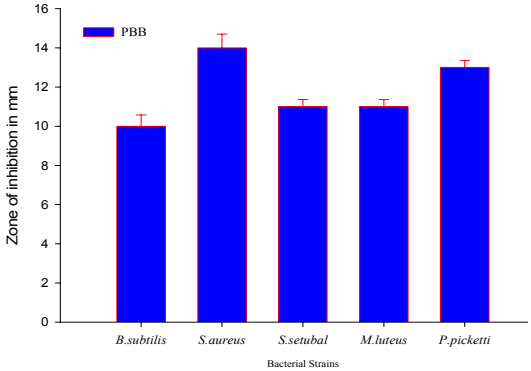

Fig. 1: Activity of PBB (*Polygonum barbatum* butanol) and standard against bacterial strains.

*To whom all correspondence should be addressed.

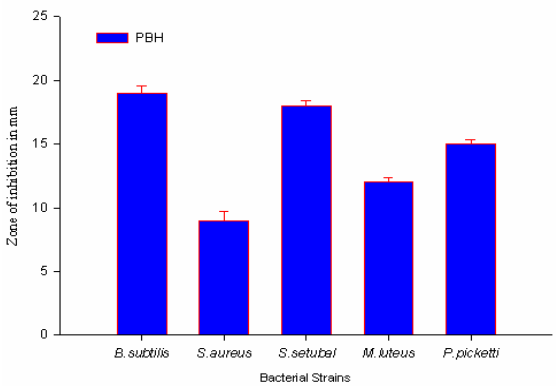

Fig. 2: Activity of PBH (*Polygonum barbatum n*-hexane) against bacterial strains.

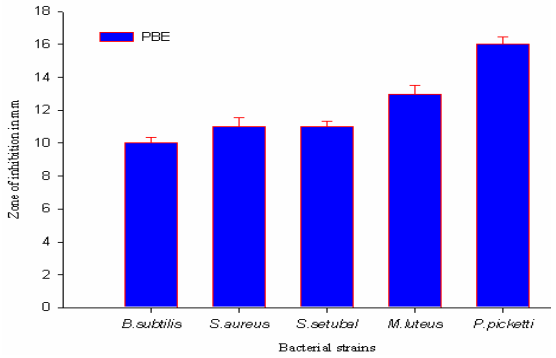

Fig. 3: Activity of PBE (*Polygonum barbatum* EtOAc) against bacterial strains.

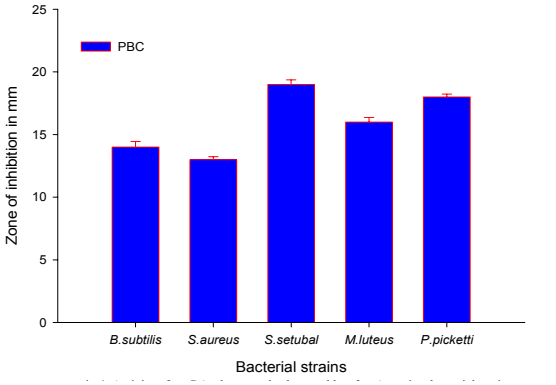

Fig. 4: Activity of PBC (*Polydonum barbatum* chloroform) against bacterial strain.

Activities of all fractions against bacterial strains compared to standard (Fig. 5) depicted that chloroform and *n*-hexane fraction were active against *S. setubal* and *n*-hexane fraction was good enough in inhibiting the growth of *B. subtilus* whereas other fractions reacted mildly toward this strain in particular. The *n*-BuOH fraction was active against *S. aureus* but was least active toward all other strains. EtOAc fraction was only fairly active against *P. picketti*. Results suggest that each fraction contains active compound responsible for antimicrobial activity to different concentration, concluding that the response of less polar fraction toward bacterial strains was better as compared to highly polar fraction like EtOAc, and *n*-BuOH. So from this data it can be assumed that antibacterial bioactive components are more abundant in non-polar fractions of plant as compared to polar fractions.

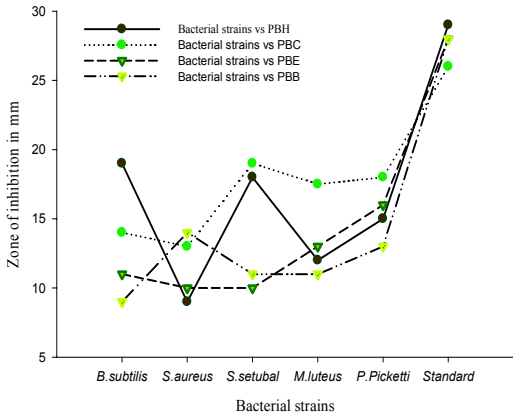

Fig. 5: Comparison of all data for antibacterial activity with slandered.

Antifungal Activity

Similarly comparing antifungal activity of plant fraction against fungal strains (Fig. 6) showed good activity of *n*-BuOH fraction against *Aspergillus flavus* and *Aspergillus fumigatus* whereas other fraction were little or ineffective compared to standard which was sufficiently active against both fungal strains.

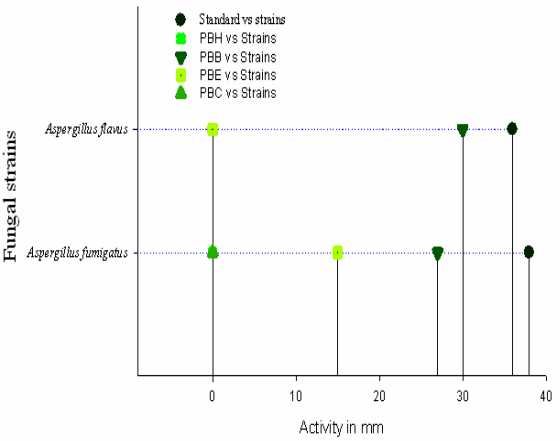

Fig. 6: Antifungal activity of fractions and standard against fungal strains.

Experimental

Materials and Methods

The whole plant of *Polygonum barbatum* was collected from District Abbottabad in May 2012. It was shade dried and soaked in methanol, then solvent removed on rotary evaporator. Crude extract obtained was partitioned in *n*-hexane, chloroform, EtOAc, and *n*-BuOH fractions. All the fractions were examined for antimicrobial screening using various types of fungi and bacteria employing AWD (agar well diffusion) method and DD (disc diffusion) method.

Tested Microorganisms

The plant fractions were examined for possible activity against (Gram+ive) *B.subtilis*, *M luteus*, *S aureus* and (Gram-ive) *P picketti*, *S setubal*, and two fungal strains *i.e*., *Aspergillus fumigatus* and *Aspergillus flavus*.

Media

The medium used in the assay for the antibacterial test was Mueller Hinton agar (MHA, Merck, Germany) and for the antifungal test was Sabouraud Dextrose agar (SDA, Merck, Germany). Dimethylsulfoxide (DMSO; Sigma-Aldrich) was used to dilute all the tested samples.

Screening for Antibacterial Activity

Fractions of *Polygonum barbatum* were screened separately, by AWD and DD method [20-22]. Sterilization of the medium at 120°C was done using autoclave. After that 30 mL of agar medium was added to sterilized petri plates aseptically, in laminar flow hood to avoid contamination till plates were settled. Then, bacterial strains were spread on agar plates with sterile cotton swab. An 8mm well was made with the help of a sterile cork borer. 3mg of each fraction was dissolved in 1mL 10 % DMSO. The 20µL of standard drug and extracts of the plant were poured in 8mm diameter well and the extracts were dissolved in DMSO. The incubation of assay plates were carried out at 37°C ± 2°C for 24h in incubator. Ciprofloxacin (50µg per disc) in standard disc taken as positive control for antibacterial activity and DMSO was used as negative control. Zone of inhibition (ZOI), measured in mm, displayed in Fig. 1-4.

Calculation of Minimum Inhibitory Concentration (MIC)

The MIC values were taken as the lowest concentration of fractions, which checked the growth of the tested organisms after 24 h of incubation at 37 °C. Serial dilutions of each fractions (3mg/mL) in DMSO were prepared to get 0.75, 0.375, 0.187 µg/mL concentration. An aliquot of 100 µL from each diluted fraction was added to wells in sterilized plates pre-inoculated with test bacterial strains. Experiment was repeated thrice and results obtained are in Table-1.

Table-1: MIC values of *Polygonum barbatum* fractions at different concentrations.

Extract	Pathogenic bacteria				
	B.subtilis	*S.aureus*	*M.leu*	*S.setubal*	*P.picketti*
PBE	0.375	1.50	0.375	1.50	0.375
PBB	0.75	0.187	1.50	0.375	0.75
PBH	0.187	0.75	0.187	0.187	0.375
PBC	0.75	0.187	0.187	0.75	0.375

B.subtilis= Bacillus subtilis, *S. aureus= Staphylococcus aureus*, *M.leu=Micrococcus luteus*, *S.set=Salmonella Setubal*, *P.picketti= Pseudomonas picketti*.

Screening of Antifungal Activity

Antifungal activity was carried out by DD method [23]. SDA petri plates were pre-inoculated with fungal strains by point inoculation. Then 6mm diameter filter paper disc impregnated with 1 mg/mL of the fractions were placed on each petri plates. DMSO was used as solvent. Blank disc impregnated with DMSO was used as negative control and Tetracyclin was taken as positive control.

After 72 h of incubation at 28 °C the activity was determined by measuring ZOI in mm. Antifungal activity of fractions was carried out by AWD method to calculate MIC value (Table-2). Serial dilutions of fractions (1mg/mL) ranging from 1.50-0.187 µL were prepared in DMSO. An aliquot of 100 µL of each dilution of the fractions was placed in agar plates pre inoculated with test fungal strains. Final results were taken after incubation for 72 h at 28 °C Fig. 6.

Table-2: MIC values for each fraction against fungal strains.

Extract	Pathogenic Fungi	
	Aspergillus flavus	*Aspergillus fumigatus*
PBE	1.5	-
PBB	1.5	1.5
PBH	-	-
PBC	-	

Physio-chemical Analysis

Qualitative analysis of plant fractions to identify the constituents was carried out, using standard procedures, Table-3 [24-26].

Table-3: Qualitative analysis of crude extract of *Polygonum barbatum*

Phytochemicals-Test performed	*Polygonum barbatum*
Anthraquinones	+
Alkaloids (Dragendroff reagent)	+
Flavonoids	+
Phenolic compounds	+
Saponins	+
Tannins	+
Triterpenoids	+

Conclusion

The present study shows that *Polygonum barbatum* has good antimicrobial activity and it can be employed as potent source of antimicrobial agents. However detail analysis is required to isolate and characterize compounds that are responsible for antimicrobial activity.

References

1. R. H. Mohlenbrock, *Nelumbonaceae to Vitaceae*, *Water Lotuses to Grapes*, Southern Illinois University, USA, p. 102 (2010).
2. M. A. Mazid, B. K. Datta, L. Nahar, S. A. M. Khairul Bashar, S. C. Bachar and S. D. Sarker, *Brazilian Journal of Pharmacognosy*, **19**, 749 (2009).
3. B. K. Datta, S. K. Datta, M. A. Rashid and S. D. Sarker, *Biochemical Systematics and Ecology*, **30**, 693 (2002).
4. B. K. Datta, S. K. Datta, M. M. Chaudhry, T. H. Khan, J. K. Kundu, M. A. Rashid, L. Nahar and S. D. Sarker, *Pharmazie*, **59**, 222 (2004).
5. M. A. Mazid, B. K. Datta, L. Nahar, S. A. M. KhairulBashar, S. C. Bachar and S. D. Sarker, *Records of Natural Products*, **5**, 143 (2011).
6. S. A. Ghazal, M. Abuzarqa and A. M. Mahasneh, *Phytotherapy Research*, **6**, 265 (1992).
7. H. Haraguchi, K. Hashimoto and A. Yagi, *Journal of Agricultural and Food Chemistry,* **40**, 1349 (1992).
8. Y. C. Kuo, H. C. Meng and W. J. Tsai, *Inflammation Research*, **50**, 73 (2001).
9. S. P. Ip, A. S. M. Tse, M. K. T. Poon, K. M. Ko and C. Y. Ma, *Phytotherapy Research*, **11**, 42 (1997).
10. Y. C. Kuo, C. M. Sun, J. C. Ou and W. J. Tsai, *Life Sciences*, **61**, 2335 (1997).
11. M. A. Mazid, L. Nahar, B. K. Datta, S. A. M. KhairulBashar and S. D. Sarker, *Archives of Biological Sciences*, **63**, 465 (2011).
12. *GRIN Taxonomy Database USDA*, ARS, National Genetic Resources Program, Germplasm Resources Information Network - (GRIN), National Germplasm Resources Laboratory, Beltsville, Maryland (2010).
13. G. Kunkel, *Plants for Human Consumption*, Koeltz Scientific Books (1984).
14. M. B. Siddique, *Ancient Science of Life*, **22**, 95 (2003).
15. R. N. Chopra, S. L. Nayar and I. C. Chopra, *Glossary of Indian Medicinal Plants*, Council of Scientific and Industrial Research, New Delhi (1986).
16. N. P. Manandhar, *Plants and People of Nepal*, Timber Press (2002).
17. A. Jain, S. S. Khatewa, S. K. Sharma, P. Galav and V. Jains, *Indian Journal of Traditional Knowledge*, **10**, 258 (2011).
18. B. A. Chaudhry, M. Y. Syad, K. H. Janbaz, A. A. Dasti and B. A. Loothar, *Journal of Research* (*Science*), **14**, 169 (2003).
19. X. Q. R. Sheela and V. Alex. Ramani, *International Journal of Current Pharmaceutical Research*, **3**, (2011).
20. C. Perez, M. Paul and P. Bazerque, *Acta Biologica Medica Exp*, **15**, 113 (1990).
21. P. R. Murry, E. J. Baron, M. A. Pfaller, F. C. Tenover and H. R. Yolken, *Manual of clinical Microbiology*, 6th edition. ASM Press, Washington, DC, p. 15 (1995).
22. P. F. Olurinola, *A Laboratory Manual of Pharmaceutical Microbiology*, Idu, Abuja, Nigeria, p. 69 (1996).
23. R. S. L. Taylor, N. P. Manandhar, J. B. Hudson and G. H. N. Towers, *Journal of Ethnopharmacology*, **546**, 153 (1995).
24. H. O. Edeoga, D. E. Okwu and B. O. Mbaebie, *African Journal of Biotechnology*, **4**, 685 (2005).
25. R. D. Amarasingham, N. G. Bisset, A. H. Millard and M. C. Woods, *Economic Botany*, **18**, 270 (1964).
26. Santaram and J. B. Harborne, *Phytochemical Method*, A Chaman and Hall, London, (1984).