Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.apjtb.com

Document heading doi:10.12980/APJTB.4.201414B182 © 2014 by the Asian Pacific Journal of Tropical Biomedicine. All rights reserved.

Evaluation of antifungal and antioxidant potential of two medicinal plants: *Aconitum heterophyllum* and *Polygonum bistorta*

Neelma Munir[1*], Wasqa Ijaz[1], Imran Altaf[2], Shagufta Naz[1]

[1]*Department of Biotechnology, Lahore College for Women University, Lahore, Pakistan*

[2]*QOL, WTO, Laboratories, University of Veterinary and Animal Sciences, Lahore, Pakistan*

ARTICLE INFO

Article history:
Received 5 May 2014
Received in revised form 10 Jun 2014
Accepted 27 Jun 2014
Available online 2 Jul 2014

Keywords:
Medicinal plants
Antifungal
Antioxidant
Biomedicines

ABSTRACT

Objective: To focus on the evaluation of antimicrobial and antioxidant activity of two endangered medicinal plants *Aconitum heterophyllum* (*A. heterophyllum*) and *Polygonum bistorta* (*P. bistorta*).
Materials: Plant extracts were obtained by using microwave assisted extraction method. The *in vitro* antifungal activity of *A. heterophyllum* and *P. bistorta* extracts were determined by measuring diameters of inhibitory zones of these extracts against *Aspergillus niger* and *Alternalia solani*.
Results: Methanolic extract of *A. heterophyllum* showed significant ($P\leqslant0.05$) antifungal activity against both the tested organisms. It was also observed that ethanolic extracts of *P. bistorta* also had good antifungal activity against the tested fungal strains as compared to the methanolic extracts. It showed significant antifungal activity ($P\leqslant0.05$) against both the tested strains. Antioxidant activity of methanolic and ethanolic extracts of *A. heterophyllum* and *P. bistorta* were also measured using a radical scavenging method. Ascorbic acid was used as a standard.
Conclusions: It was observed that *A. heterophyllum* and *P. bistorta* have significant antioxidant activity. Higher antioxidant activity was recorded in methanolic extract of *A. heterophyllum* as compared to its ethanolic extract. However, in case of *P. bistorta* ethanolic extract of the plant exhibited higher antioxidant potential than methanolic extracts. Hence both of these plants have significant antimicrobial as well as antioxidant potential.

1. Introduction

As long as human beings have been living on earth they use remedies to improve their health and cure illnesses. In the 16th century until the advent of iatrochemistry, plants had been the main and important source of prophylaxis and treatment[1]. Due to the lower efficiency of the synthetic drugs and the increasing contravention of their utilization, natural drugs are using again for treatment. Bioactive compounds produced by plants have toxicological or pharmacological effects on animals and man. A variety of biochemical products are synthesized and preserved by green plants, from which some are extractable and used as raw material for various scientific investigations or as chemical feed stocks[2].

For the production of Japanese and Chinese traditional medicine, *Aconitum heterophyllum* (*A. heterophyllum*) is considered as a necessary component. This plant has many pharmaceutical characteristics like cardio tonic effect, analgesic, anesthetics, anti–inflammatory effect and blood pressure elevation[3]. In Chinese herbal medicine, many diseases are treated with plant tubers and roots, like syncope, painful joints, collapse, rheumatic fever, edema gastroenteritis, edema, tumors, bronchial asthma and some endocrinal disorders such as irregular menstruation. However, the quantity in which this plant should be used is very important because there are also some side effects for its constituents. The drug is dangerous for heart and brain if used in an improper way.

Polygonum bistorta (*Bistorta major*) (*P. bistorta*) is a perennial herb and it belongs to family Polygonaceae. It is

*Corresponding author: Neelma Munir, Department of Biotechnology, Lahore College for Women University, Lahore, Pakistan.
Tel: +92–4299203801–9
E–mail: neelma.munir@yahoo.com

called Anjbaar (Urdu) locally, Bistort in English. It is found in Europe, Iraq, Pakistan, India, Siberia and Iran[4]. It is wide spread in northwest and northeast regions, and also in Jiangsu, Shandong and Hubei Provinces of China. The roots of the plant were used for treatments of carbuncle, dysentery, hemorrhoids, acute gastroenteritis, snake bite, severe respiratory infection with scrofula, cough, aphthous ulcer, hemorrhoidal bleeding, carbuncles and epistaxis[5].

A. heterophyllum also shows a wide range of antifungal, antibiotic and anticancer activity. The stem of plant is an astringent and utilized in treatment of fever, tumors, headache, skin diseases, diarrhea and diseases of the blood and dysentery[6].

Among all the herbs, *P. bistorta* is one of the most strongly acerbic and used to contract tissues and staunch blood flow[7]. The rhizome of *P. bistorta* is strongly demulcent, astringent, diuretic, febrifuge, laxative and powerfully styptic[8]. It is used internally as well as externally. Internal and external bleeding, dysentery, cholera, diarrhoea, *etc.* are treated by it[9]. The roots, bark, stems, leaves, flowers and fruits of *P. bistorta* contain antimicrobial compounds. The initial pharmacological studies of the plant disclosed that aqueous extract of the roots of *P. bistorta* also exhibits antimicrobial, anti-inflammatory and antiulcer activity. Anti-cancerous activity is also present in fresh rhizomes of the plant[10].

A. heterophyllum possesses free radical scavenging activity[11]. It is also used in the treatment of many bacterial, fungal and viral infections and many diseases associated to rheumatism, nervous system, fever and digestive system. It has also been reported that *P. bistorta* shows the free radical donating (antioxidant) activity. The flavonoids and tannins are the phenolic compounds and the plant phenolics are the main and important group of the compounds that perform action as the free radical scavengers and primary antioxidant[12].

Keeping in view of the significance of these medicinal plants, the present study focuses on the collection of these plants of significant medicinal potential and evaluation of their antimicrobial and antioxidant potential.

2. Materials and methods

2.1. Microwave assisted extraction

The roots from the collected plants were selected and dried in shady places in Lahore College for Women University. The plant was incubated in oven for 1 d at 37 °C for complete drying. The roots were ground in grinder to make powder. Plant contents were extracted by using microwave assisted extraction method as described by Xia *et al*[13].

2.2. Estimation of antifungal activity

Plant extracts obtained by microwave extraction method were tested for their antifungal activity against various microorganisms by agar well diffusion method[14]. Methanol and ethanol were used as negative control. Diameter of inhibitory zone was calculated in centimeters with the help of scale. Each test was performed in triplicates.

2.3. Estimation of antioxidant activity

Antioxidant potential of the extract of plant and the standard was evaluated by antioxidant activity of stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity with modified method of Braca *et al*[15]. Diluted solutions of the sample extracts were made in methanol up to 1 mL. From this solution different concentrations were used. For the above prepared solution of *Aconitum* ethanolic extracts 250.00 μL, 125.00 μL, 62.50 μL and 31.25 μL were taken, and for *Aconitum* methanolic extracts 333.20 μL, 166.60 μL, 83.30 μL and 41.60 μL were used to check antioxidant activity. From the diluted *Bistorta* ethanolic extracts 200.00 μL, 100.00 μL, 50.00 μL and 25.00 μL and from *Bistorta* methanolic extracts 90.00 μL, 45.00 μL, 22.72 μL and 11.36 μL were taken. Ascorbic acid in concentrations of 1–100 μg/mL was used as a standard. In methanol prepared the solution of 0.001% DPPH and from this solution 1 mL was mixed separately with the 1 mL of the sample solution and also with standard solution. All these mixtures of the solutions were stored in the dark place for 30 min and optical density (OD) was calculated at the wave length of 517 nm by using the spectrophotometer. For blank solution 1 mL of methanol with 1 mL of 0.001% DPPH solution was used.

The OD was calculated and the % age inhibition was also calculated from the following formula:

Age inhibition of DPPH activity (%)=[(A–B)/A]×100

Where A=OD of blank, B=OD of sample.

The IC_{50} value (inhibition concentration with 50% radical scavenging activity) was calculated by linear regression analysis and expressed in μg/mL. All test samples were conducted in triplicate (n=3).

2.4. Statistical analysis

Independent t-test was applied on the results of diameter of inhibitory zones of plant extracts against *Staphylococcus aureus (S. aureus)*, *Escherichia coli* (*E. coli*) and *Salmonella typhi* and antioxidant activity of methanolic and ethanolic

plant extracts. Results were expressed as means±SD. Significance was measured at the level of $P<0.05$.

3. Results

The present study focused on the detection of bioactive compounds of two endangered medicinal plants *A. heterophyllum* and *P. bistorta*. Furthermore, the extracts of both of these plants were investigated for their anti-microbial as well as anti-oxidant potential.

3.1. Antimicrobial activity

Comparison of all the tested species of fungi indicated that the methanolic extract of it is evident from Figures 1 and 2 that extracts of *A. heterophyllum* showed significant ($P\leq0.05$) antimicrobial activity against both *Aspergillus niger* (*A. niger*) as well as *Alternaria solani* (*A. solani*). While the methanolic extract of *A. heterophyllum* has greater antifungal activity against *A. solani* as compared to *A. niger*. The maximum zone of inhibition for this plant was recorded as 2.3 cm when methanol was used as solven however at the same concentration of extract the value was 2.0 cm.

It is depicted in Figures 3 and 4 that though the methanolic extracts of *P. bistorta* had greater antifungal activity than ethanilic extracts, this plant has less antifungal activity as compared to *A. hetrophyllum*. The highest value of zone of inhibition agains *A. solani* was 1.4 cm (Figure 4).

3.2. Antioxidant activity

The antioxidant potential of the tested plants is depicted in Table 1. IC_{50} value described how stronger plant extract has antioxidant activity. Lower the IC_{50} value greater is its antioxidant activity. IC_{50} value for ascorbic acid was 40.81 μg/

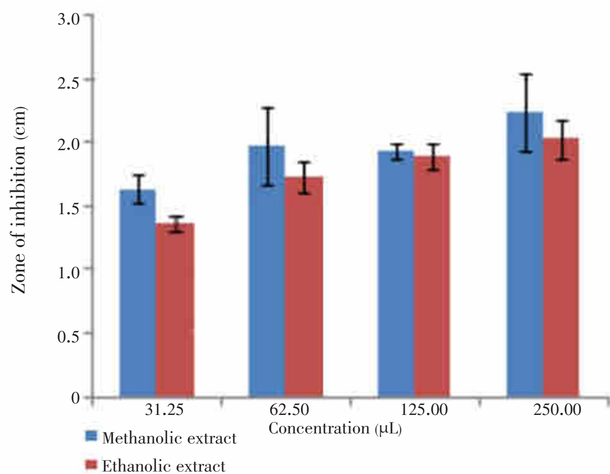

Figure 1. Comparison between inhibitory zones of methanolic and ethanolic extract of *A. heterophyllum* against *A. niger*.

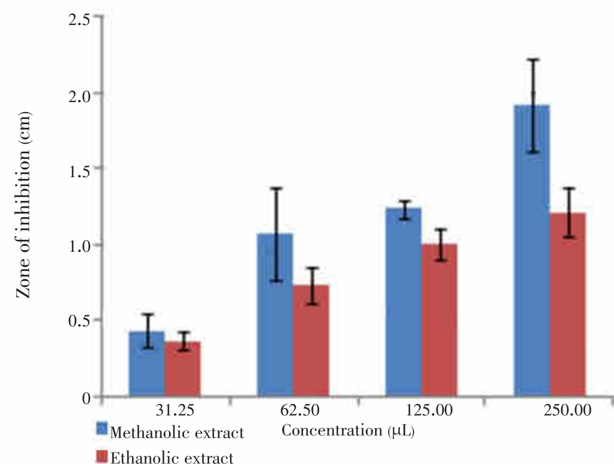

Figure 3. Comparison between inhibitory zones of methanolic and ethanolic extract of *P. bistorta* against *A. niger*.

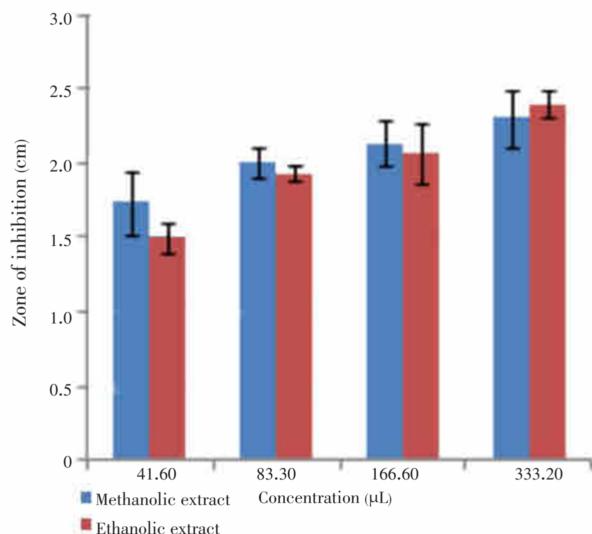

Figure 2. Comparison between inhibitory zones of methanolic and ethanolic extract of *A. heterophyllum* against *A. solani*.

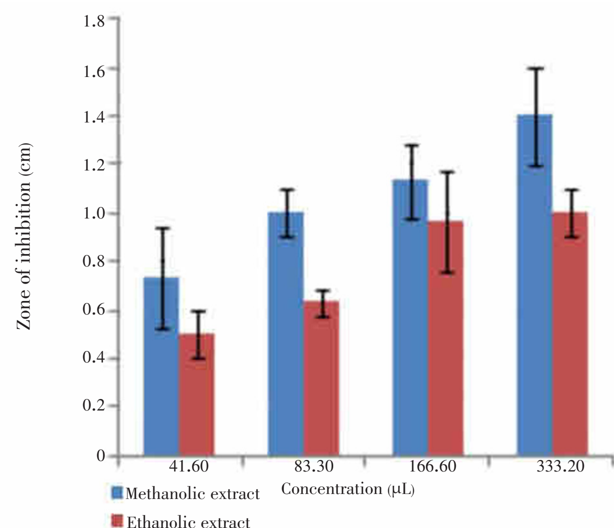

Figure 4. Comparison between inhibitory zones of methanolic and ethanolic extract of *P. bistorta* against *A. solani*.

mL on the minimum concentration so that ascorbic acid has more antioxidant activity.

IC_{50} value for ethanolic extract of *A. heterophyllum* was 83.02 μg/mL and for methanolic extract its value was 57.36 μg/mL. Hence *A. heterophyllum* in both solvents, ethanol and methanol, had antioxidant activity lesser than ascorbic acid at all the concentration. However, it was observed that the methanolic extract of *A. heterophyllum* showed high antioxidant activity than ethanolic extract of *A. heterophyllum* with significant difference (P=0.02).

IC_{50} value for methanolic extract of *P. bistorta* was 49.20 μg/mL and for ethanolic extract its value was 61.14 μg/mL. The IC_{50} values indicated that at all the concentrations and solvents tested during the present work, antioxidant activity of *P. bistorta* was lesser than ascorbic acid. The ethanolic extract of *P. bistorta* had more anti-oxidant potential with significant difference (P=0.02) than methanolic extracts.

Table 1

Percentage inhibition of methanolic and ethanolic extract of *A. heterophyllum* and *P. bistorta*.

Test compound	Concentration (μL)	Inhibition (%)	Mean IC_{50} (Y=mx+c)
Ascorbic acid as standard	31.25	74.26±0.54*	75.31
	62.50	74.88±0.78*	
	125.00	76.03±1.00*	
	250.00	76.07±0.93*	
A. heterophyllum ethanolic extract	31.25	77.82±0.41*	83.02
	62.50	81.15±0.91*	
	125.00	84.59±0.37*	
	250.00	88.51±0.47*	
Ascorbic acid as standard	41.60	46.88±0.78*	50.43
	83.30	46.93±0.89*	
	166.60	52.81±0.74*	
	333.20	55.11±1.02*	
A. heterophyllum methanolic extract	41.60	52.09±0.35*	57.36
	83.30	55.67±0.75*	
	166.60	56.84±0.78*	
	333.20	64.84±0.74*	
Ascorbic acid as standard	25.00	40.70±0.53*	51.48
	50.00	46.88±0.99*	
	100.00	56.84±0.75*	
	200.00	61.47±0.53*	
P. bistorta ethanolic extract	25.00	52.09±0.72*	61.14
	50.00	55.67±0.75*	
	100.00	67.07±0.53*	
	200.00	69.72±0.36*	
Ascorbic acid as standard	11.36	41.18±0.35*	45.40
	22.72	41.86±0.32*	
	45.00	43.08±0.91*	
	90.00	53.13±0.31*	
P. bistorta methanolic extract	11.36	42.70±0.75*	49.20
	22.72	44.84±0.66*	
	45.00	53.13±0.90*	
	90.00	56.21±0.60*	

Data of percentage inhibition are expressed as mean±SD.

4. Discussion

In the present study both the plants exhibited good antimicrobial as well as antioxidant activity.

During the present work antifungal activity was recorded by agar well diffusion method. This method has been found to be effective by many workers. Earlier it has been investigated the antifungal potential of the raw methanolic extracts of five herbs and species by agar well diffusion method.

In the present study, it was observed that methanolic extracts of *A. heterophyllum* showed antimicrobial potential against *A. niger* and *Alternaria solani*. The minimum inhibitory concentration of methanolic extract of *A. heterophyllum* obtained during the present work was also found to be greater than ethanolic extract for both the tested organisms. The alkaloids from the roots of *A. heterophyllum* have been reported to have considerable antimicrobial and enzyme inhibition activities[16]. Hence it can be suggested that the solvent greatly influence the antifungal activity of the extract. In a previous study, it was also reported that minimum inhibitory concentration of the methanolic extract of *Aconitum* has a significant antifungal activity[17]. Methanolic extract of aerial parts of *A. heterophyllum* was the more potent extract. Determination of the surviving fractions of strains against the increasing concentrations of methanol revealed that there was a considerable decrease in the surviving fractions with the increasing concentration of the extracts.

The IC_{50} values indicate that at all the concentrations and solvents tested during the present work, antioxidant activity of *A. heterophyllum* is lesser than ascorbic acid which was used as standard. *P. bistorta* in ethanol and methanol has antioxidant activity lesser than ascorbic acid compared with all the concentrations, however, IC_{50} values indicate that this plant has more activity in methanolic extracts. IC_{50} value for the extract of *P. bistorta* in ethanol has more antioxidant activity as ($P \leqslant 0.05$) than all the extracts used while extract of *A. heterophyllum* in methanol has more inhibition activity than that of in ethanol ($P \leqslant 0.05$). In an earlier study, it was found that the antioxidant activity of the *A. heterophyllum* extract using DPPH was very normal as illustrated through a low IC_{50} value compared to ascorbic acid[18]. The ethanolic exract of *A. heterophyllum* in all *in vitro* antioxidant models exhibited a low to normal antioxidant activity. While in present study the ethanolic extract of *Aconitum* also depicted a low IC_{50} value compared to the ascorbic acid IC_{50} value.

The type of method which was used for analysis of antioxidant activity during the present work was DPPH assay. A comparison has been made by Chang *et al.*

to determine the antioxidant potential of the extracts from *P. bistorta* by three methods, namely, DPPH radical scavenging, diammonium salt radical scavenging, ferric reducing or antioxidant power assay and 2,2–azino–bis–3–ethylbenzothiazoline–6–sulfonic acid assay[19]. The results were compared with 6–hydroxy–2,5,7,8–tetramethylchroman–2–carboxylic acid (trolox) and positive control 2,6–di–tert–butyl–4–methylphenol (BHT). The results obtained by all of these methods showed that *P. bistorta* had good antioxidant activity *in vitro*. The methanolic extract of *P. bistorta* showed higher DPPH and diammonium salt radical scavenging activity than that of BHT. It exhibited higher ferric reducing activity than that of BHT. Among the three extracts of *P. bistorta*, the methanolic extract showed the highest antioxidant activity, followed by ethyl acetate extract.

The present study revealed that the consumption of these medicinal plants could be beneficial to mankind by virtue of their effective antioxidant and antimicrobial activity. Plant extracts are almost safe and there is no concern about their toxicity, hence, they could be exploited as additional source of antioxidants or as nutritional supplements. Results of the findings further confirm the use of these herbs as traditional medicine and may be used as effective and potential sources of novel antioxidant and antimicrobial drugs.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Kelly K. *History of medicine*. New York: Facts on File; 2009, p. 29–50.

[2] Joy PP, Thomas J, Mathew S, Skaria BP. Medicinal plants. In: Bose TK, Kabir J, Das P, Joy PP, editors. *Tropical horticulture*. Culcutta: Naya Prakash; 2001, p. 449–632.

[3] Zhao XY, Wang Y, Li Y, Chen XQ, Yang HH, Yue JM, et al. Songorin, a diterpenoid alkaloid of the genus *Aconitum*, is a novel GABA(A) receptor antagonist in rat brain. *Neurosci Lett* 2003; **337**: 33–36.

[4] Nasir E, Ali SI. *Flora of Pakistan*. Islamabad: PanGraphics Ltd.; 1983, p. 1–22.

[5] Liu XQ, Li WW, Sheng KX, Liu J, Chen FK. Studies on the chemical constituents of the *n*–BuOH extract of *Polygonum bistorta* L. *J Shenyang Pharm Univ* 2006; **1**: 15–17.

[6] Kumar MD, Deepmala J, Sangeeta S. Hepatoprotective effects of *Polygonum bistorta* and active principles on albino rats intoxicated with carbon tetrachloride and paracetamol. *Open Access Scientific Report* 2012; doi: 10.4172/scientificreports.226.

[7] Chevallier A. *The encyclopedia of medicinal plants: a practical reference guide to over 550 herbs and their medicinal uses*. New York: DK Publishing; 1996, p. 336.

[8] Duke JA, Ayensu ES. *Medicinal plants of China*. Algonac: Reference Publ; 1985.

[9] Lust JB. *The herb book: the complete and authoritative guide to more than 500 herbs*. New York: Benedict Lust Publications; 2001, p. 626.

[10] Duwiejua M, Zeitlin IJ, Gray AI, Waterman PG. The anti–inflammatory compounds of *Polygonum bistorta*: isolation and characterisation. *Planta Med* 1999; **65**(4): 371–374.

[11] Luis JC, Valdés F, Martín R, Carmona A J, Díaz JG. DPPH radical scavenging activity of two flavonol glycosides from *Aconitum napellus* sp. lusitanicum. *Fitoterapia* 2006; **77**(6): 469–471.

[12] Polterait O. Antioxidants and free–radical scavengers of natural origin. *Curr Org Chem* 1997; **1**: 415–440.

[13] Xia EQ, Cui B, Xu XR, Song Y, Ai XX, Li HB. Microwave–assisted extraction of oxymatrine from *Sophora flavescens*. *Molecules* 2011; **16**(9): 7391–7400.

[14] Boyanova L, Gergova G, Nikolov R, Derejian S, Lazarova E, Katsarov N, et al. Activity of *Bulgarian propolis* against 94 *Helicobacter pylori* strains *in vitro* by agar–well diffusion, agar dilution and disc diffusion methods. *J Med Microbiol* 2005; **54**(Pt 5): 481–483.

[15] Braca A, Fico G, Morelli I, Simone F, Tomè F, Tommasi N. Antioxidant and free radical scavenging activity of flavonol glycosides from different *Aconitum* species. *J Ethnopharmacol* 2003; **86**(1): 63–67.

[16] Ahmad M, Ahmad W, Ahmad M, Zeeshan M, Shaheen F. *Norditerpenoid alkaloids* from roots of *Aconotum heterophyllum* wall with antibacterial activity. *J Enzyme Inhib Med Chem* 2008; **23**(6): 1018–1022.

[17] Srivastava N, Sharma V, Saraf K, Kumar A, Kamal B, Singh Jadon JS. *In vitro* antimicrobial activity of aerial parts extracts of *Aconitum heterophyllum* Wall. ex Royle. *Indian J Nat Prod Resour* 2011; **2**(4): 504–507.

[18] Prasad SK, Kumar R, Patel DK, Sahu AN, Hemalatha S. Physicochemical standardization and evaluation of *in–vitro* antioxidant activity of *Aconitum heterophyllum* Wall. *Asian Pac J Trop Biomed* 2012; **2**(Suppl 2): S526–S531.

[19] Chang X, Liu YX, Kang WY. Antioxidant activity of extracts from *Polygonum bistorta* L. *Fine Chem Interm* 2009; **39**(2): 28–31.