Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.elsevier.com/locate/JOPR

Original Article

Screening of phytochemicals, antioxidant and antibacterial activity of crude extract of *Pteridium aquilinum* Kuhn

D. Kardong, S. Upadhyaya, L.R. Saikia*

Department of Life Sciences, Dibrugarh University, Dibrugarh 786004, Assam, India

ARTICLE INFO

Article history:
Received 20 August 2012
Accepted 10 November 2012

Keywords:
P. aquilinum
Antioxidant activity
Antimicrobial activity

ABSTRACT

Leaves of *Pteridium aquilinum* collected from Dibrugarh was analysed for phytochemical constituents, antioxidant activity and antimicrobial property. The present study reveals that saponin, tannin, phenol, flavonoid, terpenoid, cardiac glycoside, alkaloid and reducing sugar were present in the sample while steroid and anthraquinone were not detected. The results revealed significant amount of antioxidant and antimicrobial activity. Ethanol and petroleum ether extracts showed antimicrobial activity against *Bacillus subtilis, Streptococcus aureus, Proteus vulgaris* and *Escherichia coli* with zones of inhibition ranging from 16 to 20 mm, but all the test organisms are highly resistant to methanol, chloroform and aqueous extract of the herb. The MIC test of the ethanolic and petroleum ether extract of *P. aquilinum* against *B. subtilis* and *S. aureus* was observed as 1 mg/ml and that for *E. coli* and *P. vulgaris* it was found as 0.8 mg/ml. Extracts of the plant were better/equally effective against tested organisms as compared to streptomycin except *P. vulgaris*. The study provides a scientific basis for the use of the plant extracts in home-made remedies and in the treatment of microbial-induced ailments.

1. Introduction

Plants are the major source of medicines and foods which play a vital role in maintenance of human health. The importance of plants in medicine remains even of greater relevance with the current global trends of shifting to obtain drugs from plant sources, as a result of which attention has been given to the medicinal value of herbal remedies for safety, efficacy, and economy.[1,2] The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body.[3] These plants are source of certain bioactive molecules which act as antioxidants and antimicrobial agents.[4–7]

Pteridium aquilinum Kuhn. belonging to family polypodiaceae grows wild in Assam. It has wide range of traditional application from use in witch craft to ethnomedicines and food additives. Leaves of the herb are used externally as painkiller, as herbal additives in traditional preparation of alcoholic beverages, and the tender leaves of the plant is used as vegetables by some ethnic communities of Assam. The present study looks into the fundamental scientific basis for the use of this herb by analysing the

* *Corresponding author.*
E-mail address: sristi_eco@rediffmail.com (S. Upadhyaya).
0974-6943/$ – see front matter
http://dx.doi.org/10.1016/j.jopr.2012.11.037

crude phytochemical constituents, antioxidant and antibacterial activity.

2. Materials and methods

Collection and processing of plant material: Leaves of *P. aquilinum* were collected from Dibrugarh in the month of March 2012, shade dried and then powdered. The powdered leaf was separately macerated with ethanol, methanol, petroleum ether, chloroform and distilled water for 48 h and filtered using Whatman filter paper No. 1. The filtrate was then evaporated at a constant temperature of 50 °C until a semi dried powder/sticky mass of plant extract was obtained which is kept in refrigerator for further use. These crude extract were dissolved separately in Dimethyl sulphoxide (DMSO) as neutral solvent to make final concentration for biochemical analysis.

2.1. Phytochemical analysis

Standard biochemical methods were followed for phytochemical analysis of the ethanolic extract of the leaves of *P. aquilinum* as described below:

2.1.1. Test for tannin

To 0.5 ml extract solution, added 1 ml distilled water and 1–2 drops of ferric chloride solution to it and observed for blue black colouration which indicates presence of tannin ii) 10% lead acetate solution was added to 0.5 ml extract solution and observed for white precipitation which indicates presence of tannin.

2.1.2. Test for saponin

0.2 g of the extract was shaken with 5 ml of distilled water and then heated to boil. Frothing shows the presence of saponin.

2.1.3. Test for flavonoid

0.2 g of the extract was dissolved in 10% NaOH solution, yellow colouration indicates the presence of flavonoid.

2.1.4. Test for phenol

To 2 ml of extract solution, added 2 ml of alcohol and few drops of ferric chloride solution and observed for colouration.

2.1.5. Test for cardiac glycoside

Five ml of each extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was under layered with 1 ml of conc. sulphuric acid. A brown ring at the interface indicated the present of cardiac glycoside. (A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may formed).

2.1.6. Test for alkaloid

0.5 g extract was boiled with conc. HCl and filtered. 0.5 ml of picric acid and Mayer's reagent was added separately to about 1 ml of the filtrate in a different test tube and observed for coloured precipitate or turbidity.

2.1.7. Test for anthraquinone

To 0.2 g of extract, added 5 ml of chloroform and 5 ml of 105 ammonia solution. The presence of bright pink colour in the aqueous layer indicated the presence of anthraquinone.

2.1.8. Test for terpenoid and steroid

Five ml of extract solution was mixed in 2 ml of chloroform, and 3 ml of conc. sulphuric acid was added to form a layer. A reddish brown colouration of the interface was formed to indicate the presence of terpenoids. Red colour at the lower surface indicates presence of steroid.

2.1.9. Test for reducing sugar

To 0.5 ml of extract solution, 1 ml of water and heated after adding 5–8 drops of Fehling's solution. Brick red precipitation indicated the presence of reducing sugar.

2.2. Antioxidant activity

2.2.1. DPPH radical scavenging activity[8]

Antioxidants react with 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) radical and convert it to 1, 1-diphenyl-2-picryl hydrazine. The degree of change in colour from purple to yellow can be used as a measure of the scavenging potential of antioxidant extracts. Aliquots of ethanol extract solutions (1 mg/ml) were taken and made up the volume to 3 ml with methanol. 0.15 ml of freshly prepared DPPH solution was added, stirred and left to stand at room temperature for 30 min in dark. The control contains only DPPH solution in methanol instead of sample while methanol served as the blank (negative control). Absorbance was noted at 517 nm using the Systronics make spectrophotometer (Visiscan 167). The capacity of scavenging free radicals was calculated as scavenging activity (%) = $[(Abs_{control} - Abs_{sample}/Abs_{control})] \times 100$ where $Abs_{control}$ is the absorbance of DPPH radical + methanol; Abs_{sample} is the absorbance of DPPH radical + sample extract/standard.

2.2.2. ABTS radical scavenging assay

The ABTS assay was carried out following the method of Re et al.[9] The stock solution included 7 mM ABTS solution and 2.4 mM potassium persulfate solution and mixed them in equal proportion then allowed to react for 12 h at room temperature in the dark and diluted by mixing 1 ml ABTS solution with 60 ml methanol to obtain an absorbance of 0.706 ± 0.001 units at 734 nm using the Systronics make spectrophotometer (Visiscan 167). ABTS solution was freshly prepared for each assay. 1.0 ml ethanol extract (1 mg/ml) was allowed to react with 1 ml of the ABTS solution and the absorbance was taken at 734 nm after 7 min using the spectrophotometer. The ABTS scavenging capacity of the extract was compared with that of ascorbic acid and calculated the percentage inhibition ABTS radical scavenging activity (%) = $[(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100$ where $Abs_{control}$ is the absorbance of ABTS radical + methanol; Abs_{sample} is the absorbance of ABTS radical + sample extract/standard.

2.3. Test for antibacterial property

The standard test organisms for antibacterial activity included the *Escherichia coli* (ATCC 10586), *Pseudomonas*

aeruginosa (ATCC 10662), *Staphylococcus aureus* (ATCC 18590), *Proteus vulgaris* (ATCC 12453) and *Bacillus subtilis* (ATCC 8590) were all pathogenic type and obtained commercially from Hi-media Pvt. Ltd and maintained at 4 °C in nutrient agar media. The subculture was done on regular interval of 2 months.

The in-vitro testing for antibacterial property of the test samples (complexes and ligands) was carried out by standard microbiological agar well method. A suspension of each bacterium with the cell density of approx. 1×10^7 colony forming units CFU/ml, prepared separately in nutrient broth media pre-sterilized at 121 °C for 20 min was used as bacterial inoculums (BI). About 1.0 ml of BI from each test organisms was transferred to different conical flask containing 50 ml pre-sterilized nutrient agar medium ($temp^r \leq 40$ °C). After proper mixing, about 20 ml of the culture media in the conical flasks was distributed in two pre-sterilized Petri plates each and then allowed to settle for solidification of the media. Wells measuring the diameter of 6.0 mm were bored at equidistant places in the nutrient agar media and each was impregnated with test compounds (100 μg/ml) dissolved in DMSO and incubated at 37 °C for 24 h. The antibacterial property was measured and expressed as diameter (mm) of the zone of inhibition (ZOI) caused by the extracts. All the observations were made in duplicate for each of the test samples. The average of two independent observations was recorded as data in the table.

The minimum inhibitory concentration (MIC) of the ethanolic extract was determined by preparing solution of varying concentration (0.2, 0.4, 0.6, 0.8 and 1 mg/ml). The streptomycin (25 mcg/disc) sensitivity of the reference bacterial strains was assessed by the disc diffusion method.

3. Results and discussion

The phytochemical characters of all the samples are summarized in Table 1. Presence of alkaloids, tannins, saponin, terpenoid, flavonoid, phenol and cardiac glycoside and absence of anthraquinone and steroid were recorded in the sample. These phytochemicals are playing vital role for the treatment of different types of diseases and therefore they are still used in modern and traditional system of medicine. Results obtained from Table 2 shows that leaves of the herb contain good amount of antioxidant activity which is comparable with that of ascorbic acid. The result of the antibacterial activity are encouraging as ethanol and petroleum ether extracts exhibited antibacterial properties against 4 tested bacteria out of 5 (Table 3). These two extracts showed antimicrobial activity against *B. subtilis*, *S. aureus*, *P. vulgaris* and *E. coli* with zones of inhibition ranging from 16 to 20 mm. *P. aeruginosa* was found to be resistant against the plant extracts. However the extraction method did effect the antibacterial activity of the plant extracts; extracts prepared in methanol, chloroform and distilled water did not show any inhibitory activity against all the test organisms. The observed difference in antibacterial activity with respect to extraction methods might be attributed to incomplete leaching of the active substances at ambient temperature and loss of active components during boiling.

The MIC test of the ethanolic and petroleum ether extract of *P. aquilinum* against bacterial pathogens – *B. subtilis* and *S. aureus* were observed as 1 mg/ml. For *E. coli* and *P. vulgaris* it was found to be 0.8 mg/ml.

The different bacterial strains responded to standard antibiotics streptomycin in a variable manner, resulting in zones of inhibition ranges from 7 to 24 mm. Present study revealed that extracts of the plant were better/equally effective against tested organisms except *P. vulgaris* as compared to streptomycin.

In conclusion, leaves of the plant exhibited certain important phytochemicals, antioxidant and broad-spectrum antibacterial activity in significant amount. This plant have been in use for years to treat various ailments. Natural antioxidants of plant origin have greater application and they can also be used as nutraceuticals and phytoceuticals as they have significant impact on the status of human health and disease prevention.[10] The inhibitory activities of the extracts live up to their potential in the treatment of bacterial induced ailments or diseased conditions, in line with the traditional use of plant extracts. This investigation thus provides a scientific basis for the use of the plant extracts in home-made remedies and their potential use in the treatment of microbial-induced ailments. Further studies may lead to their use as safe alternatives to synthetic antimicrobial drugs. Detail work by using different approaches will be the aim of further investigation.

Table 1 – Results of phytochemical screening of extracts of *P. aquilinum* leaves.

Constituents	Sample
Tannin	+
Phlobatanin	–
Saponin	+
Phenol	+
Flavonoid	+
Glycoside	+
Cardiac glycoside	+
Anthraquinone	+
Alkaloid	+
Terpenoid	+
Steroid	
Carotenoids	–
Reducing Sugar	

+ indicates presence of constituents and – indicate absence of constituents.

Table 2 – Antioxidant activities of *P. aquilinum* leaves.

Sample	Antioxidant activity (% inhibition in mg/ml)	
	DPPH radical scavenging activity	ABTS radical scavenging activity
P. aquilinum	84	73.33
Ascorbic acid	88.2	83

Table 3 – Antibacterial activity of different solvent extract of *P. aquilinum* and streptomycin against bacteria.

Bacteria	Zone of inhibition (mm)					
	Ethanol extract	Petroleum ether extract	Methanol extract	Chloroform extract	Aqueous extract	Streptomycin
E. coli	18	16	–	–	–	8
S. aureus	20	20	–	–	–	20
P. vulgaris	16	16	–	–	–	24
B. subtilis	18	22	–	–	–	7
P. aeruginosa	–	–	–	–	–	16

Conflicts of interest

All authors have none to declare.

Acknowledgement

Authors are thankful to the Dibrugarh University, Assam, India for providing necessary facilities.

REFERENCES

1. Glombitza KW, Mahran GH, Mirhom YW, Michael KG, Motawi TK. Hypoglycemic and Antihyperglycemic effects of *Zizyphus spina-christi* in rats. *Planta Med*. 1993;60:244–247.
2. Mahabir D, Gulliford MC. Use of medicinal plants for diabetes in trinidad and tobago. *Rev Panam Salud Publica*. 1997;1:1–16.
3. Edeoga HO, Okwo DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. *Afr J Biotechnol*. 2005; 4:685–688.
4. Sengul M, Yildiz H, Gungor N, Cetin B, Eser Z, Ercisli S. Total phenolics content, antioxidant and antimicrobial activities of some medicinal plants. *Pak J Pharm Sci*. 2009;22:102–106.
5. Chopra RN, Nayer SL, Chopra IC. *Glossary of Indian Medicinal Plants*. 3rd ed. New Delhi: Council of Scientific and Industrial Research; 1992.
6. Bruneton J. *Pharmacognosy Phytochemistry Medicinal Plants*. France: Lavoisiler Publishing Co; 1995.
7. Khalil MY, Moustafa AA, Naguib NY. Growth, phenolic compounds and antioxidant activity of some medicinal plants grown under organic farming condition. *World J Agric Sci*. 2007;3:451–457.
8. Stanojevic L, Stanojevic M, Nikolic V, Nikolic L, Ristic J, Canadanovic Brunet V. Antioxidant activity and total phenolic and flavonoid contents of *Hieracium pilosella* L extracts. *Sensors*. 2009;9:5702–5714.
9. Re R, Pelleorini N, Proteggente A, Pannala A, Yang M, Rice Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med*. 1999;26:1231–1237.
10. Baruah IC, Barua M. Non-conventional food plants of medicinal use in northeast India. In: Borthakur SK, Ahmed A, Gogoi P, Dutta DN, Ahmed GU, eds. *Medicinal Plants of Northeast India*. Guwahati, Assam: North Eastern Development Finance Corporation Ltd; 2010.