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Original article

Antibacterial evaluation and preliminary phytochemical screening of selected ferns from Iran

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Abstract

Background and objectives: The main aims of this study have been finding out the antibacterial activity and preliminary phytochemical screening of some fern species. **Methods:** The antimicrobial activity of the methanol extracts of *Polypodium interjectum* Shivas, *Polystichum woronowii* Fomin, *Polystichum aculeatum* (L.) Roth., *Dryopteris affinis* (Lowe) Fraser-Jenk, *Athyrium filix-femina* (L.) Roth, *Asplenium scolopendrium* L., *Asplenium adiantum-nigrum* L. and *Pteris cretica* L., was screened by measuring the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values against two Gram positive and Gram negative bacteria, *Escherichia coli* and *Staphylococcus aureus*, using standard assays. Besides, the phytochemical evaluation and measurement of the total flavonoid contents were also performed. **Results:** The maximum activity was exhibited by the extract of *Dryopteris affinis* with MIC value of 2 µg/mL. *Polystichum aculeatum* showed the same antibacterial potential against *S. aureus.* Some of the extracts had strong antibacterial activity (2-8 µg/mL) and others demonstrated moderate activity. Phytochemical analyses showed the presence of some important secondary metabolites in Iranian fern species. Triterpenoids and polyphenols were present in rhizome and aerial part of all plants. Total flavonoid contents range was 1.66 to 44.22 mg of catechin equivalents per gram of dry extract. **Conclusion:** Findings indicated that Iranian ferns have good antibacterial potential and could be a suitable source for antibiotic drug discovery.

Keywords: antibacterial, fern, phytochemical screening, total flavonoid

Introduction

Hundreds of herbs are used traditionally in Iranian folklore medicine for treatment of various diseases [1-3]. Many medicinal plants have been screened extensively for their antimicrobial potential worldwide [4]. Microbial infectious diseases and the rapid appearance of some drug resistant strains of bacteria to current antimicrobial agents have prompted the creation

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of drugs to avoid these defenses and also it has led to intensive searches for new natural antibiotics isolated from various medicinal plants to treat microbial infections [5]. Infections by *Staphylococcus aureus* are among the most common problems due to its resistance against various antibiotics. *Escherichia coli* is also responsible for serious infections and diarrhea [5]. It has been observed that ferns are not infected by microbial pathogens [6]. This group of plants has tremendous ornamental, medicinal, ethno-botanical, evolutionary, ecological and environmental significance. There are over 250 different genera of ferns and about 13000 species are distributed all around the world [7]. 2600 species of ferns grow in China [8], 1200 in India [9] and 1300 in Brazil [10]. According to the last studies on ferns of Iran, occurrence of 52 species in 26 genera and 15 families have been confirmed [11]. Many of them grow in north of Iran along the Caspian sea and in other areas restricted to shadowy and dump places, particularly in Zagros and Alborz mountains [12]. The economic importance of these ferns is mostly as important ornamental cultivars in the horticultural trade. Ferns have great potential due to their medicinal characteristics, but their chemical and biological properties relatively have not been explored [13]. Many kinds of ferns are used in Chinese, Indian and Iranian Traditional Medicine for the treatment of several illnesses [14]. They are extensively used to treat the skin tumefaction, protect the liver and treat hepatitis and they are also used as antipyretic agents [15]. About 300 kinds of ferns were used in Chinese Traditional Medicine for treatment of common cold, diarrhea, burns, trauma and bleeding while they have shown many bioactivities such as antioxidant, antimicrobial, antiviral, anti-inflammatory, antitussive, antitumor and anti-HIV properties [16]. *Polypodium vulgare* has been traditionally used for treatment of infections, asthma, and leprosy in Iran. *Asplenium adiantum-nigrum* has been used for treatment of spleen, kidney and bowel diseases. *Athyrium filix-femina* has been used as lenient and as an anti-inflammatory herb and for the treatment of ascarid disease [17]. Bioactive constituents of ferns exhibit diverse pharmacological properties which include antioxidant, antibacterial, anti-tumor, and anti-inflammatory activities [18]. Flavonoids, phenolics, alkaloids, steroids, triterpenes and polysaccharides are the isolated classes of constituents from fern species [19]. The phytochemical potential of ferns is relatively unexplored, although they possess great economic potential due to some interesting medicinal and antimicrobial properties [20]. Although there is a wide folkloric application of ferns in Iran, unfortunately no systematic report regarding their biological activities and chemical constituents could be found. So, as part of an ethnopharmacological project, searching for natural antibacterial agents from Iranian medicinal ferns, we found that the methanol extracts of several Iranian fern species possess the strong antibacterial potential. Also a phytochemical screening was performed using the methanol extracts of leaves and rhizomes of these ferns, for determination of main chemical constituents [21,22]. The total flavonoid content of the extracts was determined too. In this research 8 species from 5 families belonging to polypodialese including *Polypodium interjectum*, *Polystichum woronowii*, *Polystichum aculeatum*, *Dryopteris affinis*, *Athyrium filix-femina*, *Asplenium scolopendrium*, *Asplenium adiantum* and *Pteris cretica* (also known as leptosporangiate ferns) have been studied. The name of the plants, herbarium codes and folkloric uses [11,12] were listed in table 1. Herein we reported our findings on the antibacterial activities, qualitative phytochemical screening and total flavonoid content of eight native ferns. To the best of our knowledge, this is the first report about the biological properties and bioactive constituents of Iranian ferns.

Experimental

Plant material

The ferns were collected in October 2012 from

Table 1. List of ferns and related folkloric uses

No	Scientific name	Family	Voucher number	Folkloric uses [11,12]	Part used	Mode of use [11,12]
1	*Polypodium interjectum* Shivas	Polypodiaceae	MPH-1967	Psoriasis, bronchitis, skin disorders	Rhizome	Infusion
2	*Polystichum woronowii* Fomin	Dryopteridaceae	MPH-1968	Hepatitis, anti-inflammatory	Leaf	Decoction/ topical
3	*Polystichum aculeatum* (L.) Schott	Dryopteridaceae	MPH-1969	Anthelmintic	Leaf	Decoction
4	*Dryopteris affinis* (Lowe) Fraser-Jenk	Dryopteridaceae	MPH-1970	Antimicrobial	Leaf	Topical
5	*Athyrium filix-femina* (L.) Roth	Woodsiaceae	MPH-1971	Antiparasitic, anthelmintic	Rhizome	Decoction
6	*Asplenium scolopendrium* L.	Aspleniaceae	MPH-1972	Spleen enlargement, fever, bronchitis	Rhizome	Infusion
7	*Asplenium adiantum-nigrum* L.	Aspleniaceae	MPH-1973	Kidney disease, anti-inflammatory	Rhizome	Decoction/ topical
8	*Pteris cretica* L.	Pteridaceae	MPH-2010	Antibiotic	Leaf	Topical

the wild forests of different localities in Zirab, Mazandaran Province, north of Iran (36°16 N, 52°96´ E). The average temperature was about 16.5 °C with 725 mm average rainfall. Plants were identified by Mr. Shahram Bahadori (taxonomist) and the voucher specimens were prepared and deposited at the Herbarium of Medicinal Plants and Drugs Research Institute (MPH), Shahid Beheshti University, Tehran, Iran (table 1).

Preparation of methanol extracts

The fern bodies were cleaned to remove any residuals and then shade dried at room temperature for a period of 10 days. All powdered dried plants were extracted by cold maceration method using methanol (consecutively three times). Extraction procedure was performed at room temperature for 24 hours along with shaking. The extracts were filtered through Whatman No.1 filter paper. The solvent was removed using a rotary evaporator at 40 °C to obtain concentrated extracts.

Microbial strains

In vitro antimicrobial activity of the extracts for MIC and MBC assays were assessed against standard strains *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 as models of Gram positive and Gram negative bacteria, respectively. All strains were obtained from the Pasteur Institute of Iran (IPI).

Evaluation of antimicrobial activity

Broth micro-dilution susceptibility assay was performed as recommended by CLSI [23] with some modifications. Serial dilutions of samples were made in a concentration range of 32 to 0.5 µg/mL of the extracts in sterile 96 well trays containing Mueller-Hinton Broth supplemented with 0.5 % Tween 80. The inoculants of the microbial strains were prepared from freshly cultured bacterial strains that were adjusted to 0.5 McFarland standard turbidity using sterile normal saline, and then were further diluted (1:100) by sterile Mueller-Hinton Broth just before adding to the trays. The plates were covered with sterile sealer and minimum inhibitory concentrations (MICs) were recorded after 24 h incubation at 37 °C. MICs were recorded as concentrations which could result in complete inhibition of visible growth of the assessed microorganisms. To indicate bacterial growth, 40 µL of 0.2 mg/mL p-iodonitroterazolium chloride (INT, Sigma) was added to each well and incubated for another 30 min. Inhibition of bacterial growth was visible as a clear, colorless well and the presence of growth was detected by the presence of pink-red color. The lowest concentration showing no change in color was considered as the MIC. For determination of MBC, a loop of liquid from each well that showed no change in color was streaked onto MHA and incubated at 37 °C for 24 h. The lowest concentration that showed no

growth was taken as the MBC. Each sample was assessed in triplicate. Chloramphenicol (Sigma) was used as the standard antibiotic.

Determination of total flavonoid content

The total flavonoid content of methanol extracts was estimated according to a previously described method [24]. The absorbance was measured against a blank at 510 nm. Results were expressed as mg of (+)-catechin equivalents per gram of dried extract. Different concentrations of (+)-catechin as standard (1, 10, 20, 40, 80 µg/mL) were used to construct a calibration curve. The total flavonoid contents were calculated by the linear equation derived from calibration curve: "y= 0.003x + 0.1307" where y is absorbance and x is the flavonoid content in milligram of (+)-catechin equivalents per gram of dry extract (mg CE/g of extract). All measurements were carried out in triplicates.

Qualitative Phytochemical Screening

Phytochemical tests were done and the secondary metabolites were qualitatively tested according to the standard methods [21,22].

Results and Discussion

In order to test the antibacterial capacity of the selected ferns, two bacteria, *Escherichia coli* and *Staphylococcus aureus* were assessed. The minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of methanol extracts of eight fern species were evaluated and the results have been summarized in table 2. The methanol extracts of rhizome and leaves of *Dryopteris affinis* showed low MIC values against both *Escherichia coli* and *Staphylococcus aureus* (2 µg/mL). *Asplenium adiantum* showed MIC values against *E. coli* and *S. aureus* with concentration of 4 µg/ml. *Asplenium scolopendrium* exhibited MIC values 8 µg/mL and 4 µg/mL against Gram negative and Gram positive bacteria, respectively. Results of MBC tests (table 2) also indicated good activities for extracts of rhizome and leaves of ferns. MIC and MBC values, not greater than 32 µg/mL were recorded.

The qualitative phytochemical analysis for ten different classes of natural compounds (coumarins, triterpenoid, saponins, alkaloids, cardiac glycosides, polyphenols, tannins, anthraquinones, anthocyanins and quinones) were carried out and the results were summarized in table 3. This preliminary screening indicated the presence of active phytoconstituents in the methanol extract of the ferns. Presence of terpenoids and polyphenols were detected in all tested samples. Anthocyanins were absent in all tested plants. Quinones and anthraquinones were present in *Polystichum woronowii*, *Polystichum aculeatum* and *Dryopteris affinis*, all of them belong to Dryopteridaceae family.

Using the standard plot of (+)-catechin (y= 0.003x+0.1307, R^2 = 0.9531), the flavonoid contents of the leaves and rhizome of testing ferns were found ranging from 1.66 to 46.22 mg CE/g of dried extract as shown in table 4. The flavonoid content of the leaves of *Asplenium scolopendrium*, *Pteris cretica* and *Polypodium interjectum* was high compared to that of the rhizomes. But in other species total flavonoid content of the rhizome was higher than the leaves.

This is the first comparative assessment report of eight most common species of ferns, found in Iran. All selected fern species, especially *Polystichum aculeatum* and *Dryopteris affinis* showed potent antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. To the best of our knowledge, the antibacterial activity of these fern species and their phytoconstituents profile is reported here for the first time. The flavonoids and other phytochemicals in these species can be responsible for considerable antibacterial activity. Secondary metabolites such as polyphenols and flavonoids, which have phenolic hydroxy functional groups in their structure, may be responsible for the observed bioactivity. Some of the phenolic compounds like ellagic acid and gallic acid have also been reported for potent

Table 2. MIC and MBC values of methanol extracts of some fern species against bacterial strains (µg/mL)

Fern species	*Staphylococcus aureus*				*Escherichia coli*			
	Rhizome		Leaves		Rhizome		Leaves	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Polypodium interjectum	32	>32	32	>32	>32	>32	>32	>32
Polystichum woronowii	16	>32	4	16	4	32	32	>32
Polystichum aculeatum	2	16	8	32	8	>32	32	>32
Dryopteris affinis	2	8	2	16	16	>32	2	32
Athyrium filix-femina	8	32	32	>32	32	>32	32	>32
Asplenium scolopendrium	4	16	>32	>32	8	32	>32	>32
Asplenium adiantum	4	16	32	>32	4	32	>32	>32
Pteris cretica	4	32	32	>32	16	>32	>32	>32
Chloramphenicol	1	8	-	-	4	8	-	-

Table 3. Results of phytochemical screening**.**

Metabolite	*D. aff*		*P. wor*		*P. acu*		*A. fil*		*P. cre*		*P. int*		*A. sco*		*A. adi*	
	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R
Triterpenoids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Coumarins	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+
Saponins	+	+	-	-	-	-	-	-	+	+	+	+	-	-	-	-
Alkaloids	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-
Anthocyanins	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Polyphenols	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tannins	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-
Anthraquinones	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
Quinones	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
Cardiac glycosides	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-

+: presence, -: absence, L: leaves, R: rhizome.

antimicrobial activity [25] and flavonoids have been found to possess antimicrobial properties in various studies [26,27]. In a recent work antibacterial potential of *Blechnum orientale*, an important fern species in China, was assessed against five Gram positive and six Gram negative bacteria using disk diffusion method [28].

Table 4. Total flavonoid content of selected ferns (mg CE/g).

Fern species	Leaves	Rhizome
Polypodium interjectum	44.22±2.50	7.22±0.75
Polystichum woronowii	7.33±0.25	24.33±2.15
Polystichum aculeatum	4.44±0.4	46.22±2.95
Dryopteris affinis	1.66±0.16	5.22±0.66
Athyrium filix-femina	3.22±0.15	3.44±0.25
Asplenium scolopendrium	7.56±0.66	4.00±0.20
Asplenium adiantum	6.33±0.55	19.33±1.45
Pteris cretica	24.56±1.90	4.55±0.33

No activity was found against Gram negative bacteria tested. In another study antimicrobial activity of methanol extract of four *Adiantum* species from Indian traditional medicine, were evaluated and MIC values were determined ranging 0.48-62.5 µg/mL [6]. High phenolic contents were observed in tested ferns in that study. Antibacterial activity evaluation of four ferns from Malaysia have shown MIC values ranging 2.60-50 mg/mL [17]. In contrast to *Escherichia coli* (Gram-negative), *Staphylococcus aureus* (Gram-positive) was more sensitive to the inhibitory effects of the fern extracts. Similar observations were made in other studies which investigated the antibacterial potential of ferns [17,29]. Our results provide important information for the antibacterial efficacy of these plants from Iran. The observations of the present study indicated that a number of Iranian medicinal ferns have antibacterial potential. The results of the present study demonstrated that, these ferns could be new and an efficient source for antibiotic drug discovery and it would be worth to isolate and identify active components in the future.

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Declaration of interest
The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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