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Evaluation of phytochemicals, antioxidant activity of fern *Pteris longifolia* L.

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Abstract
The aim of this study was to find the antioxidant potential of various solvent extracts of *Pteris longifolia* was determined by using DPPH free radical scavenging method along with screening of secondary metabolites. The various extracts showed significant antioxidant potential against DPPH free radicals. The antioxidant activity of the sample was comparable with standard ascorbic acid. Phytochemical screening of fern confirms various secondary metabolites which played important role for controlling the plant and animal disease. The whole extract of *Pteris longifolia* was explored for its versatile phytochemical constituents like flavonoids, tannins, saponins, alkaloids, steroids, phenols and terpenoids (Table 1). The results of this study allow that the plant extracts have huge amount of secondary metabolites and high scavenging power of free radicles.

Keywords: Plant extract, screening, secondary metabolites, Antioxidant, terpenoids

1. Introduction
Plants have in one way to furnish the basic need of mankind – shelter, food, clothing, defense from disease causing agents and treatment of various infections and ailments since early days of human history [1]. A huge range of medicinal plants around the globe has not yet been examined to determine the claims made by traditional folks about their usefulness in treating diseases [2]. Reactive oxygen species are formed as byproducts during the metabolic processes of bio-molecules like proteins, lipids, enzymes, DNA and RNA thus involve in many chronic and degenerative diseases, such as coronary heart disease, inflammation, stroke, diabetes mellitus etc [3]. If reactive oxygen species are formed in excessive amount they can cause oxidative stress. The therapeutic potential of medicinal plants as antioxidants in decreasing oxidative stress made tissue injury [4]. Medicinal plants have infinite variety of secondary metabolites which depends on history, family and phytochemical properties. It was reported that ferns have a lot of beneficial phytochemicals (secondary metabolites) such as terpenoids, phenolic, alkaloid compounds, fatty and amino acids, flavonoid and steroids [5]. Secondary metabolites regulates the ecological interactions between plants and their environment, they are chemically different compounds and exert allopathic process which are used in veterinary, scientific research, agriculture, human therapy and interesting biological activities and much effective against various plant pathogen [6]. Pteris longifolia is a medicinal plant and widely used in herbal products their extract showed highest antioxidant activity against various pathogens.
In the present study qualitative phytochemical screening and antioxidant potential of Pteris longifolia was carried out.

2-Material and Methods
2.1 Collection and identification
Pteris longifolia L. was collected from district Hazara, KPK, and was scientifically identified and authenticated by the help of flora of Pakistan. The voucher specimen was deposited to the Department of Botany, Abdul Wali Khan University, Mardan.

2.2 Preparation of crude extracts
Collected plants were washed carefully with water and unwanted particles were removed, then shade dried at room temperature for 7 days.

The dried plants were grounded into fine powder with the help of electric blender. The dried powder was soaked in methanol, ethanol, chloroform and aqueous at room temperature for one week. The mixtures were shacked regularly during this interval. Then the solutions were filtered by means of No.1 Whatman filter paper, and this process was repeated twice. At 40oC, the filtrate was placed in a water bath and become evaporated and dry. Thus greenish semisolid crude extracts was obtained. Below 4oC, the crude extracts was stored in an airtight container and used for further analysis.

2.3 Phytochemical screening

Preliminary qualitative phytochemical screening was carried out by using standard procedure described by Evans [7].

a. Tannins- About 2.5ml of crude extracts was treated with 1ml of water and boiled on a water bath. Then solution was filtered and minute drops of 2% FeCl3 (ferric chloride) were treated. Dark green color was indicated the occurrence of tannins.
b. Flavonoids- Crude extracts of 2ml was treated with small number of magnesium ribbon. The mixture was dissolved in concentrated hydrochloric acid. After a few minutes pink scarlet color appeared which reassure the formation of flavonoids.
c. Alkaloids- Crude extracts of 1ml was treated with few drops of potassium mercuric iodide (Mayer reagent). Suddenly orange-red precipitate was seen which proved the occurrence of alkaloids.
d. Saponins- Extracts solution of (4ml) was taken in a test tube. Then 2ml of sodium carbonate were mixed and shaken carefully. Formation of forth (honey comb like mass) was appeared which show the presence of saponins.
e. Terpenoids- Plant extracts of (3ml) was treated with concentrated H2SO4 and 2.5ml of acetic anhydride solution was mixed and boiled for one minute. Blue-reddish color was not detected which prove negative result for terpenoids.
f. Phenols- Plant extracts of 2ml was dissolved in methanol. Then 1% of FeCl3 (ferric chloride) solution was added to the mixture. Formation of green-black color observed the presence of phenols.
g. Steroids- Plant extracts of 5g was added with 3ml of chloroform taken in a test tube and shaken carefully, and then few drops of H2SO4 were mixed. Presence of steroids was not detected.

Table 1: screening of *Pteris longifolia* for secondary metabolites

S. No	Secondary metabolites	Methanol	Ethanol	Aqueous
1	Tannins	+	+	+
2	Terpenes	-	-	-
3	Phenols	+	+	+
4	Flavonoids	+	+	+
5	Saponins	-	+	+
6	Steroids	-	-	-
7	Alkaloids	+	+	+

Note '+'present and '–'absent

Fig 1: *Pteris longifolia*

2.4 DPPH radical scavenging activity

Antioxidant property of plant extracts was carried out by using stable free radical DPPH Measured spectrophotometrically [8]. Different quantities of solvent extract ranges from 0.05, 0.1 and 1mg/ml were dissolved in 2ml of methanol and taken in test tubes, and then 1mg/ml DPPH solution were added to each test tube and shaken carefully. Later, the mixture was incubated and left in darkness at room temperature for 30 minutes, absorbance was kept at 517nm. The standard solutions like (vitamin C, DPPH) were prepared in methanol of similar concentrations and used as a control and measured for free radical activity. All samples were tested in triplicates. Percentage of scavenging free radical DPPH was calculated by the following equation.

Inhibition of free radical DPPH% = 100 A0control – A1 sample / A0 control

Table 2: Percentage inhibition in solvent extracts of *Pteris longifolia*

Plant extract	Concentration (mg/ml)	Mean± SD	(%) inhibition
Methanol	0.1mg/ml	0.415±0.004	58.50%
	0.05mg/ml	1.163±0.063	16.30%
	1mg/ml	0.508±0.001	49.80%
Ethanol	0.1mg/ml	1.087±0.007	8.70%
	0.05mg/ml	0.034±0.027	65.40%
	1mg/ml	0.376±0.012	62.40%
Aqueous	0.1mg/ml	0.129±0.011	87.10%
	0.05mg/ml	1.718±0.015	71.80%
	1mg/ml	0.868±0.211	13.20%

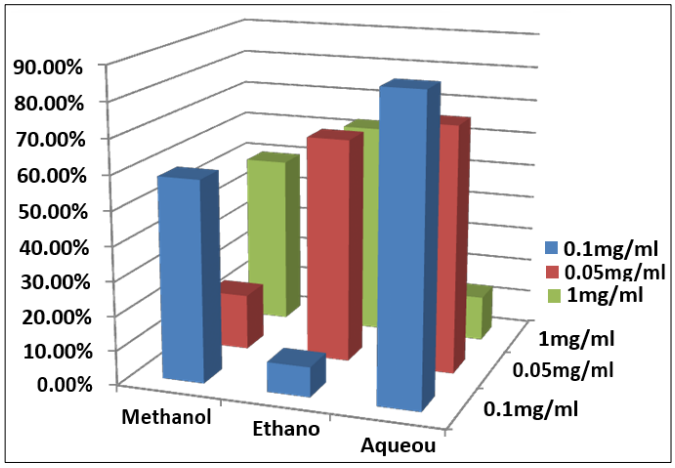

Fig 2: Percentage of antioxidant potential in various extracts of *P. longifolia* L.

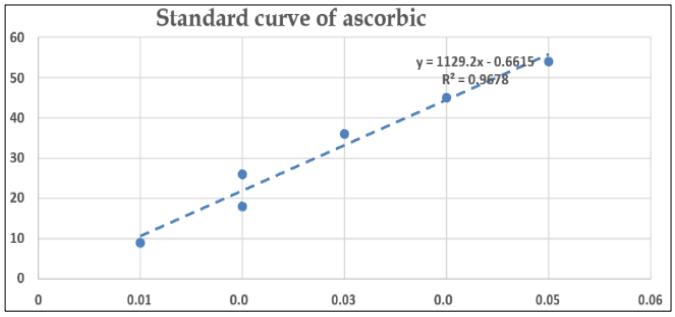

Fig 3: Standard curve of ascorbic acid for evaluating the antioxidant potential

3. Results and Discussion

A plot of antioxidant potential of free radicals versus extracts concentration shows the antioxidant activity of the extracts of Pteris longifolia based on their free radical activity. The inhibition result was presented in Fig. 3 and Table 2. The aqueous extract revealed the great antioxidant potential at all concentration it was remarkably lower ($p<0.05$) the scavenging activity of DPPH and vitamin C. Different qualitative tests were accomplished for the detection of tannins, terpenes, phenols, flavonoids, saponins, steroids and alkaloids in methanol, ethanol and aqueous extracts. Secondary metabolites like tannins, phenols, flavonoids, alkaloids were found in all solvent extracts. Saponin was detected only in ethanol and aqueous extract. But steroids presence undetected in all extracts. So the result specifies presence of metabolites in the Pteris longifolia extract has chief role against harmful pathogens. Earlier studies pointed out the importance of this metabolites such as Tannins are known to hasten the healing of wounds, flavonoids for anticancer activity and kill viral enzymes such as protease and reverse transcriptase, steroids are capable for cholesterol reducing property, saponins are responsible to coagulate the red blood cells (RBC) [9]. The phenolic compounds also help in metabolic process and showed anti-inflammatory and antioxidant activity [10]. Similarly alkaloid acts as diuretic which effects on CNS (central nervous system) and reduces appetite. The antioxidant activity of plant extract is due to the phenolic compounds which exhibit scavenging free radical activity. The phenolic compounds like flavonoids, tannins, carotenoids have the ability to donate hydrogen, quench singlet oxygen species DPPH is stable free radical molecule showed deep purple color their color is disappeared when antioxidant molecules donate hydrogen and convert them into Colorless and decrease its absorbance [11]. Hence, the occurrence of these secondary metabolites in Pteris longifolia increases the therapeutic value of this fern.

4. Conclusion

Studies on Pteris longifolia reveals this plant hold antioxidant properties and many metabolites which exist in plant extracts. This phytochemicals act against various pathogenic infectious diseases. This fern is more dependable for the extensive variety of uses in the field of drug development. So further studies based on the bioactive constituents and clinical trials will be very much needed for growth of new beneficial drugs.

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